EFFECT OF ANTIFUNGAL TREFLAN AND LENTIL ESSENTIAL OIL DISC INHIBITION METHODS ON *LAGENIDIUM CALLINECTES*

Marcellia Selvi¹, Saputra Afandi^{2*}, Ulfa Ade Maria¹

¹Department of Pharmacy, Faculty of Medicine, University of Mahalayati, Lampung, Indonesia

²Department of Aquaculture, Sekolah Tinggi Perikanan Jakarta, Indonesia

*E-mail: afandi.saputra@yahoo.co.id

ABSTRACT

Lagenidium callinectes is marine phycomycetous parasitic fungus capable to spread disease in marine Crustacea eggs and larvae. Treflan (trifluralin, Elanco) is used in penaeid shrimp larval mycosis treatment. The disease was caused by the phycomycetous fungi *Lagenidium* sp. and *Sirolopidium* sp. The study aimed to determine the efficacy of Treflan and Lentil Essential Oil to *Lagenidium callinectes* growth inhibition in Potatoes Dextrose Agar (PDA) + 2% NaCl. This study was observational analytical research utilizing experimental design. The data were obtained from two treatment group. Treatment Group 1 used Lentil Essential Oil and Treatment Group 2 used Treflan product. Each treatment dilution 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} were dripped onto disc. The control group was immersed in sterile seawater. The inhibition zone diameters from each plate were measured. The treflan treatment group exhibited *Lagenidium* sp. 11.08 cm growth inhibition zone in the second day of incubation and 8.27 cm growth inhibition zone in the third day of incubation on dilution 10^{-1} (100.000 ppm). Lentil Essential Oil treatment group exhibited *Lagenidium* sp 8.38 cm growth inhibition zone on the second day of incubation on dilution 10^{-1} (100000 ppm). The study indicated that Treflan and Lentil Essential Oils are capable to inhibit *Lagenidium* sp. growth as these contain antifungal compounds.

KEY WORDS

Lagenidium sp., Treflan product, lentil essential oil, growth inhibition.

Lagenidium callinectes is a marine phycomycetous parasitic fungus. *Lagenidium callinectes* or similar species have been reported spread disease on marine Crustacea eggs and larvae. The *Lagenidium* exhibits similar pathology in all host species. In wild crustacean populations, it affects the eggs. In aquaculture, however, it affects both eggs and larvae [1].

Lagenidium callinectes hyphae forms are contorted, irregularly branched, sparingly septate. It possesses cell wall and membrane, vacuoles, mitochondria, ribosomes, small and large vesicles, and woronin bodies. *Lagenidium callinectes* spore is singular or in pairs. The fungal Mycelium could either invade or infect larval *Penaeus monodon* muscle tissues [2].

Treflan (trifluralin, Elanco) is used in penaeid shrimp larval mycosis treatment. The disease was caused by the phycomycetous fungi *Lagenidium* sp. and *Sirolopidium* sp. Some culturists have reported treatments using Mfluralin to be ineffective while others have found it to be quite efficacious [3].

Lentil Essential Oil (LEO) contains sweet almond oil, lavender oil, coconut oil, olive oil, peppermint oil extract. These extracts are used as antifungal and antibacterial.

The specific objectives of this study were to determine the efficacy of LEO and Treflan product to inhibit pathogenic fungus growth in agar media.

MATERIALS AND METHODS OF RESEARCH

The fungus was obtained from AHS Lab. These samples were identified, purified, certificated, and assessed by a Professor. Other materials used were Lentil Essential Oil. Treflan Product, PDA medium, Incubator, Petri dish.

Agar media used in this experiment was PDA+2% NaCl. Agar media was kept in 4°C temperature prior to the experiment.

Pure fungus isolate was obtained from PDA agar, which was procured from AHS Lab. Fungus culture used were 5-7 days old. The fungal colony was suspended in 50 ml of sterile seawater 20 ppt in a beaker and incubated at 30°C for 48 h to induce zoospore formation. Zoospore densities were estimated using hemocytometer light microscopy [4]. After determining zoospore densities, based on the modification of the method by Tidaporn, Spores was transferred to agar media (SDA+2% NaCl+ 100 mg gentamicin) [5]. Treatment Group 1 used 15 ul Lentil Essential Oil dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} . The dilution was dripped onto a disc and was incubated at 25-27°C. Treatment Group 2 would be used 15 ul Treflan product dilution 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} . The dilution was dripped onto a disc and incubated at 25-27°C. The control group used sterile seawater. After 24 hours of incubation, the inhibition zone diameters from each plate were measured.

