1980

In-vitro effect of fungicides on hyphal growth and sporogenesis of Lagenidium sp. isolated from Penaeus monodon larvae and Scylla serrata eggs

Lio-Po, G. D.

Aquaculture Department, Southeast Asian Fisheries Development Center

Lio-Po, G. D., Sanvictores, M. E. G., Baticados, M. C. L., & Lavilla, C. R. (1980). In-vitro effect of fungicides on hyphal growth and sporogenesis of Lagenidium sp. isolated from Penaeus monodon larvae and Scylla serrata eggs. SEAFDEC Aquaculture Department Quarterly Research Report, 4(4), 1–6.

http://hdl.handle.net/10862/2377

In-vitro effect of fungicides on hyphal growth and sporogenesis of *Lagenidium* sp. isolated from *Penaeus monodon* larvae and *Scylla serrata* eggs

G.D. Lio-Po, M.E.G. Sanvictores, M.C.L. Baticados and C.R. Lavilla

The sensitivity of *Lagenidium* sp. isolated from *Penaeus monodon* (isolate F16-30) and *Scylla serrata* (isolate F111-22) to thirty-four antimycotic compounds was determined. Mycostatic effects were evaluated from observations of chemicals on the development of vesicles, zoospores and mycelial growth.

The rates of mycelial growth after 48 hr incubation in four different media are presented in Table 1. Analysis of variance of the growth rates of test fungi showed significant differences ($P < 0.01$). Duncan's multiple range test ($P < 0.05$) showed that nutrient agar is the common medium that allowed accelerated growth for both isolates.

Experiments with organic solvents used in subsequent trials revealed that ethanol and dimethylformamide possess inherent antimycotic properties (Table 2). Their use, therefore, was limited to maximum levels of 5,000 mg/l for dimethylformamide and 500 mg/l for ethanol to minimize contributory inhibiting effects that might mask the relative fungitoxic activity of compounds being assayed.

The specific activities of the 34 test chemicals on vesicle formation, sporulation and mycelial growth of *Lagenidium* sp. are presented in Table 3. A "no effect" dose was one that did not inhibit development of vesicles, zoospores or mycelial growth. Mycostatic effects of the therapeutants tested were grouped into: A, doses that allowed vesicle formation but no zoospores were detected; B, doses that inhibited both vesicle and zoospore development; and C, doses that inhibited the development of the said sexual reproductive units and mycelia but which after 48 hours allowed mycelia to continue to grow. Mycocidal doses are those that killed the fungus as evidenced by the absence of hyphal development even after seven days incubation under optimal conditions.

It is apparent from the data that fungitoxic effects of the tested chemicals resulted in inhibition or reduction of either vesicles formed, motile zoospores, or hyphal germination. An interesting observation is that, with most of the chemicals tested, the lowest dose that repressed hyphal growth did not necessarily inhibit sexual reproduction.

Table 1. Mycelial growth of *Lagenidium* sp in different media after 48 hours incubation.

Culture Medium	Mycelium size (mm)[a] F 16–30[b]	F 111–22[c]
Nutrient agar (NA)	12	9
Brain Heart Infusion agar (BHIA)	8	5
Peptone-yeast extract-glucose agar (PYGA)	6	6
Sabourauda's dextrose agar (SDA)	0	0

[a]Mean of three replicates

[b]F value: 10.43 (significant at 1% level)

[c]F value: 9.96 (significant at 1% level)

Potassium permanganate and methylene blue affected both zoospore release and hyphal growth of isolate F16-30 at the least inhibitory doses of 10 mg/l and 0.5 mg/l, respectively. Econazole nitrate yielded similar effects with isolate F111-22 at 0.5 mg/l. A pattern of progressive reduction in the relative number of swimming zoospores and in the size of hyphal development was observed as the dose increased. This pattern was consistently seen in tests on treflan, methylene blue, hydrogen peroxide, clotrimazole, furanace, malachite green, basic fuchsin, benlate, calcium hypochlorite, potassium permanganate and formalin (Table 3). Relationships between dose level and the inhibition rate (%) estimated by the least squares regression analysis revealed linear relationships in benlate, calcium hypochlorite, potassium permanganate, formalin, benzalkonium chloride, 2,4-D, econazole nitrate, ethanol, dimethylformamide, phenol, PVP-iodine and resiguard. On the other hand, very poor antimycotic effects were observed with iodine, triacetin, amphotericin and nystatin. Inhibition of zoospore and vesicle formation by boric acid and fungitox were initially noted at 500 mg/l and at 10 mg/l by griseofulvin. Suppression of mycelial growth by the latter chemicals was practically nil. Lastly, a fluctuating effect on the size of mycelial growth was demonstrated by pimaricin although zoospores were inhibited at doses of 50 mg/l and 100 mg/l.

