Evaluation of aqueous extract of robusta coffee (\textit{Coffea canephora}) leaves for controlling \textit{Argulus japonicus} infestation on common carp seed

N Afifah\textsuperscript{1}, Kismiyati\textsuperscript{2} and H Kencono\textsuperscript{3,4}

\textsuperscript{1}Study Program of Aquaculture, Campus Banyuwangi, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia
\textsuperscript{2}Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia
\textsuperscript{3}Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Kampus Banyuwangi, Universitas Airlangga, Indonesia
\textsuperscript{4}Corresponding author :hapsari@fpk.unair.ac.id

Abstract. Argulus \textit{japonicus} is common crustacea ectoparasite that infest in freshwater fish. The heavy infestation may trigger other fish pathogen infection which lead to mass mortality. Common carp seed has been known as the most vulnerable host for \textit{A. japonicus} infestation due to their immature immune system. Generally, \textit{A. japonicus} infestation could be controlled by chemical pesticides but its long-term use could create a harmful effect on the environment. Therefore, an eco-friendly natural product is needed to replace chemical pesticide. The purpose of this study was to evaluate the effectivity of water extract of robusta coffee leaves to control \textit{A. japonicus} infestation. Water extract of robusta coffee leaves was obtained from decoction method. The fish were infected with \textit{A. japonicus} by artificial infestation using cohabitation method. The infected fish were dipped at different concentration 400 (P1), 800 (P2), 1200 (P3) and 1600 ppm (P4) of water extract for 30 minutes. The result presented that the water extract of robusta coffee leaves was effective to control \textit{A. japonicus} infestation in 1600 ppm. This study revealed that robusta coffee leaves have anti-parasitic activity against \textit{A. japonicus}. Hence, it can be used as an alternative natural product for controlling \textit{A. japonicus} infestation in fish.

1. Introduction
Common carp is one of the biggest commodities that mostly cultivated in Indonesia [1]. The production of common carp has increased throughout the year. Data from the Ministry of Marine Affairs and Fisheries Republic of Indonesia [2] showed that an increase of common carp production in a row from 2012 to 2016 was 374,366 tons, 412,703 tons, 434,653 tons, 461,107 tons, and 498,297 tons. However, in 2017 it decreased with the total production of 312,954 tons. The decrease of common carp production is caused by several factors, one of which is seed deaths due to the disease outbreaks. Disease can be happened due to the poor quality of aquatic environment and lack of biosecurity management. This condition will make the immunosuppression in fish while the pathogen will grow rapidly [3]. Common carp seed has been known as the most vulnerable host for pathogen due to their immature immune system. One of the pathogen that generally attack the common carp seeds is Argulus [4].
*Argulus japonicus* is one of Argulus species that native to Asia [5]. The heavy infestation of *A. japonicus* on seeds may trigger secondary infection by viruses, bacteria, or fungi which lead to mass mortality[6], therefore it needs to be controlled. *A. japonicus* infestation could be controlled by chemical pesticides, but its long-term use could create a harmful effect on the environment. Therefore, an eco-friendly natural product is needed to replace chemical pesticide [7].

One of the natural product that can be used as an alternative is robusta coffee leaves. Some studies suggest that robusta coffee leaves contain metabolites with antiparasitic activities such as caffeine [8]. The caffeine tested on *Drosophila melanogaster* and *Hypothenemus hampei* showed the inhibition of the delivering impulses process to the nervous system [9]. In our knowledge, the application of robusta coffee leaves to control *Argulus* infestation is still limited. Nevertheless, based on activity from phytochemical compound contained in robusta coffee leaves, it is necessary to evaluate its effect against the infestation of *A. japonicus*.

2. **Methods**

2.1. **Research method**

The experiment was conducted at Laboratories of Universitas Airlangga Campus Banyuwangi. The research method using experimental method with Completely Randomized Design (CRD) as the experimental design.

2.2. **Materials**

Sixty common carp seeds sized 7-10 cm were obtained from Unit Pengembangan Budidaya Air Tawar (UPBAT) Punten, Malang. *A. japonicus* were obtained from Balai Benih Ikan (BBI) Plalangan, Jember. Sample of coffee leaves were collected from coffee plantation in the Kelir Village, Banyuwangi.

2.3. **Research procedure**

2.3.1. **Preparation of water extract of coffee leaves**

The coffee leaves were cleaned and cut into small pieces, then dried at room temperature between 20-25°C for 7 days. The decoction process were carried out as previous method with some modifications [10]. This step begins with ground the dried coffee leaves into powder, then boiled with distilled water with a ratio of 1:15 (every 1 gram of coffee leaves boiled with 15 ml of distilled water) using a hot plate at 60ºC for 6 hours. Then, it filtered to get the filtrate.

2.3.2. **Preliminary research**

The preliminary research was conducted to evaluate the safe concentration of aqueous extract on common carp seeds. The safe concentration was determined by LC50 value. As many as 10 healthy common carp seeds were dipping in each concentration of coffee leaves water extract (0 ppm/without extract, 2000 ppm, 4000 ppm, 6000 ppm, and 8000 ppm) for 96 hours. The LC50 was determined using a probit analysis 5%, IBM SPSS 20.

