Note

Toxicity of Five Fungicides Against the Ciliate *Colpoda aspera*

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The acute toxicity levels of 5 kinds of fungicide against the free-living protozoal ciliate *Colpoda aspera* were tested in cocultivation with *Alcaligenes faecalis* IFO13111. The fungicides used were products containing the following effective components: dimethylimol (DMM), admixture of ziram, thiram and polyoxin (ZTP), flutolanil (FLN), streptomycin (SM), or 8-hydroxyquinoline copper and captan (CHC). Each fungicide was added at varying concentrations, and the effective concentration (EC_{50}) at which the specific growth rate of *C. aspera* was reduced to 50% after 24 h culture was defined as the acute toxicity of the fungicide tested. It was found that ZTP had the highest level of toxicity, followed by CHC, SM, DMM and FLN. When the fungicides were evaluated in terms of safety on the basis of indices obtained by dividing normally applicable concentrations (NC) by EC_{50} , the safety levels of SM, DMM and FLN were high compared to those of ZTP and CHC.

Key words : *Colpoda aspera*/Fungicide/Toxicity/Median effective concentration.

Various fungicides are used in agricultural production for the prevention of a productivity drop caused by pathogenic microorganisms. If the fungicides used are washed out with rain water into the soil and bodies of water, ecological systems may be affected. Among microfauna, protozoal ciliates are known to be helpful in the purification of water.

A variety of methods have been introduced to test the acute toxicity of poisons on free-living protozoal ciliates (Kakiichi, 1993b; Persoone and Dive, 1978). We previously tested the acute toxicity of 13 kinds of disinfectant and 13 kinds of insecticide against the ciliate *Colpoda aspera* in cocultivation with *Alcaligenes faecalis* (Kakiichi et al., 1991, 1993b, 1995, 1996). The median effective concentration (EC_{50}) at which the specific growth rate of this ciliate

was decreased to 50% was defined as the acute toxicity (Huber et al, 1990; Kakiichi et al, 1993a; Persoone and Dive, 1978). Reasons why acute toxicity were based on EC_{50} were explained in our previous reports (Kakiichi et al., 1991, 1993b, 1995, 1996). This study was performed to examine the acute toxicity of fungicides against the ciliate *C. aspera* for the environmental protection of water bodies.

The following 5 kinds of fungicide were used: Milcarb®, Polymet-P®, Moncut®, Phytomycin® and Fujiokishiran® of Nihon Nohyaku Co., Ltd. Milcarb is a liquid preparation containing 12.5% (w/v) dimethylimol (DMM). Polymet-P is a wettable powder preparation containing 41% (w/w) ziram, 25% (w/w) thiram and 5% (w/w) polyoxin (ZTP). Moncut is a wettable powder preparation containing 25% (w/w) flutolanil (FLN). Phytomycin is a liquid preparation containing 5% (w/v) streptomycin (SM). Fujiokishiran is a wettable powder preparation containing

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30% (w/w) 8-hydroxyquinoline copper and 20% (w/w) captan (CHC).

The ciliate *Colpoda aspera* was isolated from activated sludge at our laboratory. Cocultivation with *Alcaligenes faecalis* IFO 13111 was carried out using an egg-lettuce medium (EL medium) (Curds, 1963; Kakiichi et al., 1991; Sudo and Aiba, 1971). The same methods as described previously (Kakiichi et al., 1991, 1993b, 1995, 1996) were used for the storage and growth culture of *A. faecalis* and *C. aspera*.

The minimum inhibitory concentrations (MICs) of the fungicides against *A. faecalis* were determined as previously described (Kakiichi et al., 1991, 1993b, 1995, 1996). The toxicity tests of the fungicides against *C. aspera* were carried out by the methods previously described (Kakiichi, 1993a).

The concentrations of the fungicides used in this experiment represent the percent contents of the principal ingredient(s) as mentioned above. For example, EC₁₀ and EC₅₀ of Milcarb against *C. aspera* and its MIC against *A. faecalis* were determined on the basis of the DMM content (12.5%, w/v) of this fungicide. For the mixture preparations containing two or three kinds of ingredients, corresponding values were determined on the basis of the total content of the principal ingredients. Regarding Porymet-P, for example, the total

content of the principal ingredients was 71% (w/w) (41% ziram, 25% thiram and 5% polyoxin).

