2

by Sudarno Sudarno

Submission date: 05-Nov-2022 12:15PM (UTC+0800)
Submission ID: 1945116733
File name: 2._IOP_2017_Sudarno.doc (81.5K)
Word count: 2699
Character count: 13722

The Effectiveness of Extracts Basil Leaves (*Ocimum sanctum* Linn) against *Saprolegnia* sp. by in Vitro

Sudarno[1], Muhammad Luthfi Hakim[2] and Rahayu Kusdarwati[3]

[1]Department of Fish Health Management and Aquaculture Faculty of Fisheries and Marine, Airlangga University- 031-5914151,
[2] Study Program Aquaculture Faculty of Fisheries and Marine, Airlangga University
[3]Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Airlangga University

darnosudarno1@gmail.com

Abstract. *Saprolegnia* SP. is a fungi which is opportunistic and generally as a secondary pathogen on fish. *Saprolegnia* sp. infects epidermis tissue that begins at the head or fins and can spread over the entire surface of the body. The result of the using of chemicals to control infections of *Saprolegnia* spp. can cause pollution of the environment and harm the consumer. The purpose of this research was to determine the potential and the minimum concentration of extracts basil leaves (*Ocimum sanctum* Linn) as antifungi against the growth of *Saprolegnia* sp. by vitro. The research was held in Fish Quarantine Kelas I Juanda Suarabaya in January 2015. A positive result was obtained in the test of the effectiveness of basil leaves in inhibiting the growth of the fungus *Saprolegnia* sp. Concentration of the extract given to treatment 90% and 100% was able to inhibit the growth of *Saprolegnia* sp., indicated by the formation of the inhibitory zones at a concentration of treatment, and had the best results on the concentration of 100%.

1. Introduction

Fish disease is one of the serious problems that must be faced in the aquaculture business. One of the diseases that attack the fish cultivation is caused by fungus, *Saprolegnia* sp. [1]. This fungal infection can be triggered by several factors, one of which is the high fish density. Saprolegniasis is the disease in fish and fish eggs caused by *Saprolegnia* SP. or watermolds. The infected fish show the presence of white patches on the body and there is a bloody wound observed on the gills and skin. These fungi attack may cause death in fish eggs or fish itself which is significantly harmful to the survival of the fish farming business. Many farmers and businessmen fish use a variety of chemicals such as formaldehyde or antibiotics in fish disease control. However, the use of chemicals and antibiotics is continuous with the dose or concentration that is not appropriate. It will cause a problem recently in the form of increased resistance to microorganisms against these materials. In addition, another issue is the danger posed to the environment surrounding it, the fish is concerned, and human consumption [2]. Therefore, it is an alternative way to control the disease. The use of medicinal plants is a safe way to inhibit microbial growth and to kill as well as to be environmentally friendly. One of them is by using plant basil leaves (*Ocimum sanctum* Linn). The part that can be used is part of the leaf. Basil leaves can be used as antifungi because it contains essential oils with a population amounting to 0.8 mg/100 g. The content of essential oil compounds in basil leaves

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

as methyl chavicol antifungi and linalool which reacts with the cell membrane and a significant reduction in the amount of ergosterol. Ergosterol is a vital component of fungal cell membranes that keep [3]. Other compounds in the basil leaves are alkaloids, tannins, flavonoids and saponins [4]. On the research that has been done from extracts of essential oils essential oil, kemangi leaves have antifungi activity against *Fusarium solani*, *Penicillium funicolusum*, *Trichorderma reesi,* and *Rhizomucor auricus* [5], and *Aspergillus fumigatus* and *Aspergillus niger* [6]. Based on the background, this research aims to discover the influence of extracts of basil leaves (Ocimum sanctum Linn) against the growth of the fungus *Saprolegnia* sp. in vitro.

2. Materials and Methods

The research was conducted in the laboratory of Fish Quarantine Kelas I Juanda Surabaya in January 2015.

2.1. Equipments and materials

2.1.1. Equipments

Equipment needed in this study are reaction tubes, racks of reaction tubes, petri dish, bunsen burners, ose, microscopes, scalpel, glass objects, cover glass, autoclave, hot plate, paper discs, a micropipette, pipette drops, mixer, erlenmeyer flask, measuring cup, haemocytometer, digital scales and Rotary vacum evaporator.