Table 1 – Experimental Design

Location	Group	Rep	Dilution	Product
Lab Fungus	Treatment (Disc)	3	10^{-1}	Lentil Essential Oil
			10^{-2}	
			10^{-3}	
			10^{-4}	
			10^{-5}	
			10^{-6}	
Lab Fungus	Treatment (Disc)	3	10^{-1}	Treflan
			10^{-2}	
			10^{-3}	
			10^{-4}	
			10^{-5}	
			10^{-6}	
Lab Fungus	Control	3	20 ppt	Sterile sea water

The Fungi growth Inhibition Test was conducted at Integrated Laboratory in June 2016.

RESULTS OF STUDY

Table 1 exhibits the efficacy of each Lentil Essential Oil and Treflan product on *Lagenidium* sp. Lentil Essential Oil and Treflan at 10^{-1} dilution inhibited the growth of *Lagenidium* sp.

Table 1 – The efficacy of the Lentil Essential Oil and Treflan Product on the fungi growth inhibition and minimum inhibitory concentration (MIC)

Treatment	<i>Lagenidium</i> sp. Growth Inhibition			MIC
	DOI 2	DOI 3	DOI 4	
Treflan	11,08±1,04	8,27±0,10	0,00±0,00	10^{-1}
Lentil Essential Oil	8,38±0,13	0,00±0,00	0,00±0,00	10^{-1}
Sea Water (control)	0,00±0,00	0,00±0,00	0,00±0,00	

NB: DOI (Day of Incubation) Average ± SD.

DISCUSSION OF RESULTS

Lagenidium sp. is parasite species in arthropods class, known to attack *L. Vannamei* shrimps. A fungal disease caused by *L. callinectes* was reported from larval *P. monodon* from India for the first time. The morphology of the hyphae and spores described in the study is very similar to the description of the fungus in *P. Setiferus* [6].

Lagenidium sp growth was assessed using Treflan. It exhibited 11.08 cm inhibition zone on the second day of incubation and 8.27 cm inhibition zone in the third day of incubation. This occurred on dilution 10^{-1} (100.000 ppm) (Figures 1-2). Treflan contains trifluralin (dinitroaniline) or organic herbicide. Dinitroaniline herbicide would be active when

applied the growing media before the fungal hyphae grew. Dinitroaniline herbicide worked as mitotic toxic that inhibits the fungal hyphae growth.

Figure 1 – Inhibition zone in Treflan group on the second day of incubation (1A) Treflan Replicate A; (1B) Treflan Replicate B; (1C) Treflan Replicate C

Figure 2 – Inhibition zone of Treflan group on the third day of incubation (2A) Treflan Replicate A; (2B) Treflan Replicate B; (2C) Treflan Replicate C

Figure 3 – Inhibition zone in Lentil Essential Oil (LEO) group on the second day of incubation (3A) Lentil Essential Oil Replicate A; (3B) Lentil Essential Oil Replicate B; (3C) Lentil Essential Oil Replicate C

Treflan products reduced damage caused by *Lagenidium* sp [2, 7, 8]. Some culturists have reported trifluralin to be efficacious while others have found it to be ineffective. At the E.R.L.-Marine Culture Facility at Kahuku, Oahu, Hawaii, periodic treatments with trifluralin were less effective compared to a continuous drip system. 0,5 ppm Treflan treatment inhibited *Lagenidium* growth and spread. It reduced the mortality rate of the infected *P. monodon* larval compared to the less effective 0,1 ppm Treflan treatment [9, 10]. In a previous study, Baticados recommended a 20 ppm Treflan treatment for 2 hours. Boonyaratpalin recommended a daily treatment of 0,01-0,05 ppm of Treflan [11, 12]. Treflan treatment should be at 10 ppb every 4 h to contain *Lagenidium* infections [13].

Lentil Essential Oil assessment on *Lagenidium* exhibited 8.38 cm inhibition zone on the second day of incubation on dilution 10^{-1} (100000 ppm). Lentil Essential Oil contains sweet almond oil, lavender oil, coconut oil, olive oil, peppermint oil which functions as antifungal and antimicrobial.

Treflan product and Lentil Essential Oil assessment *Lagenidium* sp. growth exhibited that treflan product is more effective. The Treflan product produces an inhibition zone up to the third day of incubation. On the other hand, Lentil Essential Oil product exhibited an inhibition zone up to the second day of incubation.

CONCLUSION

The study exhibited that Treflan and Lentil Essential Oils are capable to inhibit *Lagenidium* sp growth. Both Treflan and Lentil Essential Oils contain antifungal compounds. Treflan product contains Trifluralin. Lentil Essential Oil contains extraction of sweet almond oil, lavender oil, coconut oil, olive oil, peppermint oil. These are especially effective to inhibit fungal hyphae growth.

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