Effectiveness of Pepaya Leaf Extract (*Carica Papaya* L.) to Control Ectoparasite *Argulus* on Common Carp (*Cyprinus Carpio*)

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**Abstract.** *Argulus* controls on common carp can be done by using natural ingredients. However, due to the lack of information and products of natural ingredients, consumers choose to use chemicals products. One of natural ingredients that can be used is papaya leaf extract. Papaya leaves contain active ingredient *alkaloid carpain*, which is a vegetable insecticide and a potential antiparasite especially against *Argulus*. The method used in this extraction was the maseration method, which is a soaking process that continues to the treatment stage. The research design used was Completely Randomized Design with P0 control (without papaya leaf extract), 2 ppt, 4 ppt, and 6 ppt, and it was repeated five times. The main parameter in this study was the number of *Argulus* detached from goldfish. The data were then analyzed using Variance Analysis (ANAVA), then continued with Duncan Multiple Range Test with the lapse of the significance at 0.05%. The results showed that papaya leaf extract can control *Argulus* infestation on common carp and detach it from common carp. Treatment P0 (control) with an average percentage of the amount of *Argulus* that had been isolated was 0%, treatment P1 (2 ppt) equals to 24%, treatment P3 (4 ppt) equals to 52%, treatment P3 (6 ppt) equals to 88%. Treatments P1, P2, and P3 were not significantly different (P> 0.05), whereas P1, P2, and P3 were significantly different from P0 (P> 0.05). The effective dose of papaya leaf extract to control the *Argulus* infestation common carp was 6 ppt, which can isolate *Argulus* from common carp as much as 88%.

**Keywords.** Control *Argulus*, Papaya Leaf Extract, Alkaloid Carpain, Common Carp.

1. **Introduction**

The development of goldfish cultivation has a future potential prospect. The achievement of goldfish production in 2010-2013 was 282,695 tons, 332,206 tons, 374,366 tons, and 340,863 tons respectively (Directorate General of Aquaculture, 2013). The common problem in fish farming is disease outbreak, which can cause a decrease in the number of goldfish production (Sumiati and Aryati, 2010). One of the diseases that commonly strikes carp cultivation (*Cyprinus carpio*) is the attack of ectoparasites *Argulus*, generally attacking fins, gills, skin, and operculum (Suhendra, 2006).

The outbreak of *Argulus* can result in the production of excess mucus followed by increasingly weak fish conditions and disruption of the fish growth (Huda, 2008). These parasitic attacks generally cause secondary infections that can cause mass death of fish, so that the level of survival of fish is low (Tam, 2005). Until now, the control carried out on *Argulus* in carp is still done in a simple method, which is removing one by one of the attached parasites. Some carp farmers also overcome *Argulus* outbreak using chemicals that can damage the environment and surrounding ecosystems (Walker, 2008).

An alternative solution to overcome this problem is to use natural ingredients like herbs, which are environmentally friendly, safe, easily available, and potential to treat parasitic *Argulus* in carp. One of the plants that can be used to control the *Argulus* parasite in fish is papaya leaves that contain alkaloid...
carpain (Muhlisah, 2001). Papaya leaf extract containing alkaloid carpain is expected to be used to control Argulus, so it can help the problems of carp farmers, especially in controlling the attack of Argulus ectoparasites.

The objective of this research is to determine and analyze the effectiveness of papaya leaf extract (Carica payaya L.) on the infestation of Argulus on carp (Cyprinus carpio) and find out the optimal and appropriate dose of papaya leaf extract (Carica payaya L.) for controlling the infestation of Argulus in carp (Cyprinus carpio). The benefit of this research is that it provides and complements scientific information about the effectiveness of papaya leaf extract on the amount of Argulus detached from the body of the carp. The results of this research are beneficial to determine the optimal and appropriate dose of papaya leaf extract (Carica payaya L.) to control Argulus infestation in carp (Cyprinus carpio).

2. Method
The research was conducted in February 2018. The research method used was the experimental method using test animals of carp (Cyprinus carpio) as the object of research. Experimental research was carried out by giving certain treatments to research objects under controlled conditions (Jaedun, 2011) to determine the effect of papaya leaf extract on the amount of Argulus isolated from carp.