2.3.3. **Artificial infestation**

Artificial infestations were carried out based on procedures reported by Ghazali et al. [11]. *A. japonicus* was fasted by being kept separately from the host for 24 hours to create a hungry condition for *A. japonicus* so it will be easier to attach to the new host. Artificial infestations were performed by inserting fifteen *A. japonicus* into plastic jars containing 3 common carp seeds. Then, wait for 15 minutes until all Argulus sticks.

2.3.4. **Treatment**

Twenty plastic jar containing coffee leaves aqueous extract with various concentration, i.e without extract as control (P0), 400 ppm (P1), 800 ppm (P2), 1200 ppm (P3) and 1600 ppm (P4) were prepared. Each plastic jar was filled with 1 liter of aqueous extract. Then, 3 common carp seeds that
had been infesting with *A. japonicus* were put in a jar. The dipping process is performed for 30 minutes. Then, each fish was removed from this jar and all *A. japonicus* found in fish body were counted. Antiparasitic efficacy of coffee leaves aqueous extract were calculated according to Wang et al. [12]:

\[
AE = \frac{[B - T]}{B} \times 100\%
\]

Note \( AE \) = Antiparasitic efficacy; \( B \) = number of surviving *A. japonicus* in control treatment; \( T \) = number of surviving *A. japonicus* in treatment.

2.3.5. Data analysis

Data were analyzed using ANOVA statistical analysis (Analysis of Variance) at the 5% significance level, then followed by the Duncans Multiple Range Test (DMRT). This analysis were performed by using SPSS Version 20.

3. Result and discussion

Decoction is a conventional extraction method that used distilled water solvent. Some studies suggest that distilled water solvents can dissolve the active compounds contained in robusta coffee leaves such as mangiferin, isomangiferin, and tannins from polyphenols, caffeine and trigonelline from alkaloid, and routine from flavonoid [13,14]. Alkaloids have been investigated the ability to control *Argulus* infestations. Pricilia *et al.* [15] explained that in banana plant stems extracted using distilled water solvents there are alkaloid compounds that can reduce of *Argulus* infestation. The ability of phytochemical compounds contained in aqueous extract of robusta coffee leaves to control *Argulus* infestation showed in Figure 1.

![Figure 1](image_url)

**Figure 1.** Antiparasitic efficacy of robusta coffee leaves aqueous extract on *A. japonicus*

There is a significant difference between the control treatment (without water extract of robusta coffee leaf) with treatment P1, P2, P3, and P4 (containing water extract of robusta coffee leaf). *A. japonicus* in the control treatment remained attached to the common carp seeds until the end of the dipping time, while in treatments P1, P2, P3, and P4 showed the release of *A. japonicus*. After DMRT further testing with a significance level of 5%, it was found that the lowest percentage of *A. japonicus* release was in the control treatment, while the highest percentage was found in treatment P4. The
release of *Argulus* in the treatment of P1, P2, P3, and P4 suspected due to the antiparasitic activity of various phytochemical compounds contained in the water extract of robusta coffee leaves. Some studies mention the alkaloid contained in robusta coffee leaves has the activity to control *Argulus* infestation.

Alkaloids are polar compounds that can weaken the parasitic nervous system. The derivatives of alkaloid compounds contained in robusta coffee leaves, caffeine can inhibit the working system of the enzyme acetylcholinesterase [16]. The acetylcholinesterase enzyme plays a role in stopping nerve impulses by hydrolyzing acetylcholine. Acetylcholine is a neurotransmitter compound that acts as a conduit for nerve impulses. Inhibition of the work system of the acetylcholinesterase enzyme causes accumulation of acetylcholine at nerve synapses, this causes nerve impulses to move continuously and the nervous system experiences excessive stimulation. This condition can cause damage to the central nervous system and cause organ paralysis. Apart from caffeine, flavonoids also affect the nervous system. Nugraha et al. [17] said that flavonoid compounds attack nerve parts in several vital organs which can lead to nerve weakness. *Argulus* has an organ, namely maxilla, which its function is to attach the hosts [18]. Because of the flavonoid content, the paralysis can be occurred in the maxilla. In consequence, *A. japonicus* lose their inherent ability to attach the host, so they couldn’t stick in fish body.

The release of *A. japonicus* from common carp due to paralysis showed that aqueous extract of robusta coffee leaves was effective to control *A. japonicus* infestation. Moreover, the aqueous extract of robusta coffee leaves is more effective than other extract, such as noni juice and papaya leaves. Noni fruit juice were able to control 49.5% *Argulus* at a dose of 3.5% (35,000 ppm) with dipping for 15 minutes [11]. Papaya leaves were able to control 88% *Argulus* at a dose of 30% with soaking for 20 minutes [19], while the aqueous extract of robusta coffee leaf was able to control 51.67% at a dose of 1,600 ppm with soaking for 30 minutes. The effectiveness of a compound from extract material is influenced by the dose used and the length of time of exposure. Andiarsa, [20] states that the smaller the concentration of a drug and the shorter the exposure time needed to cause death in parasites, it can be said that the drug is effective in controlling parasites.

4. **Conclusion**

Aqueous extract of robusta coffee (*Coffea canephora*) leaves is effective in controlling the infestation of *Argulus japonicus* in carp (*Cyprinus carpio*) with an optimal dose of 1600 ppm. Based on this study, it is recommended to test the compound content of coffee leaves which are soluble in distilled water solvents.

5. **References**

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