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FUNGICIDE EFFECT OF TRITON-N ON PHLYCTIDIUM

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SUMMARY

Triton-N surfactants can combat algal parasites *Phlyctidium scenedesmi (Fott)* and *Phlyctidium sp.* Triton N-57 inhibits temporarily the growth of *Scenedesmus acutus Meyen* and *Scenedesmus acuminatus (Lagerh./Shod.)* and later stimulates it. This surfactant can be used in producing healthy inoculum as a starting material for mass algal cultures. A medium formulation was developed for maintenance of parasites in laboratory.

INTRODUCTION

The scarcity of data about the fungi belonging to the genus *Phlyctidium* is due to difficulties in maintaining laboratory cultures for periods longer than a few weeks (Abeliovich and Dickbuck, 1977; Richmond, 1986). We report here a suitable medium formulation for the maintenance of algo-fungal cultures. Our several year's experience proves that the formulation is successful.

Open-field algal cultures can be seriously affected or completely destroyed by fungal parasites (Ilkov, 1980,; Richmond 1986). Very often some parasite cells survive the chemical treatments and restore the infection. On the other hand some chemicals restrict algal growth for long time or are very toxic. We report here that some ethoxylated nonylphenols can be effective fungicides in producing a healthy inoculum as a starting material for mass algal cultures.

MATERIALS AND METHODS

Strains. The green algae *Scenedesmus acutus (Meyen)*, strain *Tomasselli 8* and *Scenedesmus acuminatus (Lagerh./Shod.)*, received from the Center for Investigations of Autotrophic Organisms, Florence, Italy and from the Hydrobiological Laboratory, Institute of Botany, Trebon, Czechoslovakia, respectively were used. The two chitridial strains, *Phlyctidium scenedesmi (Fott)*, strain P1 and *Phlyctidium sp.* strain P2 which are obligate parasites on *Sc. acutus* and *Sc. acuminatus* respectively, were isolated from heavily infected mass algal cultures in Roupite, Bulgaria.

Nutrient medium. Algo-fungal cultures were maintained on mineral water having the following composition in mg/l : F^-=5.80; Cl^-=35.78; I^-=0.04; SO_4^{2-}=123.04; HCO_3^-=1444.95,

HPO_4^{2-}=0.05; HS^-=1.09; S_2O_3=7.36; Na^+=549.28; K^+=41.40; Ca^{2+}=31.43; Mg^{2+}=12.91; Al^{3+}=0.74. This mineral water was enriched with the following nutrients in mg/l : NH_4NO_3=800; $MgSO_4$.$7H_2O$=988; KH_2PO_4=340; $Fe_2(SO_4).9H_2O$=14.05; H_3BO_4=3.09; $MnSO_4.4H_2O$=1.11; $CuSO_4.5H_2O$=1.24; $ZnSO_4.7H_2O$=1.40; $(NH_4)_2$ $MoO_4.2H_2O$=1.21; EDTA (sodium salt)=300.

Culture conditions. Continuous illumination (8OOO lux) was supplied from one side of 200 ml culture vessels by 4 x 40 W daylight fluorescent lamps at constant temperature (29°C) and aeration 100 l/m^3h of air enriched with 2% CO_2. Algae from exponentially growing cultures were used as inoculum in all experiments. The effect of Triton on algal yield gain was registered by daily correction of suspension density to 0.5 g/l and addition of lacking nutrients . The death of all parasite cells was verified by semicontinuous cultivation over ten days.

Development of infection. Direct microscopic counts of infected cells were performed with a hemocytometer. Healthy algal cultures (0.5 g/l) were innoculated either with free zoospores or with heavily infected cultures to achieve 2 % infected to healthy algal cells.

Yield gain was determined by dry weight.

Chemicals were analytical grade. Each result is the mean of four trials.

