Preface

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It's such a great pleasure for me to welcoming all of you on behalf of Faculty of Fisheries and Marine Universitas Airlangga, for the 2nd international conference on fisheries and marine science. The 1st International Conference on Fisheries and Marine Sciences (InCoFiMS) 2018 has been successfully carried out which facilitated hundreds of publications to the Scopus-indexed proceeding of IOP and connected many researchers. The prior experience encourages to improve the quality of the conference through The 2nd InCoFiMS with the broader topic called "Sustainable Fisheries and Marine Development and Management". This expanded level of this conference with the theme of "Sustainable Fisheries and Marine Development and Management" is expected capable of connecting students, lecturers, researchers, government and professionals from across the world to meet, greet, share and discuss about the potential and best practices in the field of fisheries and marine during the period of focusing on SDG's.

The aims of this conference is to developed and improve the goals of Universitas Airlangga to be of the Top 500 University in the world by improving aquaculture and Fisheries Sustainable sector. For this conference, we also cooperate with Scopus Indexed Publisher. In order to assist students, lecturers and researchers in disseminating their findings, to publish selected papers which are expected helping societies to implement the findings in the focus on developing aquaculture and fisheries sustainable.

I strongly hope that all of the participants from around the world enjoy the conference at the Historical City of Surabaya, the second biggest city in Indonesia with competitive economic activities for the future of Fisheries and Marine development.

Once again, I am most grateful for your participant and your support. Thank you

Dr. Ahmad Shofy Mubarok

Chief of 2ND INCOFIMS

Peer review statement

To cite this article: 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **441** 011002

Peer review statement

All papers published in this volume of *IOP Conference Series: Earth and Environmental Science* have been peer reviewed through processes administered by the proceedings Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

Different concentration influence of *Moringa oleifera* leaf aqueous extract immersion against *Argulus japonicus* egg damage

Different concentration influence of *Moringa oleifera* leaf aqueous extract immersion against *Argulus japonicus* egg damage

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Abstract. *Argulus japonicus* is an ectoparasite that invades freshwater fish, causing ulceration and bleeding, besides opening the secondary infections access for bacteria, fungi, viruses and triggering fish death. *A. japonicus* efficient control can be done by cutting the ectoparasite life cycle, specifically the egg stage, using natural insecticide. One of the natural insecticides is *M. oleifera* leaves as it has phytochemical contents, i.e., tannins, flavonoids, alkaloids, and toxic saponins for eggs *A. japonicus.* The study aimed to analyze the immersion effect of *M, oleifera* leaf aqueous extract under different concentrations against *A. japonicus* egg damage and determine the optimal concentration of *M. oleifera* leaf aqueous extract that significantly damaged *A. japonicus* egg until 100%. This study used four aqueous extract treatment, namely 0% (A), 4% (B), 6% (C), and 8% (D) with five times repetitions. The results showed that each treatment had significant influence against *A. japonicus* egg damage ($P < 0.05$). The lowest *A. japonicus* egg damage was found at A treatment, while the highest was at D treatment. *M. oleifera* leaf aqueous extract can be utilized as a natural insecticide to disrupt the egg phase of *A. japonicus* with 8% concentration in aqueous extract.

1. Introduction

Fish disease is one of the obstacles in aquaculture business due to a disease outbreak that can cause fish death. The disease is the result of an imbalance among the host, pathogen, and environment. One of the most common pathogens in Cyprinidae family fish is parasite [1].

Parasites are organisms that live on or in other organisms (hosts), which get some or all parts of organism nutrients and cause a high damaging degree of the organism [2]. *A. japonicus* parasites are found all over the world and infect freshwater aquaculture [3]. According to [4], the predilections of *A. japonicus* are on the caudal fins, body surface, pectoral fins, bottom ventral fin, dorsal fin, operculum and head. *A. japanicus* becomes parasitic by sucking the host blood, causing damage to the host skin through Maxillule and preoral stylet [5]. Wounds caused by *A. japonicus* on fish body result in injuries that make secondary infections [6]. Secondary infections of the fish that have been wound to the skin and body surface can cause high mortality [7].

