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EFFICACY OF SOME ANTIBIOTICS AND SODIUM HYPOCHLORITE AS ANTIMICROBIAL CHEMICALS IN BIOLOGICAL CONTROL STUDIES OF NEMATODES

by

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Summary. *In-vitro* experiments were conducted to evaluate the effect of sodium hypochlorite (NaOCl), chloramphenicol and streptomycin sulphate on the parasitism of *Meloidogyne javanica* eggs and growth of the nematode egg parasite, *Paecilomyces lilacinus*. The percentage of eggs exhibiting fungal parasitism, radial growth of *P. lilacinus* and mycelia dry weights of the fungus decreased significantly with increasing concentrations of NaOCl. The effect of 0.5% NaOCl was not significantly different from the control. Chloramphenicol significantly inhibited egg parasitism and fungal growth less than streptomycin sulphate. Thus, streptomycin sulphate and 0.5% NaOCl may provide long-term antimicrobial protection in biological control studies without affecting the antagonistic potential and growth of fungal antagonists.

Studies on the management of root-knot nematodes using nematode egg parasites have increased in the last ten years (Kerry, 1990; Oduor-Owino *et al.*, 1993). However, further studies have been inhibited by the lack of appropriate bioassay techniques. Chemicals such as sodium hypochlorite (NaOCl) and antibiotics are often used in biocontrol studies associated with fungal-nematode interactions (Barker, 1985; Freire and Bridge, 1985; Oduor-Owino and Waudo 1994), but their effects on the efficacy and viability of fungal egg pathogens have not been adequately studied. Inconsistencies in the use of these chemicals affect the screening and detection of appropriate fungal egg pathogens for use in biological control investigations.

The concentration of NaOCl used to extract nematodes for biological control studies has been varied (Esser, 1972; Oduor-Owino, 1993). Antibiotics have also been used inconsistently in studies on fungal antagonists (Kerry and Crump, 1977). Some investigators (Clovis and Nolan, 1983) have used a mixture of penicillin and streptomycin sulphate, while others have utilized streptomycin sulphate, chloramphenicol or aureomycin (Oduor-Owino and Waudo, 1994).

Since NaOCl is a strong oxidizing agent (Esser, 1972), and some fungi are sensitive to antibiotics (Orion and Kritzman, 1991; Pope and Hill, 1991), it is possible that these chemicals affect fungal growth and fungal-nematode interactions, and may interfere with estimations of fungal antagonistic potentials and/or selection of 'active' fungal isolates for use in biological control studies. Information on the effects of different bioassay chemicals on nematophagous fungi is vital for rational screening and isolation of nematophagous fungi. The data may also be

useful for choosing appropriate chemicals for use in biocontrol studies.

The objectives of this study were to determine the effects of NaOCl, streptomycin sulphate and chloramphenicol on fungal growth from naturally parasitized *Meloidogyne javanica* (Treub) Chitw. eggs and radial growth of *Paecilomyces lilacinus* (Thom). Samson Schlecht, a fungal egg pathogen isolated from root-knot nematodes eggs in Kenya (Odour-Owino *et al.*, 1993).

Material and methods

Fifty egg masses of *M. javanica* were hand picked from the roots of tomato (*Lycopersicon esculentum* Mill) cv. Moneymaker, grown in a 15 cm diameter plastic pots filled with naturally infested soil. They were homogenized in a blender containing 10 ml of distilled water for three min (Freire and Bridge, 1985) and the released eggs were collected on a 0.02 mm sieve, washed five times with sterilized distilled water and resuspended in 10 ml of sterilized distilled water. One ml of the egg suspension containing 500 eggs per ml was poured on to 1.5% water agar (WA) plates in Petri dishes which were incubated on the bench at room temperature. Three days later, individual eggs were aseptically transferred to new WA plates and incubated. Fungi isolated from them were tested against *M. javanica* eggs. *P. lilacinus* was frequently isolated and infected a significantly higher number of eggs on WA (Oduor-Owino *et al.*, 1993); it was therefore selected and cultured on potato dextrose agar medium (PDA) in 9 cm Petri dishes for 10 days at 20 °C. Pure cultures were stored on PDA slants at 4 °C and used in three experiments.