2.1.2. Materials

Materials required are pure *Saprolegnia* sp. isolates obtained from the laboratory of Fish Quarantine Kelas I Juanda Surabaya, basil leaves (*Ocimum sanctum* L.), aquades, ethanol 96%, Formalin, Dimethyl sulfoxide (DMSO) 10% , Sabouraud Dextrose Agar (SDA), and antibiotics.

2.2. Media Preparation

Use of the media is very important for the isolation, identification or differentiation to get a suitable environment for the growth of fungi. Erlenmeyer flask is placed in an autoclave along with petri dish at a temperature of 121 ˚ C 1 atm pressure for 15 minutes. The SDA materials in liquid state included as many as 20 ml in each Petri dish.

2.3. Solvent extracts of basil leaves

Basil leaves extracts is diluted with the solvent Dimethyl sulfoxide (DMSO). The solvent DMSO used is concentrations of 10%, to make the solution 10 ml of DMSO is required 100% added to 90 ml of aquades [7]. Basil leaves is dried by Sun light for 3 days and mashed into powder of basil. Basil powder as much as 500 grams is macerated using ethanol 96% over the 3 x 24 hours in room temperature. Maceration is a way of extracting the most simple because the materials would be extracted simply dissolved in the solvent. Besides solvent used in the study is 96% ethanol. especially those that have antifungal properties. The solution obtained is then filtered using filter paper and then evaporated using a Rotary evaporator vacum. The resulting extract is then diluted in accordance with the concentration 100% i.e. 5 ml ingredient extracts of basil, 90% i.e. 4.5 ml ingredients plus 0.5 ml of DMSO 10%, 80% i.e. 4 ml ingredients plus 1 ml of DMSO, 70% that is 3.5 ml ingredients coupled with 1.5 ml of DMSO, 60% that is 3 ml ingredients coupled with 2 ml of DMSO, 50% i.e. 2.5 ml ingredients coupled with 2.5 ml of DMSO, 40% IE 2 ml ingredients coupled with 3 ml of DMSO, 30% i.e. 1.5 ml 3.5 ml plus material of DMSO, 20% i.e. 1 ml ingredients coupled with 4 ml of DMSO, 10% i.e. 0.5 ml ingredients coupled with 4.5 ml of DMSO, control the positive i.e. 0.2 ml formalin and control the negative i.e. 5 ml DMSO.

2.4. Culture and identification of *Saprolegnia* sp.

Saprolegnia sp. obtained from laboratory stocks of Fish Quarantine Kelas I Juanda Surabaya reproduced by culturing mildew on SDA media. The process begins with the way of inoculating mushroom on one SDA media made by using a scalpel in aseptis.

The purification process begins by taking one type of colony using a scalpel on the old SDA media which has a similar color and texture then inoculated on SDA new media and incubated at temperatures of 25° C for 2-7 days to obtain pure isolates. After done, culture media of water for easy observation. Start with cutting edge of jelly that has a colony of mold approximately 0.5-1.0 cm using a scalpel blade set on fire and then move jelly piece to aquades in a petri dish and incubate for several days at temperatures of 25° C [8].

2.5. Yeast Suspension

Isolate pure fungi *Saprolegnia* SP. which has been cultured on the media of jelly to be moved in a tube.Tto get the suspense of mushrooms, spores calculation is done using haemocytometer. The density of spores at a minimum to be used is around 10^5 sel/ml [8].

2.6. The Effectiveness of Antifungal

Testing of the effectiveness of the disc diffusion method used antifungal. Testing is conducted to find out the minimum concentration of a solution of an antifungal that may inhibit the growth of mold. Paper disc diffusion method begins by soaking paper discs that will be used during CA. 10 minutes into the basil leaf extract with a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% with a size paper discs of 6 mm, media control (-) is given a 10% DMSO. Then the study used the concentration of 0.4% in controls (+). Incubation is done at a temperature of 25 ˚ C for 48 hours. The magnitude of the clear zones produced antifungal potential comparable to that produced by the active substances contained in materials and measured using a ruler.

2.7. Data Analysis

Data research results are in the form of drag zone diameter growth of mold *Saprolegnia* sp. by basil leaves extracts antifungi (*Ocimum sanctum* Linn) and formalin 0.4%. The diameter of the zones of drag is the diameter of the area which is not covered by fungus around the paper discs with a diameter reduced paper discs.