Fresh papaya leaves were washed. The papaya leaves used were the old ones with a dark green color taken from the stem to 3 or 4 papaya trees from below. Old papaya leaves according to Olafsdottir et al., (2002), are rich in karpain alkaloids, which is about 0.4%. Carp used in the study must be acclimatized first in maintaining the aquarium (Elkajuli, 2005). The extraction method used was the maceration method, which is soaking extract material with a solvent ratio of 1: 5 (Suriaman, 2017). Extraction started from washing and weighing. Papaya leaves were cut into small pieces and then dried at room temperature 30-35oC in ± 3-4 days. After that, it was grinded to form a powder grinding machine. ± 500 gram papaya leaves were macerated using 96% ethanol solution 2.5 liters for ± 3 days while stirring occasionally. The results of maceration were then filtered with a Buchner funnel, to get the filtrate. Following that, the filtrate was evaporated in the rotary evaporator at a temperature of ± 60 ° C, 40 rpm in ± 1 hour to obtain 100% concentrated extract (Sulistianto, 2004). The total yield of papaya leaf extract was calculated by comparing the weight of powder with the total weight of total papaya leaf extract (Department of Health, 2000). This research used goldfish with length of 7-10 cm. Argulus was first separated from the host (fish) for 2-3 hours, so that in the artificial infestation process it would be faster to attach fish to obtain nutrients in the new host. The artificial infestation method Argulus refers to (Kismiyati). , (2009).

Based on preliminary research, the results of the dosage reference of papaya leaf extract that can be used in the treatment were P0 control (without papaya leaf extract), P1 (2 ppt papaya leaf extract), P2 (papaya leaf extract 4 ppt), and P3 (papaya leaf 6 ppt extract). Each carp was infested with five Argulus, then observed for 60 minutes. After that, the percentage of detached Argulus from carp was calculated. Research on papaya leaf extract (Carica payaya L.) to control Argulus infestation in carp (Cyprinus carpio) was analyzed by analysis of variance (ANOVA). Further test employed Duncan's Multiple Distance Test with a significance of 5% (Kusriningrum, 2008).

3. Result and discussion
3.1. Result
3.1.1. Extraction of Papaya Leaves (Carica payaya L.)
Papaya leaf extract (Carica payaya L.) by maceration method produced 64.97 gram extract with a yield of 12.9%. The yield value was influenced by the particle size or simplicia powder of the material used. This is because finer powder will be more easily extracted because the surface of the simplicia powder is in contact with the wider liquid canvas. Meanwhile, the more crude the simplicia powder, the more cells that must be penetrated by the solvent. The smaller the size of the simplicia powder, the greater the yield of the extract is (Indonesian Ministry of Health, 2000). The effect of papaya leaf extract can be seen in Table 1.
3.1.2. Fish behavior

Observation of fish behavior was carried out before, during and after treatment. The behavior observed was the movement and metabolism of fish. Changes in behavior in carp show clinical symptoms caused by Argulus, which were excessive mucus production, a weakened state of the fish and the rubbing of the body on rough surfaces (Kismiyati et al., 2011).

Table 2. Fish behavior.

| No | Treatment | Fish Behavior |
|----|------------|---------------|
|    |            | Before Treatment | During Treatment | After Treatment |
| 1  | PO (control) | Swam actively, bright body color. | Fish was its body against aquarium wall, fish were at the bottom of the aquarium, and excessive mucus production | swam actively |
| 2  | P1 (2 ppt) | Swam actively, bright body color | Fish was its body against aquarium wall, excessive mucus production | Swam actively |
| 3  | P2 (4 ppt) | Swam actively, bright body color | Fish tended to stay at the bottom of aquarium and produced feces and mucus | Swam actively |
| 4  | P3 (<6 ppt) | Swam actively, bright body color | Passive movement, feces excretion, excessive mucus production, fish tended to swim at the surface | Swam actively |

3.1.3. Water Quality Observation

Table 3. Value of water quality.

| Treatment | Standard. Ivlutu CPP- Number S2 Year 2001 | P^t?jn eter ECuslitas A ii |
|-----------|------------------------------------------|--------------------------|
| PO H.Loll L1 | P2. (4 ppt) | P3 ppO |
| t>1> | pi d Ppt> | |
| Before Treatment | | |
| S | ■ & ■ | o s |
The results observed water quality did not differ between before and after treatment. DO observations of treatments P1, P2, and P3 experienced a slight decrease after treatment. Observation of treatment temperatures P1, P2, and P3 experienced a slight increase after treatment. Meanwhile, the overall pH value were likely to be stable after treatment.

3.2. Discussion

3.2.1. Extraction of papaya leaves

Based on the extraction results, the value produced was 12.9%, influenced by the type of solvent used. The selection of ethanol solvents in this study was due to the fact that ethanol is a polar solvent. Therefore, active compounds such as alkaloids, flavonoids, saponins, etc. in papaya leaves, which are also polar, can dissolve well (MOH, 2000). The results of extraction, were then calculated of its yield value. The value is the number of compounds extracted by solvents and can be calculated by comparing the extracted extracts with the initial weight of papaya leaf powder (MOH, 2000). The value is influenced by the particle size or simplicia powder of the material used. Finer powders will be easier to extract because the surface of the simp lysia powder that is in contact with the fluid of the dancer becomes wider. Meanwhile, the more crude the simplicia powder, the more cells the solvent must penetrate. As a result, the smaller the size of the simplicia powder, the greater the amount of the extract is.

