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The effect of *Melastoma malabathricum* leaf extract on growth and spawning of blue swimming crab *(Portunus pelagicus)*

The effect of *Melastoma malabathricum* leaf extract on growth and spawning of blue swimming crab (*Portunus pelagicus*)

N Alam[1], Y Fujaya[1], Haryati[1], D K Sari[2], M Achmad[1], M Rusdi[3], N Farizah[4]

[1]Fisheries Department, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, South Sulawesi Indonesia;
[2] Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia
[3] Faculty of Medicine and Health Sciences, Alauddin State Islamic University, Makassar, Indonesia
[4]Aquaculture Department, Faculty of Fisheries and Marine Sciences, Borneo University, Tarakan, North Kalimantan, Indonesia

Email: yushinta.fmuskar@gmail.com, yushinta.fujaya@unhas.ac.id

Abstract. The aim of this study was to evaluate the effect of *Melastoma malabathricum* leaf extract on growth and spawning of the blue swimming crab *Portunus pelagicus*. This research was conducted from March to May 2019 at the Hasanuddin University mini hatchery in Barru Regency, South Sulawesi Province, Indonesia. The study used five treatment doses of *M malabathricum* leaf extract: 0, 5, 10, 15, and 20µg/g of female crab body weight. The extract was dissolved in water and an aliquot of 0.1 mL was injected into each crab. The results showed that there was a decrease in growth in all treatments within the first 10 days after injection, but an increase after the 20th day. Doses of 15 and 20 ug/g resulted in the highest spawning rate, ie 100% after 30 days. Crabs with no extract treatment had the lowest spawning rate, which was only 33%. The conclusion of this study is that an appropriate dose of *M. malabathricum* leaf extract can be used to stimulate spawning of Portunus pelagicus.

1. Introduction

The blue swimming crab (*Portunus pelagicus*) is one of the marine fisheries commodities that can be cultivated but aquaculture of this species is not as yet well developed. One of the problems faced in aquaculture is the availability of seeds in sufficient quantities and quality. Today, crabs can be bred in the hatchery but the outputs are not sufficient. The survival of larvae and crablets is still low [1]. The mortality rate of larvae is influenced by broodstock, water, and feed quality. The quality of the crab broodstock used in hatcheries is often still low and information about good quality broodstock is still limited.

Control of gonadal maturation, particularly ovary maturation, is a major problem for the development of crustacean culture, since in several species the gonads do not mature without external manipulation [2]. Gonadal maturation in crustaceans (shrimp and crabs) can be stimulated by hormonal approaches, nutrition [3] and the environment [4]. The hormonal approach most commonly used for ovarian maturation in crustaceans is eye stalk ablation [5]. This method effectively stimulates gonadal maturation, but in the long term it can reduce larval quality, as well as being contrary to animal welfare, which has recently become a strong issue in aquaculture.

Nowadays herbs or herbal products also have a significant role in aquaculture [6]. There are advantages in using herbs that are environmentally safe and are not hazardous for the cultivated organisms. The active compounds in herbal products include phenols, polyphenols, alkaloids, quinones, triterpenoids, steroids, lectins, and polypeptides [7,8]. These substances have the ability to act as tonics to enhance the immune system, as growth promoters to increase the growth rate [9], as appetite stimulators, as an inducer or aphrodisiac in gonadal maturation [10] and as antifertility agents [11].

Melastoma malabathricum, known as Malabar melastome, Indian rhododendron, Singapore rhododendron and senduduk, is a flowering plant in the family Melastomataceae with a number of medicinal uses, including the ability to promote fertility [12]. The aim of this study was to evaluate the effect of *M. malabathricum* leaf extract on growth and spawning of *P. pelagicus*. The results of this study are expected to be useful as information regarding the use of herbal extracts when preparing broodstock in crab hatcheries.

2. Materials and method

This research was conducted in March - May 2019 at the mini Hatchery of Hasanuddin University, Barru Regency, South Sulawesi Province, Indonesia.