Resporulation of washed and subcultured test fungi that did not sporulate during exposure to the chemicals showed that majority of these recovered from repressive action of the drug. F16-30 isolates exposed to ethanol, basic fuchsin, acetic acid and malachite green, however, showed no reproductive responses possibly due to permanent damage by these chemicals. Daconil and PVP-iodine seemed to have equal effects on treated colonies of F111-22.

Table 2. Fungitoxic activity of dimethylformamide and ethanol on vesicle formation (V), motile zoospores (Z) and mycelial growth inhibition (MG)[d] of *Lagenidium* sp.

Concentration (mg/l)	Structure affected	Dimethylformamide F16–30	Dimethylformamide F111–22	Ethanol F16–30	Ethanol F111–22
	V	+	+	+	++++
	Z	++	+	+++	++++
	MG	0	0	0	0
	V	++++	++	+	++ ++
500	Z	++	++++	+++	+++
	MG	0	0	0	0
	V	+	+	+	–
1000	Z	+++	+	+	–
	MG	0	0	0	0
	V	+	+	–	–
5000	Z	+++	+	–	–
	MG	0	0	0	0
	V	+	+	–	–
10000	Z	+	+	–	–
	MG	0	14	0	7
	V	–	–	–	–
50000	Z	–	–	–**	–
	MG	86	48	100	45

d – mean of three replicates

** – washed and subcultured mycellium did not resporulate.

Among the systemic antibiotics tested, amphotericin and nystatin exhibited very poor mycostatic effects. This may imply that *Lagenidium* sp. cells have no sterol-containing membrane in which these compounds could induce adverse changes (Gale, 1978). Griseofulvin and furanace exhibited low levels of mycostatic doses. The latter seems promising because of its accompanying antibacterial action (Delves-Broughton & Poupard, 1976; Shimizu & Takase, 1967). On *P. monodon* larvae, however, furanace has been found to adversely affect morphological development (Gacutan & Llobrera, 1977).

Table 3. Mycostatic and mycocidal levels (mg/l) of 34 antifungalagents on *Lagenidium* sp after 24 hours exposure

Chemical	F 16–30					F 111–32				
	"No effect"	Mycostatic dose			Mycocidal	"No effect"	Mycostatic dose			Mycocidal
	Dose	A	B	C	dose	dose	A	B	C	dose
Acetic acid, glacial	100				500	100				500
Amphotericin B	>100					>100				
Benlate	10	50	100		500	10	50	100		500
Benzalkonium chloride	1		5	10	50	0.5	1	5	10	50
Boric acid	100	500	1000			500	1000			
Calcium hypochlorite	100				500	100				500
Clotrimazole	0.1		0.5–1		5	0.1		0.5–1	5–10	50
Copper sulfate	1		5–100		500	0.1	0.5–1	5–100		
Crystal violet	0.1			0.5	1	0.1			0.5–1	5
2, 4 D	1	5	10–100	500		0.1	0.5–1	5–100	500	1000
Dacomil	1	5–10	50–500			1	5–10	50–500		
Detergent	1		5–10		50	1		5–50		100
Dimethylformamide			50000			5000	10000	50000		
Econazole nitrate	0.1		0.5–500	1000		0.1		0.5–100	500–1000	
Ethanol	1000		5000–10000	50000		500		1000–50000		
Formalin	5		10		50	5		10		50
Fuehsin (basic)	1		5–10		50	1	5		10	50
Fungitox	100		500–1000			50		100–1000		
Furanace	0.5	1	5	10	50	1		5–10		
Griseofulvin	5	10	50–1000			5		10–1000		
Hydrogen peroxide	100		500–1000			10		50		100
Iodine	>10					>5				
Malachite green	0.05		0.1	0.5	1	0.5	0.1		0.5	1
Methylene blue	0.1	0.5	1–10		50	1				5
Nystatin*	1000					500	1000			
Phenol	10		50–1000			10		50–1000		
Pimaricin	10	50–>100				50	100–>100			
Potassium permanganate	5	10	50		100	5	10	50		100
PVP-Iodine	100		500	1000		50		100–500	1000	
Resiguard	1	5–10			50	50				100
Tolnaftate	5		10–100			0.5	5–50	50–100		
Triacetin	>100					>50				
Treflan-R	0.5	0.5–1				0.05	0.1–5			
Trifluralin	0.001	0.005–0.1	0.5–10			0.001	0.005–0.1	0.5–5		

A — dose at which vesicles developed but no motile zoospores were detected.