3. Result and Discussion

3.1. Identification of *Saprolegnia* sp.

Identification process is done to ensure the truth against fungal isolates obtained from Fish Quarantine Hall class I Surabaya I that the fungus is a fungus *Saprolegnia* SP. Identification can be done by looking at the macroscopic and microscopically [9].

Identification of the fungus microscopically can be done by looking at the morphology of *Saprolegnia* sp. i.e. aseptat and Hypha, has tube-shaped, with a system of branching in sporangial (internal proliferation), and has two forms of zoospore (*dimorphic*), namely primary and secondary zoospore. Primay zoospore is pyriform-shaped with no flagellate. Secondary zoospore is cysts-shaped (reniform) like nuts with flagella and swim more strongly than primary zoospore. The characterisitics of the suit stated by [10].

Macroscopicly *Saprolegnia sp*. can be seen with the characteristics of a colony of white-yellowish with forms such as cotton. The growth of a colony is very fast (optimum at 15-30 ° C) [11]. Observation of

results of test extract potential basil leaves against the growth of *Saprolegnia* SP. is done by looking at the onset of drag that results from the zone mechanism of antifungi.

3.2. The Minimum Inhibitory Concentration (MIC)

Observation of results of test extract potential kemangi leaves against the growth of *Saprolegnia* SP. is done by looking at the onset of drag that results from the zone mechanism of antifungi in inhibiting the growth of fungus. The results obtained in this study demonstrate positive results because it occurs at concentrations inhibitory zones given. Basil leaf extract that is produced is able to inhibit the growth of *Saprolegnia* sp. It was indicated by the formation of the inhibitory zones at a concentration of 90% and 100% control in conjunction with the positive (+) in the form of a solution of formalin 0.4% which is able to inhibit the *Saprolegnia* sp. by 1 cm.

Table 1. The results of observations of the formation of the inhibitory zones basil leaves extracts against *Saprolegnia* sp.

No.	Concentration basil leaves extracts (%)	Inhibitory zone diameter (cm)
1.	10	0,0
2.	20	0,0
3.	30	0,0
4.	40	0,0
5.	50	0,0
6.	60	0,0
7.	70	0,0
8.	80	0,0
9.	90	0,8
10.	100	1,7
11.	K (-)	0,0
12.	K (+)	1,0

The results of the assay activity antifungi extracts of basil leaves (Ocimum sanctum Linn) against the fungus *Saprolegnia* sp. has been carried out in vitro. Antifungi activity test includes testing the Minimum Inhibitory Concentration (MIC) concentration of 10% up to 100% concentration. The method used is the method of diffusion paper discs that show Basil leaf extract has the ability to inhibit the growth of mold *Saprolegnia* sp. This can be seen with the formation of the zone around the mushroom paper drag discs, these results correspond to the positive (+) control as a comparison which is also visible for the presence of inhibitory zones around the paper discs.

The difference between the concentration of inhibitory power extracts are inoculated with the fungus and results positive control then, approaching the concentration of extract, basil leaves are considered capable of inhibiting the growth of *Saprolegnia* sp. [7]. The greater the concentration of the extract of the basil leaves, the greater the drag the extract power anyway against the growth of *Saprolegnia* sp. [12] state that the higher the concentration of extract, the bigger the death or inhibition of the growth of a fungus.

From the pictures the minimum inhibitory concentration test results show that the growth of *Saprolegnia* sp. colonies. Is white like cotton and dominions of the round. This is in accordance with [10] that the colony of *Saprolegnia* sp. is white with yellowish surface such as cotton, round and prominent. The growth of colonies of different sizes is due to the difference in concentration of the basil leaves extract that can inhibit the growth of *Saprolegnia* sp. the greater the concentration of extract the basil leaves, the smaller the size of the colony of *Saprolegnia* sp. or not even growing colonies.

Not growing colonies of fungus on the positive control containing 0, 2 ml formalin 10% proves that formaldehyde has the ability in inhibitting and killing the fungus. Formaldehyde (formalin) is highly reactive chemicals that interact with proteins, DNA, and RNA in vitro. Formaldehyde was chosen because according to [13] that formaldehyde is a versatile antimicrobial compounds, one of which can kill fungus. Formaldehyde is considered sporicidal based on its ability to penetrate into the inside of the mold spores. Formaldehyde also reacts extensively with nucleic acids [14].