RESULTS AND DISCUSSION

The above mentioned formulation of nutrient medium ensured high virulence of both investigated parasite strains; 48 hours after the inoculation most of the algal cells were infected or killed (lysed) by the parasites. Weaker virulence was maintained at dimmer light and scant aeration. Diurnal and seasonal changes in virulence were noticed. The heaviest fungal infections of mass algal cultures in field conditions (Roupite, Bulgaria) were noticed during the hottest months of the year. The virulence was stimulated by light and suppressed temporarily by the antioxidants α-tocopherol (0.24 mM) and butylated hydroxytoluene (1.1 mM) suggesting that free radical processes might be involved, but light-sensitive anti-algal toxin was not found (unpublished results). DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea (10 mM) stopped algal growth and minimized oxygen production but did not affect virulence, suggesting that oxygen-dependent chemotaxis did not help parasite zoospores to find their hosts in the light (results are not shown). Surfactants can inhibit the growth of many aquatic organisms (Caux et al, 1988; Weinberger et al, 1987). No data concerning the toxicity of Tritons and nonylphenol to the parasite *Phycomycetes* are available. Data concerning *Phlyctidium scenedesmi* (Table 1) show that ethanol has no fungicide effect but it enhances the fungicide effect of Triton N-57, obviously due to enhanced solubility of nonylphenol. The fungicide effect of Tritons depended on their hydrophilic - lipophilic balance. The higher ethoxylate Triton N-101 had weaker fungicide effect - its higher emulsifying capacity led to concentration of active substance over the suspension so diminishing the surfactant concentration in it.

Table 1

Effect of the degree of ethoxylation and solubility in water of Tritons on their fungicidal activity to *Phlyctidium scenedesmi*

	Chemicals					
	Triton N-57	Nonylphenol	Polyethylene-glycol	Triton N-101	Ethanol	Triton-57 +Ethanol
Concentration (mg/l)	30.00	14.57	15 43	43.60	1.40	21.00+1 40
Fungicidal effect (%)	100	96	0	58	0	100

Note: Triton N-57 and Triton N-101 were used in concentrations ensuring equal nonylphenol quantity. Nonylphenol and polyethyleneglycol concentrations equalled their corresponding ontent in Triton N-57. The fingicidal effect of 21 mg Triton N-57/l was 73 %.

Higher concentration of Triton N-57 (260 mg/l) had to be applied to kill *Phlyctidium sp.*The developmental stage of infection was important: when more than 30% of the algal cells were infected, the above mentioned concentrations of the fungicide were insufficient to control the infection. The growth of pure uninfected cultures of *Scenedesmus acutus* and *Sc. acuminatus* was affected differently by Triton N-57 (Table 2).

Table 2

Effect of Triton N-57 concentration on the yield gain of *Scenedesmus acutus* and *Scenedesmus acuminatus after 30 hours*

Scenedesmus acutus				Scenedesmus acuminatus			
Concentration of Triton N-57 (%)							
3×10^{-3}	3×10^{-4}	3×10^{-5}	0	3×10^{-3}	3×10^{-4}	3×10^{-5}	0
Yield gain (g/l)							
0 77	0.89	1.08	1 65	0.95	1 96	2 06	1.86

Low concentration (3 x 10^{-5} %) of Triton N-57 stimulated the growth of *Sc. acuminatus* and at all the tested concentrations of the surfactant this alga grew better than *Sc.acutus*. The growth of algae was temporarily suppressed by Triton treatment, but later a stimulation of growth was noticed.The stimulative effect of Triton N-57 is illustrated at Table 3.

Table 3

Effect of Triton N-57 on the yield gain of *Scenedesmus acutus* in a semi-continous culture. At the zero hour, 30 mg of fungicide/l were added.

Treatment	Time, (h)			
	0	24	48	72
	Yield gain (g/l day)			
Control	1	1	1	1
Triton N-57	1	0.7	1.2	1.7

Some authors (Heussler et al., 1978 and Grobbelaar, 1981) believe that suboptimal growth conditions lower the resistance of algae to infections. The present study shows that healthy fast growing (about two doublings per day) cultures of Sc.acutus and *Sc. acuminatus* are easily lysed by *Phlyctidium*. In addition - as a result of technical problems and climatic factors, optimal growth conditions cannot be always maintained in open-field algal cultures. So evidently the parasite problems in these cultures are unavoidable. Our results indicate that a cheap surfactant, Triton N-57, can be used for combatting some strains of *Phlyctidium* and if necessary, in the production of a healthy inoculum as the starting material for algal mass cultures. The net yield gain of algae is not expected to diminish.

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