A. japonicus is an easily reproduced ectoparasite with a direct life cycle that requires a host to develop from larvae to adult [7]. *A. japonicus* breed by laying eggs approximately 20- 250 eggs [8]. Population control of *A. japonicus* should be done to prevent the fish from being infected by these

parasites. A control population of *A. japonicus* can be done by reducing the population initiated at the egg stage, therefore disrupting *A. japonicus* life cycle.

M. oleifera leaves are natural ingredients that contain phytochemical compounds. Phytochemical compounds that function as insecticides include saponins, tannins, flavonoids, alkaloids, terpenoids, steroids, sterols, phenols, polyphenols and essential oils [9,10]. According to [11], the content of active ingredients in plants such as flavonoids, saponin, alkaloids, and tannins are pharmacologically toxic. Therefore, further studies on the active ingredient of *M. oleifera* leaves against *A. japonicus* egg damage is necessary to be conducted.

The purposes of this study were to analyze the immersion effect of *M. oleifera* leaves under different concentrations against *A. japonicus* egg damage and determine the optimal concentration of *M. oleifera* leaves that can damage *A. japonicus* egg until 100%. This study is expected to prevent *A. japonicus* parasitic infection in freshwater ornamental fish culture in Indonesia.

2. Material and methods

2.1 Study design

This study was held in April, 2019 in the laboratory of anatomy and aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was conducted using a completely randomized design experimental method to determine the immersion influence of *M. oleifera* leaf against *A. japonicus* egg damage. Four treatments with five replications (20 experiment units) were given in this study, namely: (1) **A** as control treatment containing *A. japonicus* egg and 1000 ml water, (2) B contains *A. japonicus* egg with 40 ml *M. oleifera* leaf aqueous extract and 960 ml water (4%), (3) C contains *A. japonicus* egg with 60 ml *M. oleifera* leaf aqueous extract and 940 ml water (6%), (4) D contains *A. japonicus* egg with 80 ml *M. oleifera* leaf aqueous extract and 920 ml water (8%).

2.2 Sterilization

Equipment used were firstly cleaned from the dust and dirt with soap, then dried. Chlorine was also utilized to sterilize aquaria and water used, then dried under the sunlight.

2.3 A. japonicas cultivation

A. japonicus males and females used in this study were sized 6 mm for males and 8-9 mm for females [12]. According to [13], *A. japonicus* had an egg pocket on the ovary along the body midline. Males and females of *A. japonicus* can also be characterized by the existence of seminal receptacle (Spermateca) in females and a pair of testicles on the abdomen posterior part in males. Furthermore, the gender selection of *A. japonicus* can be seen on the abdominal lobes or posterior part of the body.

2.4 M. oleifera leaf aqueous extract preparation

M. oleifera leaves used were old *M. oleifera* leaves with dark green color and stiff to hard texture. Old *M. oleifera* kelor leaves have more tannins content with a bitter taste [14]. Fresh *M. oleifera* leaves were measured 1500 grams, then washed using running water and dried under the sunlight. *M. oleifera* leaves were cut into small pieces and mashed using a blender until slurry shape had been required. *M. oleifera* leaves were squeezed using clean fabric equipment and filtered using filter paper. The total of aqueous extract produced was 250 ml, with 100% concentration.

2.5 A. japonicus egg preparation

Aquarium (sized 50x30x40 cm^3) with water and aeration stone inside filled was filled with *A. japonicus* males and females. Fifteen *A. japonicus* males and females in each aquarium were placed with a 5 cm goldfish (*Carassius auratus*). *A. japonicus* would lay eggs on the stone that had been prepared in the aquarium. Egg attached to the stone was removed from the aquarium for further study.

2.6 A. japonicus egg immersion in M. oleifera leaf aqueous extract

A. japonicus eggs were removed from the aquarium and immersed in *M. oleifera* leaf aqueous extract based on the treatments given. Eggs were observed first using microscope dissecting stereo, before immersed in the extract to calculate the initial amount of eggs containing embryos, besides determining damage and good eggs. Eggs development can be seen as the existence of eye spots on the egg [5]. Treatments were given when most eye spots were discovered on the egg.

A. japonicus eggs were immersed in *M. oleifera* leaves aqueous extract for eight days with the water temperature used approximately 29^{O}C on each aquarium with the stocking density of 40 eggs.