A method for calculating EC₅₀ has been described in detail (Kakiichi, 1993a; Kakiichi et al., 1991). The specific growth rates of *C. aspera* in media containing different concentrations of fungicides (μ_{tox}) and in those containing none (μ_{cont}) were applied to calculate EC₅₀ and EC₁₀, the concentrations at which μ_{cont} was decreased to 50% and by 10%, respectively.

Table 1 shows the EC₅₀ and EC₁₀ against *C. aspera* and the MIC against *A. faecalis* for the 5 fungicides. ZTP had the lowest value of both EC₁₀ and EC₅₀ against *C. aspera*. It was followed in order by CHC, SM, DMM and FLN. ZTP also had the lowest MIC against *A. faecalis*. It was followed in order by SM, DMM, CHC and FLN. The MIC/EC₅₀ value of SM was 1.3, but the corresponding values of the other fungicides were greater than 3.5. An EC₅₀ test for *C. aspera* was achieved by calculation of the specific growth rates of *C. aspera* in cocultivation with *A. faecalis*. If the MIC against *A. faecalis* and EC₅₀ against *C. aspera* were close in value, it was likely that the EC₅₀ value did not represent EC₅₀ resulting from the direct toxic action of the fungicide used, but could be interpreted as reflecting the decrease or death of the bacteria on which *C. aspera* feeds. The lowest MIC/EC₅₀ value of

TABLE 1. EC₁₀ and EC₅₀ against *C. aspera* and MIC against *A. faecalis* for five fungicides.

Fungicide	<i>C. aspera</i>		<i>A. faecalis</i>
	EC ₁₀ ^a (mg/l)	EC ₅₀ ^a (mg/l)	MIC (mg/l)
Dimethirimol	46.27 ± 11.65	201.56 ± 23.86	700
Ziram + Thiram + Polyoxin	0.16 ± 0.02	0.81 ± 0.11	20
Flutolanil	212.52 ± 12.95	445.11 ± 28.61	>25,000
Streptomycin	33.82 ± 6.54	78.16 ± 9.95	100
8-Hydroxyquinoline copper + Captan	0.48 ± 0.04	1.53 ± 0.24	1,000

^a Values are mean ± S.D.

TABLE 2. Relationship between the normal applicable concentration (NC) and the EC₅₀ of five fungicides.

Fungicide	NC	EC ₅₀	Dilution ^a
	mg/l	mg/l	NC/EC ₅₀
Dimethirimol	42 - 1,250	201.6	> 0.2 - 6.2
Ziram + Thiram + Polyoxin	875	0.8	> 1,094
Flutolanil	250 - 5,000	445.1	> 0.5 - 11.2
Streptomycin	125	78.2	> 1.6
8-Hydroxyquinoline copper + Captan	625 - 1,250	1.5	> 417 - 833

^a Dilution required to keep concentration below EC₅₀.

SM may lead to the presumption that EC_{50} of SM against *C. aspera* reflects the inhibitory influence of SM against *A. faecalis*. This suggested that determination of MIC against bacteria that are used as food is necessary when determination of EC_{50} against ciliates is undertaken in cocultivation with the bacteria.

Table 2 shows the relation between the normal applicable concentration (NC) and EC_{50} of the 5 fungicides. The safety of the fungicides was assessed by dividing NC by EC_{50} . Safety increases with the decrease in NC/ EC_{50} . On calculating NC/ EC_{50} ratios using the highest NC values, SM had the lowest value. It was followed in order by DMM, FLN, CHC and ZTP. SM, DMM and FLN were relatively safe fungicides.