The effect of NaOCl, streptomycin and chloramphenicol on the growth of the fungus from eggs was assessed on WA plates. Eggs of *M. javanica* were extracted from tomato roots as described above, and suspended in 60 ml of distilled water. Two mls of the egg suspension containing 2000 eggs were transferred to glass vials containing either 0.0 (control), 0.5, 1.5, 3 or 5% NaOCl in distilled water. Control vials contained 5 ml of sterilized distilled water. All of the vials were shaken vigorously for 2 minutes then the eggs were collected on 0.02 mm sieves, washed with distilled water and resuspended in 80 ml of water in 300 ml conical flasks. Each flask received 8 ml of either 1% chloramphenicol or 0.5% streptomycin sulphate resulting in concentrations of 0.1% chloramphenicol or 0.05% streptomycin sulphate, respectively. Flasks without antibiotic served as controls. One ml aliquot containing approximately 300 eggs was poured on to each Petri dish containing 1.5% WA. All Petri plates were kept on benches in a randomized design with eight replicates per treatment. The percentage of eggs exhibiting fungal growth was determined three days later by randomly examining 100 eggs per plate at x 100 magnification as described by Kerry and Crump (1977).

Effects of NaOCl, streptomycin sulphate and chloramphenicol on the growth of *P. lilacinus* was tested on WA plates. A 13% NaOCl solution was added to 1.5% WA just prior to pouring on to plates to make concentrations of 0.5, 1.5, 3, or 5% NaOCl (v/v). These media were further supplemented with either chloramphenicol or streptomycin sulphate to yield concentrations of 0.1 and 0.05% respectively, as recommended by Kerry (1987). Media without NaOCl or antibiotic served as controls. The agar surface was allowed to dry for 48 hrs before placing 9 mm diameter plugs, cut from the peripheries of 10 day old fungal colonies, at the centre of each plate. The plates were incubated at room temperature and colony radial growth measured 20 days after inoculation. There were eight replications per treatment.

Effects of antibiotics and NaOCl on fungal dry weights were determined using potato dextrose broth (PDB) as the medium. Three fungal plugs (9 mm diameter) were aseptically transferred to sterile 100 ml bottles each containing 60 ml of PDB and either 0.0 (control) 0.5, 1.5, 3,

or 5% NaOCl (v/v). In addition, chloramphenicol (0.1%) or streptomycin sulphate (0.05%) was added to each media after passing it through Whatman No. 1 filter paper and through 0.2 µm millipore filters. Media without antibiotic served as controls. All treatments were replicated eight times and placed on a rotary shaker at 100 rpm for 20 days at room temperature. After the incubation period, fungal colonies were collected by vacuum filtration through Miracloth, dried at 100 °C for 72 hrs and weighed. Fungal radial growth values, dry weights, and the proportion of eggs exhibiting fungal parasitism were subjected to statistical analysis using Analysis of variance (ANOVA) (Freund and Littell, 1981). Treatment means were compared using Duncans multiple Range Test.

Results and discussion

The percentage of parasitized eggs, mycelial dry weights and radial growth of the fungus were significantly greater (P = 0.05) on plates supplemented with 0.5% NaOCl than on plates treated with 1.5, 3 or 5% NaOCl (Tables I, II and III). Increased fungal inhibition with increasing NaOCl concentration could be attributed, in part, to the oxidative effects of this chemical on nematode eggs and the associated fungi (Esser, 1972). Nematode egg shells are composed of chitin and albuminoid material (Chitwood, 1938) that serve as nutrient sources for fungal antagonists (Morgan-Jones and Rodriguez-Kabana, 1985). It is possible that the oxidative effects of NaOCl interfered with this nutritional relationship, resulting in low egg parasitism and growth of *P. lilacinus* on WA. Since high concentrations of NaOCl may also inhibit infectivity of *Meloidogyne* spp. juveniles (Kanwar *et al.*, 1991), a concentration of 2%-5% NaOCl may therefore not be appropriate for ne-

TABLE I - *Percentage of* Meloidogyne javanica *eggs exhibiting fungal parasitism three days after treatment with antibiotics and sodium hypochlorite.*

NaOCl conc. (%)	Percentage infected eggs		
	Chloramphenicol (0.1%)	Streptomycin sulphate (0.05%)	Control
0.0 (control)	17.8 c	24.8 c	23.4 c
0.5	17.0 c	23.3 c	22.8 c
1.5	12.1 b	16.3 b	15.3 b
3.0	9.0 ab	12.6 ab	13.4 ab
5.0	6.7 a	11.3 a	12.3 a

Means with similar letters are not significantly different by Duncan's Multiple Range Test for P = 0.05.