2.7 A. japonicus egg damage

Egg damage was observed as the loss of mucous wrapping the egg on the outer structure, thereby causing the extract entered to the egg and becoming wrinkle and black [5]. According to [15], damaged *A. japonicus* egg is visually visible in dark black, wrinkled or empty shells. Unhatched *A. japonicus* egg occured as a result of the reaction in *M. oleifera* leaf aqueous extract which destroyed the egg-cell membrane. Cell membrane serves to keep the fluid stability in cell, therefore as the cell membrane disrupts, *A. japonicus* egg is unable to develop. The calculation of egg damage percentage can be calculated by the formula:

$$\text{Damaged egg} = \frac{\text{Total of egg damage}}{\text{Egg Total before treatment}} \times 100\%$$

2.8 Water quality measurement

Physical and chemical parameters of water quality measured were temperature, pH and DO. Temperature measurement was performed using thermometer. pH and DO was measured using pH meter and DO meter. Measurements using DO meter should be done carefully as dissolved oxygen in the aquarium is very volatile due to the aeration administration.

2.9 Data analysis

Data obtained in this study were analyzed using Analysis of Variance (ANOVA) with *SPSS 16.0*. Data were continuously analyzed using Duncan's multiple range test, whether indicating significant difference in all treatments to determine the difference among treatments [16].

3. Result and discussion

3.1 A. japonicus egg damage

A. japonicus egg damage can be seen visually as white to black discoloration occured [15]. *japonicus* egg damage observation was performed for 8 days. The data retrieval of *A. japonicus* egg damage percentage conducted at the end of the study can be seen in Table 1.

Table 1. he percentage of *A. japonicus* egg damage

Treatment	Damaged egg (%) ± SD
A	0.00^{d} ± 0.00000
B	54.40^{c} ± 0.09099
C	80.60^{b} ± 0.23797
D	100.00^{a} ± 0.00000

Note: Different superscript notation on one column shows significant difference ($p<0.05$), A = 0% *M. oleifera* leaf aqueous extract , B = 4% *M. oleifera* leaf aqueous extract, C = 6% *M. oleifera* leaf aqueous extract, D = 8% *M. oleifera* leaf aqueous extract

A. japonicus egg damage percentage data was then analyzed using ANOVA. ANOVA resulted that each immersion treatment of *M. oleifera* leaf aqueous extract provided significant effect on *A. japonicus* egg damage ($P < 0.05$). ANOVA result was continously analyzed using Duncan's multiple range test with 0.05 degree of confidence. Duncan's multiple range test result indicated the highest significant egg damage was observed at D treatment with 8% *M. oleifera* leaf aqueous extract immersion.

3.2 Water quality

Water quality is the supporting factor for *A. japonicus* egg development. The water quality parameters measured in this study were pH, Disolved Oxygen (DO) (mg/l), and temperature (^{o}C). The water quality parameter measurement result is shown in Table 2.

Table 2. Water quality during treatments immersion period

Parameter	Treatment			
	A (0 %)	B (4%)	C (6%)	D (8%)
pH	8.00	8.00	8.00	8.00
DO (mg/l)	3.37	3.00	3.17	3.05
Temprature (^{o}C)	30.00	30.00	30.00	30.00

Note: A = 0% *M. oleifera* leaf aqueous extract , B = 4% *M. oleifera* leafaqueous extract, C = 6% *M. oleifera* leaf aqueous extract, D = 8% *M. oleifera* leaf aqueous extract

3.3 Discussion

According to [17], *A. japonicus* is an easily reproduced ectoparasite through sexual and internally fertilization. *A. japonicus* eggs were placed in rows or groups with neatly arranged and slight cavity of each other. Eggs on the substrate will be coated with gelatin substance then hardened when contact with water, thus attaching firmly to the substrate [5]. The next stage is the growing egg characterized as the firstly development of eye spots, then the organs attached to thorax development, and the third stage is the embryo movement at 24-48 hours before hatching [5].