Of the fungicides used in this study, only thiram, a constituent component of ZTP, had been tested previously, and its minimum active dose (MAD) against *Colpidium campylum* had been reported to be 0.3 mg/l (Dive and Leclerc, 1975). The 48h- LC_{50} of ziram, thiram and polyoxin against *Oryzias latipes* have been reported to be 0.056, 75 and >40 mg/l, respectively, and their 3h- LC_{50} against *Daphnia pulex* to be 0.45, 10-23 and >40 mg/l, respectively (Nishiuchi, 1983; Tanaka, 1978). Frear and Boyd (1967) described the 24h- LC_{50} of ziram and thiram against *Daphnia magna* to be 0.016 and 1.30 mg/l, respectively. The toxicity levels of ziram, thiram and polyoxin against fish are ranked C, B and A, respectively (Nihon Nohyaku Co., Ltd., 1995). Of the 3 fungicides, ziram has the highest toxicity against aquatic animals. Of the 5 fungicides used in this study, ZTP had the lowest EC_{50} against *C. aspera*. Ziram was presumed to have largely contributed to the high toxicity of ZTP.

The 48h- LC_{50} of DMM against *O. latipes*, its 3h- LC_{50} against *D. pulex* and its 48h- LC_{60} against *Sympetrum frequens* in the larval stage have been reported to be 74, 350 and >40 mg/l, respectively (Nishiuchi, 1983; Tanaka, 1978). DMM is ranked A in the toxicity list (Nihon Nohyaku Co., Ltd., 1995). The EC_{50} (201.6 mg/l) of DMM against *C. aspera* is close to its 3h- LC_{50} against *D. pulex*, and DMM seems to have a low toxicity against aquatic animals. The fish-toxicity of FLN is in the B rank. The 48h- LC_{50} of FLN against *Cyprinus carpio* has been reported to be 2.4 mg/l (Nihon Nohyaku Co., Ltd., 1995). FLN seems to have a lower toxicity against *C. aspera* than against fish, because its EC_{50} against *C. aspera* was high (445 mg/l).

SM is an antibiotic effective against gram-positive and -negative bacteria and ineffective against anaerobic bacteria. The fish-toxicity of SM is in the A rank, and its 48h- LC_{50} against *C. carpio* and *O. latipes* have been reported to be both >40 mg/l and its 3h- LC_{50} against *D. pulex* to be >40 mg/l (Nihon Nohyaku

Co., Ltd., 1995; Nishiuchi, 1983; Tanaka, 1978). The EC_{50} of SM against *C. aspera* was 78.2 mg/l in this study. This EC_{50} level might have resulted from the bactericidal activity of SM against *A. faecalis* which served as food for *C. aspera*. From this, SM is presumed to have only a minor impact on aquatic animals.

CHC is an admixture of captan and 8-hydroxyquinoline copper. These are both ranked C in the fish-toxicity table and exert a strong effect on aquatic animals. The 48h- LC_{50} s of captan against *C. carpio*, *O. latipes* and *S. frequens* of the larval stage have been reported to be 0.25, 1.0-2.2 and $>1,000$ mg/l, respectively, and its 3h- LC_{50} against *D. pulex* to range from 5.5 to 8.4 mg/l (Nihon Nohyaku Co., Ltd., 1995; Nishiuchi, 1983; Tanaka, 1978). Frear and Boyd (1967) reported that the 26h- LC_{50} of captan against *D. magna* was 1.30 mg/l. The 48h- LC_{50} s of 8-hydroxyquinoline copper against *O. carpio* and *O. latipes* have been reported to be 0.18 and 0.08 mg/l, respectively (Nihon Nohyaku Co., Ltd., 1995; Nishiuchi, 1983). This fungicide has been described as having a 3h- LC_{50} value of 10-23 mg/l against *D. pulex* and a 48h- LC_{50} value of >40 mg/l against *S. frequens* (Nishiuchi, 1983; Tanaka, 1978). The EC_{50} of CHC against *C. aspera* was 1.53 mg/l in this study. This suggests that CHC has the highest toxicity against fish, approximately the same degree of toxicity against ciliates and water fleas and the lowest toxicity against insects.

The results of this study have shown that ZTP had the highest toxicity against *C. aspera*, followed in order by CHC, SM, DMM and FLN. The safety levels of SM, FLN and DMM were almost equally high and those of ZTP and CHC were low. These findings suggest that adequate caution is required to prevent the flow of ZTP and CHC into bodies of water.

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