2.1. *Preparation of M. malabathricum leaf extract*

Fresh *Melastoma malabathricum* leaf samples were obtained from Pinrang Regency in South Sulawesi Province. Approximately 2 kg of fresh leaves were taken and washed and then dried in an oven at 40°C. After drying the leaves were blended and then macerated using 80% ethanol with a ratio of 1: 5 (100 g of blended leaves in 500 mL of 80% ethanol). The maceration procedure was repeated 3 times (3x24 hours). The resulting ethanol extract was then concentrated in a rotary evaporator at 40°C. The concentrated ethanol extract produced was used for testing on *Portunus pelagicus* broodstock.

2.2. *Experimental animals.*

Immature female blue swimming crabs *Portunus pelagicus* were used for *in vivo* assay. There were 80 female crabs used in this study obtained from fishermen operating in the waters of Suppa, Pinrang Regency. The body weight was between 61-72 g and carapace width between 72-92 mm. The crabs were adapted to the rearing environment for 2-3 days before extract injection.

2.3. *Experimental design.*

The treatments applied were five different doses of *M. malabathricum* leaf extract: 0, 5, 10, 15, and 20 µg/g of female crab body weight. The extract was dissolved in water and an aliquot of 0.1 mL was injected into each crab.

The crabs were kept in concrete tanks with sand as a substrate. The depth of sea water in the tanks was approximately 80 cm. The crabs were fed with fish and squid at a rate of approximately 10% of crab body weight with two feeding times per day. Water quality was maintained at: dissolved oxygen > 6; pH 7-8; salinity ≈32 ppt and temperature ≈30°C.

2.4. *Parameters measured and data analysis.*

The parameters measured were crab growth and spawning rate. Growth was measured on the 10th and 20th days after extract injection, while the spawning rate was observed until the 30th day after extract injection. The growth was calculated as the difference between the weight on day n and the initial weight at the time of injection. Spawning rate was the percentage of crabs that had spawned. The data were tabulated; the mean and standard deviation (SD) were calculated. Data were presented as mean ± SD. Statistical analysis performed was quadratic regression of the parameter values against the dose of leaf extract injected.

3. Results

3.1. Crab growth

The response of *P. pelagicus* to injection with *Melastoma malabathricum* leaf extract varied depending on the dose of the extract which had been injected (Figure 1). During the first 10 days after injection, crab growth was lower in injected crabs than in the controls without extract treatment (R^2 = 0.717). However, by the 20th day, the growth of crabs treated with *M. malabathricum* leaf extract was higher than that of the controls (R^2 = 0.674).

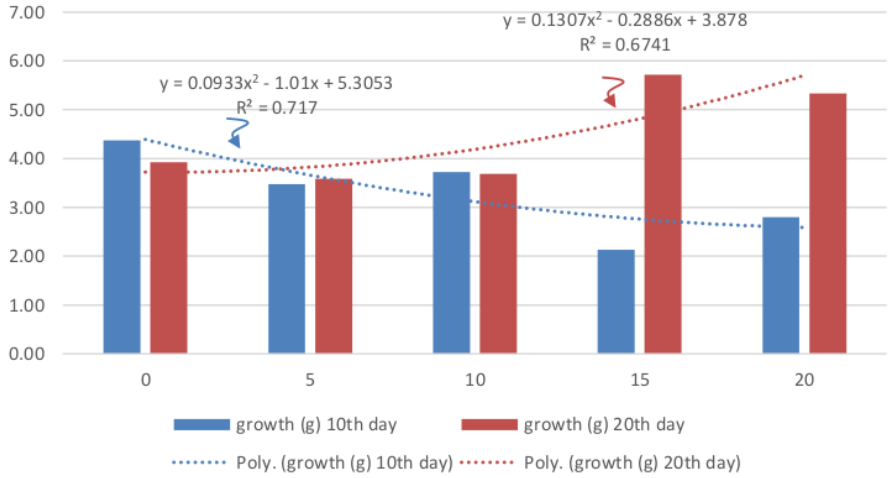

Figure 1. Growth of *P. pelagicus* at 10 and 20 days after injection with *M malabathricum* leaf extract at different concentrations (0, 5, 10, 15 and 20 ug/g of crab body weight)

3.2. Spawning Rate

Blue swimming crab *P. pelagicus* spawning rate was strongly influenced by the injection of *M. malabathricum* leaf extract (R^2 = 0918). Spawning rate was highest (100%) in crabs treated with injections of 15 and 20 ug/g body weight. Without extract injection, only 33% of the control crabs spawned within the study period (Figure 2).