A. japonicus egg development was disrupted after *M. oleifera* leaf aqueous extract immersion. According to [18], damaged *A. japonicus* eggs are marked with black and wrinkle egg with mucus loss that envelops the outer layer of the egg and decreased egg wall density. According to [10], *M. oleifera* leaves contain phytochemical compounds, i.e. flavonoids, saponins, steroids, terpenoids, and tannins. Tannins are natural chemicals found in most leaves that have antiparasitic activity [19]. Tannins are compound that can react with protein [20]. Tannins are protein-binding agent to interfere with the protein absorption process on the embryo, resulting undeveloped *A. japonicus* embryo [5]. Tannins are also suspected to shrink cell membrane, thus disrupting the cell permeability [5]. Saponins are toxic compound [20]. Saponins perform an inhibition activity by forming complex compounds with cell membranes through hydrogen bonds, thereby destroying the cell membrane permeability and inflicting cell death [5]. The egg wall structure consists of several layers composed by wax and lipid layer [21]. Saponins are one of triterpenoid compound groups that can inhibit the development of eggs by damaging the egg membrane, changing the egg wall cell structure composed by wax and lipid layer, disrupting the cell permeability, therefore draining the fluid inside the cell and dehydrating the cell. Cell dehydration will result in eggs failure to hatch, because the egg development requires a fluid containing nutrients [22]. Another compound that can damage *A. japonicus* egg is flavonoids. Flavonoids are able to damage cell membranes that contribute to cell integrity by denaturing the protein in cell membranes, increasing cell membrane permeability and leaking cell content [5]. Alkaloid compound has an inhibitory mechanism by disrupting the peptidoglycan constituent component in cells, so that the cell wall layer is not formed and causes the cell death [23]. According to [24], eggs of *A. japonicus* is coated with mucus that serves as a protector. Mucus will coat all parts of the egg shell and unite the egg with the other eggs by forming a firmly attached circuit on the substrate surface and maintain the balance of hydrominerals in eggs [13]. Phytochemical compounds are tannins, saponins, alkaloids and flavonoids will scrape mucus and egg cell walls, causing wrinkles and fluid leaks occur and interfere the egg permeability. *M. oleifera* leaves that enter the egg cause imbalanced hydromineral content, therefore the embryo becomes undeveloped and turns into black due to the decay process.

The efficient control of *A. japonicus* can be done by cutting the life cycle, specifically during the egg stage [25]. According to [26], newly hatched *A. japonicus* is directly parasitic. According to [27], *A. japonicus* may damage the integument part as the first defense system, resulting in the emergence of secondary infections, i.e viruses, fungi, and bacteria. This secondary infection can lead to death for cultured fish.

A. japonicus egg immersion on *M. oleifera* leaf aqueous extract indicated the significant difference occured among treatments against damaged egg. The study result showed that 8% immersion concentration of *M. oleifera* leaf aqueous extract produced the largest percentage of egg damage with 100%, followed with 6% (80.6%), 4% (54.4%), while the lowest egg damage percentage was found at 0% concentration as there were no influence of *M. oleifera* leaf aqueous extract. This means that the highest level of flavonoids, saponin, and tannins was observed at 8% concentration, thus working optimally to disrupt *A. japonicus* egg development among other treatments. Therefore, the optimum concentration to produce high *A. japonicus* egg damage is 8% due to the ability to

produce 100% egg damage.

The most influential water quality parameters for *A. japonicus* development are temperature, dissolved oxygen, and pH. According to [13], *A. japonicus* can live within wide temperature range, thus called as cosmopolitan species. Water quality measurement result showed that the condition of water quality was still in the normal range with 30^{o}C temperature and 3 mg/L DO, which means the water quality condition is suitable for *A. japonicus*, however pH value reached at the base range. This condition occurs as alkaloids presented in plants. *M. oleifera* leaves have neutral to base pH (7.0-8.0) [28]. Alkaloids are base properties depending on the electron pair in the nitrogen [29]. pH obtained from *M. oleifera* leaf aqueous extract was 8. According to [30], suitable pH for all fish ranges 6.7-8.2. pH 8 is good for the fish development, therefore the application aqueous extract can be delivered before or after the fish are stocked.

4. Conclusion

The immersion of *M. oleifera* leaf aqueous extract with different concentration affected significantlly against *A. japonicus* egg damage. The optimum concentration of *M. oleifera* leaf aqueous extract that damage *A. japonicus* egg until 100% is 8% concentration. Further studies about the specific phytochemical content of *M. oleifera* leaf and the observation of leaf toxicity in fish and environment are necessarily to be done in order to be optimized application.

5. References

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Acknowledgments
Authors would like to thank parents, family, and colleagues as the best support for the assistance to complete this manuscript.