While the growth of a colony of mold on negative control containing 5 ml of DMSO 10% indicates that the solvent DMSO does not affect the growth of mold. This is in accordance with [15], States that DMSO is not toxic and carcinogenic at concentrations of 5%-10% so that DMSO does not enter reacts with microbial testing.

Basil leaves were chosen because it has the active ingredient as antifungi. Content of active ingredients in basil leaves already examined to be able to fight fungi is essential oil, flavonoids, and saponins. On the research that has been done from extracts of essential oil of basil leaves, exposing the antifungi activity against *Fusarium solani*, *Penicillium funicolusum*, *Trichorderma reesi* and *Rhizomucor auricus* [5]. The active compounds that can inhibit and kill *Saprolegnia* sp. obtained from results of extraction using ethanol. Essential oils in basil leaves most of the lot contains ethyl p-metoksisinamat (EPMS) are generally insoluble in solvents of ethanol, ethyl acetate, methanol and heksan [16].

Flavonoid is compound of polar so that flavonoids are soluble in polar solvents such as ethanol, methanol, acetone, dimethyl sulfoksida (DMSO) and dimethyl fonfamida (DMF) [17]. According to [19] it is a secondary metabolite compound found in plants. Flavonoids found in plants are flavones and a flavonol. Besides flavonoids have compound genestein function to inhibit the cleavage of cell proliferation or mushrooms. These compounds bind to proteins on microtubules in the cell and disrupts the function of mitosis giving rise to inhibition of fungal growth. Influence of phenol compounds as an antifungal protein bonding denature is with the membranes of the cell so that the cell membrane Lysis and allowed the phenol to penetrate into the cell nucleus that cause fungi does not develop [18]. The Saponins are a work mechanism with antifungi that can form a complex with sterol that is lowering the surface tension of the membrane permeability of the cell membrane so that the sterol molds and yeasts [19]. This result in the cell walls becomes permeable and fungal cell structure is destroyed due to the antifungal compounds inhibiting ergosterol biosynthesis of sterols which is to maintain the integrity of the cell membrane of yeast [20].

4. Conclusion

Based on the results of this research, it can be concluded that solution of extracts of basil leaves (Ocimum sanctum Linn) has the potential of antifungi against *Saprolegnia* sp. Concentration of 90% (0.9 g/ml) with zone drag 0.8 cm and 100% (10 g/ml) with zone drag 1.7 cm from extracts of basil leaves is able to inhibit the growth of *Saprolegnia* sp. while formalin 0.4% as control (+) is able to inhibit the inhibitory zones with a 1 cm.

ORIGINALITY REPORT

10%	5%	8%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.nveo.org Internet Source	3%
2	Anindya Wirasatriya, Hiroshi Kawamura, Muhammad Helmi, Denny Nugroho Sugianto et al. "Thermal structure of hot events and their possible role in maintaining the warm isothermal layer in the Western Pacific warm pool", Ocean Dynamics, 2020 Publication	2%
3	dspace.brunel.ac.uk Internet Source	2%
4	Rahayu Kusdarwati, Kismiyati, Sudarno, Hendi Kurniawan, Yudha Teguh Prayogi. " Isolation and Identification of and sp. on Catfish ( ) in Floating cages in Bozem Moro Krembangan Surabaya ", IOP Conference Series: Earth and Environmental Science, 2017 Publication	1%
5	Ismalia Husna, Endah Setyaningrum, Tundjung Tripeni Handayani, Yogi Kurnia et al. " Utilization of Basil Leaf Extract as Anti-	1%

Mosquito Repellent: A Case Study of Total Mosquito Mortality (3 Instar) ", Journal of Physics: Conference Series, 2020
Publication

6	acikbilim.yok.gov.tr Internet Source	<1 %
7	Akhmad Taufiq Mukti, Odang Carman, Alimuddin, Muhammad Zairin. " A rapid chromosome preparation technique without metaphase arrest for ploidy determination in Nile tilapia, ", Caryologia, 2016 Publication	<1 %
8	Chen, Xiao-jun, Zhi-yuan Meng, Ya-jun Ren, Hao-tian Gu, and Chun-liang Lu. "Effects of ZnO Nanoparticle on Photo-Protection and Insecticidal Synergism of Rotenone", Journal of Agricultural Science, 2016. Publication	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		