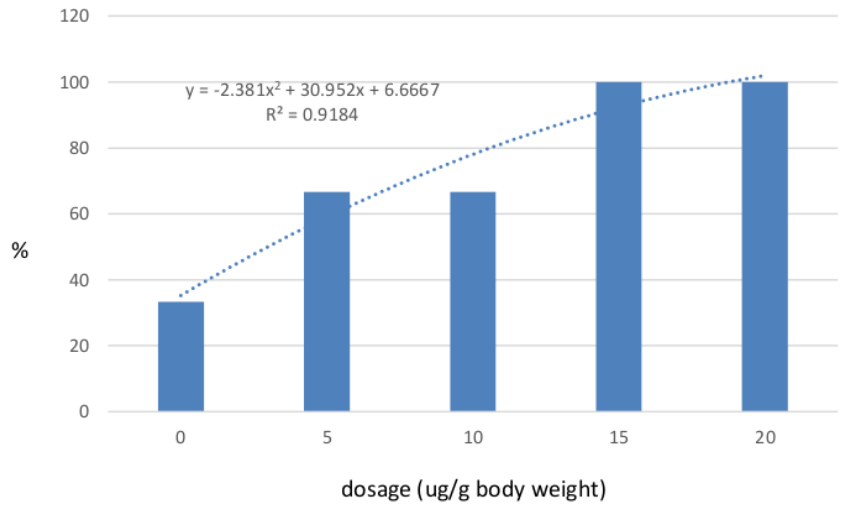

Figure 2. Spawning rate of *P. pelagicus* 30 days after *M. malabathricum* extract injection

4. Discussion

The results of this study indicate that M. *malabathricum* leaf extract can be used to stimulate spawning in female blue swimming crabs *P. pelagicus*. However, there is a need to be careful about the dosage. There is an optimal dose that has a physiological effect. If the dosage is too low, it will not be effective; however, if it is too high it can cause inhibition [13]. In the mangrove crab *Scylla* sp., a dose of 1 mg/g of body weight acts as a stimulant on the reproduction process, whereas a dose of 2 mg/g of body weight acts as an inhibitor.

M. malabathricum extract administered to rats is safe even at a high dose of 5 mg/g of body weight and has no oral toxicity [14]. An acute toxicity study on rats at doses of 2 mg/g and 5 mg/g did not result in any mortality in the treated rats and no toxic effects were observed throughout the 14 days study period. Physical observations of the rats indicated no signs of change in skin and fur, eyes and mucus membrane, behaviour patterns, tremors, salivation, diarrheal and sleep. The body weight of the treated male and female rats increased gradually but was not significantly different compared to those of the control rats. Gross necropsy findings did not reveal any changes in the organs. The clinical observations and biochemical measurements reflected normal status of the kidney and liver functions, and histopathological evaluations of these organs all together revealed that there were no significant differences between the control and the test groups. Although the doses used in this study were much lower than those in the study on rats, there is a need for more in-depth research regarding the possible toxicity of *M. malabathricum* to crabs.

The effect of the M. *malabathricum* leaf extract on the growth and spawning of blue swimming crabs can be assumed to be caused by the active substances contained in the leaves. Although in this study no active substances which might be influential were measured, previous studies have identified an analogestrogen in *M. malabathricum*. Farizah et al. reported an increase estradiol concentrations in the hemolymph of mangrove crabs after the application of *M. malabathricum* extract [10]. Estradiol injection was thought to indirectly stimulate ovarian development and vitellogenesis in female *Portunus trituberculatus*, a crab closely related to *P. pelagicus*, by mediating the secretion of hormones and gene expression in the endocrine organs (e.g. eyestalk and mandibular organs) [14].

5. Conclusion

The main conclusion drawn from this study is that the right dose of *M. malabathricum* leaf extract could be used to stimulate spawning in *P. pelagicus*. The 15 ug/g of crab body weight was found to be the best dose in terms of crab growth and spawning.

Acknowledgement

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