B — dose that inhibited vesicle formation

C — dose that inhibit mycelial growth for 48 hours

* — dose computed as units/ml

The broad spectrum activity of clotrimazole has been demonstrated with *Candida albicans* (Iwata & Yamaguchi, 1975). Pimaricin, however, required high doses to be mycostatic in the present study. The poor antimycotic activity of triacetin may be attributed to the absence of esterases in seawater which catalyzes its conversion to the actively antifungal derivative.

Disinfectants tested, i.e., benzalkonium chloride, detergent (Tide), formalin, phenol. potassium permanganate and resiguard were mycocidal at low concentrations and may be considered for disinfection of equipment and facilities used in crustacean culture. Acetic acid, boric acid, calcium hypochlorite, hydrogen peroxide, iodine and PVP-iodine required such high levels that they are not economically suitable for disinfection.

The poor sporulation response of some treated isolates may infer partial or permanent damage of sporogenesis in *Lagenidium* sp. exposed to these fungicides. The absence of correlation between both test fungi must be regarded with skepticism until more extensive studies are made. A consistent observation in the present experiment was the apparently relative sensitivity of *Lagenidium* sp. hyphae to the test fungicides even at very low doses as evidenced by the degree of inhibition rates. That these inhibition rates are not correspondingly accompanied by effects on sporogenesis leads to the conclusion that fungal mycelia of *Lagenidium* sp. are probably affected by fungicidal action even at very low doses. Although comparative studies on mycostatic and sporostatic activities of antifungals showed no evidence that the latter is stronger than the former, in general, fungistatic activity of various substances seems to have more effect on mycelial growth than on germ tube emergence (Yanagita & Yamagishi, 1958).

Although mycocidal levels of the chemicals tested will be ideal for lethal treatment or control of *Lagenidium* sp. the high dose required may be lethal to the host. The use of the mycostatic concentrations therefore is a practical approach. Based on this study, clotrimazole, crystal violet, econozole nitrate, malachite green, treflan and trifluralin should be considered first in priority since these exhibit mycostatic effects at less than 1 mg/l dose. In addition, other chemicals such as benzalkonium chloride, formalin and resiguard may be included because of their relatively low cost. Treatments of rearing water containing larvae, adult shrimps or crabs, however, should be done only after preliminary tolerance experiments using at least the mycostatic dose prove to be safe for the hosts. Mycocidal doses on the other hand, can be useful for determining disinfection doses of equipment and facilities used in rearing procedures as well as for destroying batches of infected larvae.

Literature cited:

Delves-Broughton D.J., and Poupard C.W. 1976. Disease problems of prawns in recirculating systems in the U.K. Aquaculture 7, 201-217.

Gacutan R.Q. and Llobrera A.T. 1977. Effects of furanace on the zoeae and mysis of *Penaeus monodon*. Kalikasan, Phil. Jour. of Biol. 6, 263-268.

Gale E.F. 1978. Polyene antibiotics. From Symposium papers on antifungal antibiotics and other antifungal agents by A. Trinci. British Mycological Society Bulletin 11, 136-144.

Iwata K. and Yamaguchi H. 1975. Clotrimazole (Canesten) – mode of action in broad spectrum antimycotic Canesten, Bayer pp. 6-10.

Shimizu M. and Takase Y. 1967. A potent chemotherapeutic agent against fish diseases, 6-hydroxymethyl-2 (2-(5-nitro-2-furyl) vinyl) pyridine, (P-7138). Bulletin of the Japanese Society of Scientific Fisheries 33, 544-554.

Yanagita T. and Yamagishi S. 1958. Comparative and quantitative studies of fungi toxicity against spores and mycelia. 6, 375-381.