3.2.2. Effect of papaya leaf extract (Carica papaya L.) on detachment of argulus in carp (Cyprinus carpio)

The control method used was dipping, which is soaking fish in papaya leaf extract. Dipping is an effective method for controlling Argulus infestation because karpain compounds are soluble in water (Whindholz et al., 1989), so that karpain compounds can be in direct contact with parasites on the surface of the fish body. Papaya leaf extract can prevent infestation of Argulus in carp because there are karpain, benzilisothiocyanate, benzylglucosinolate, glucotropacoline, benzylthiourea, caricin and myrosin enzymes (Boshra and Tajul, 2013). Of all the active ingredients possessed by papaya leaves, karpain is a compound that can be used as an antibacterial or antiparasitic (Krishna et. Al., 2008). Karpain on papaya leaves is a class of alkaloid with levels of 1000-1500 ppm (Duke, 2002).

The results showed that the dose of 6 ppt was able to control the most Argulus infestation, around 4-5 in 1 goldfish. Thus, the greater the dose of papaya leaf extract given, the less Argulus infestation and the more it causes the isolation of Argulus from carp. However, excessive dose can cause death in carp. The duration of goldfish immersion for one hour also did not cause death, but it weakened the movement of the fish. The one-hour immersion time was sufficient time for Argulus infestation in carp because the artificial infestation time on Argulus carried out by Kismiyati (2009) is 15 minutes.

The P3 treatment showed the smallest percentage of infestation Argulus compared to treatment P1 and P2 because the effect of karpain worked to suppress the central nervous system (Nur, 2002). Duke (2002) said, karpain has a suppressing activity on the Central Nervous system (CNS) by binding Na + ions to the nerves. The Na + ion functions to deliver nerve impulses to action. Argulus nerves relate to a sucker that functions to attach to something. The pressed nerve cannot deliver nerve impulses to the
sucker, resulting in Argulus being unable to infest carp.

3.2.3. Carp behavior
Carp in treatment P0 showed the behavior of rubbing its body against the wall of the treatment tub. The fish stayed at the bottom of the tub and secreted excessive mucus. The P0 treatment was a control treatment, meaning that behavior changes showed clinical symptoms caused by Argulus. Treatment P1 showed the behavior of fish rubbing the body against the wall of the tub and producing excessive mucus. The P2 treatment showed fish behavior that tend to stay at the bottom of the tub, removing feces and mucus. On the other hand, treatment P3 showed behavior that was not much different from passive fish movements, excessive mucus and feces. Excessive mucus production was seen by the presence of white clumps like fog found in the tail and body surface of the fish, causing the color of the fish's body to turn pale. After treatment the fish was put into a tub filled with clean water and the carp returned to active swimming.

3.2.4. Water quality
Water quality was measured before and after treatment to determine changes in water quality due to papaya leaf extract. Water quality measurements in the study were DO, temperature and pH. The results of measurements of water temperature before and after treatment were not different, at 29°C in treatment P0. The treatments P1, P2, and P3, which experienced changes in temperature, ranged between 29°C and 30°C. According to Latha and Lipton (2007), carp can live at a temperature of 23-30°C. Observation of DO showed a decrease in treatment P1, P2, and P3 from 8 mg/L in each treatment to 5.6 mg/L, 5.6 mg/L, and 5.8 mg/L. In contrast, treatment P0 did not change DO because treatment P0 was a control. Decreasing DO occurred along with the increasing water temperature. Effendi (2003) said an increase in temperature of 1°C would increase oxygen consumption by around 10%. Changes in DO can still be considered to be good because carp can live with DO ranges from 4-7 mg/L (Latha and Lipton, 2007). Observation of pH before and after treatment was 7, meaning that papaya leaf extract has no effect on pH stability. Carp can grow well in the pH range 6-8.3 (Latha and Lipton, 2007). The results of this water quality measurement can be taken into consideration that papaya leaf extract can be used as control of Argulus infestation in carp.

4. Conclusion and recommendation
4.1. Conclusion
1) Papaya leaf extract (Carica papaya L.) has the potential to control Argulus infestation and isolate Argulus from carp (Cyprinus carpio).
2) The optimal dosage of papaya leaf extract (Carica papaya L.) to control Argulus infestation in carp (Cyprinus carpio) is 6 ppt, which can prevent Argulus infestation by 88% with 60 minutes soaking time.

4.2. Recommendation
Prevention of infestation Argulus in carp with size of 7-10 cm in length can be carried out using papaya leaf extract with a concentration of 6 ppt and 60 minutes immersion time. The use of concentrations above 6 ppt and long soaking above 60 minutes can cause fish to die. There needs to be a further research regarding the utilization of papaya leaf extract (Carica papaya L.) against other parasites that might attack carp (Cyprinus carpio).

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