TABLE II - *Mean colony radial growth (mm) of* Paecilomyces lilacinus *on water agar supplemented with antibiotics and sodium hypochlorite.*

NaOCl conc. (%)	Colony radial growth		
	Chloramphenicol (0.1%)	Streptomycin sulphate (0.05%)	Control
0.0 (control)	30.3 d	47.1 d	48.1 e
0.5	30.1 d	47.3 d	46.8 e
1.5	24.3 c	30.1 c	38.4 d
3.0	20.1 ab	22.1 b	26.3 bc
5.0	19.8 ab	17.3 a	21.1 a

Means with similar letters are not significantly different by Duncan's Multiple Range Test for P = 0.05.

TABLE III - *Mean mycelial dry weights (g) of* P. lilacinus *grown for twenty days in potato dextrose broth amended with sodium hypochlorite and different antibiotics.*

NaOCl conc. (%)	Mycelial dry weight		
	Chloramphenicol (0.1%)	Streptomycin sulphate (0.05%)	Control
0.0 (control	3.4 c	3.3 d	3.2 de
0.5	3.1 c	3.2 cd	2.8 d
1.5	1.8 b	2.1 b	1.6 bc
3.0	1.5 b	1.8 b	1.4 b
5.0	1.2 a	1.1 a	0.3 a

Means with similar letters are not significantly different by Duncan's Multiple Range Test for P = 0.05.

matode and/or fungal isolation for use in biological control studies. The 0.5% NaOCl concentration would be preferred under these conditions because it had no inhibitory effect on egg parasitism, or on the growth of *P. lilacinus*.

The effects of streptomycin sulphate were not significantly different from controls, indicating that it is a better antibotic for screening tests than chloramphenicol. Since the inhibitory effects of chloramphenical against bacteria and fungi is well documented (Orion and Kritzman, 1991), it still remains appropriate for use as an antimicrobial agent in most *in vitro* biocontrol tests (Pope and Hill, 1991), but may not be appropriate in studies involving fungal-nematode interactions. As there is a need for the production of large amounts of fungal mycelia for use in field biocontrol experiments, it is important to have prior knowledge of the effects of various chemicals and antibiotics on the efficacy of fungal antagonists against nematodes. Until this information is available, production of fungal antagonists in liquid media and evaluation of natural egg parasitism and screening for biologically active fungal isolates will continue to rely on intuition rather than fact. However, it is worth noting that results from this study may not necessarily apply to all nematophagous fungi. Efforts to evaluate the effects of NaOCl and antibiotics on a wide range of biocontrol organisms need to be intensified to ease selection of bioassay and antimicrobial chemicals.

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Literature cited

BARKER K. R., 1985. Nematode extraction and bioassay, pp. 19-38. In: *An advanced Treatise on Meloidogyne*, Vol. II Methodology, K. R. BARKER C. C., CARTER and J. N. SASSER Eds). North Carolina State University graphics.

CHITWOOD B. G., 1938. Further studies on nemic skeletoids and their significance in the chemical control of nemic pests. *Proceedings of the Helminthological Society of Washington, 5*: 68-70.

CLOVIS C. J. and NOLAN R. A., 1983. Fungi associated with cysts, eggs and juveniles of the golden nematode (*Globodera rostochiensis*) in Newfoundland. *Nematologica, 29*: 346-356.

ESSER R. P., 1972. Effect of sodium hypochlorite concentration on selected genera of nematodes. *Proceedings of the Helminthological Society of Washington, 39*: 108-114.

FREIRE C. C. and BRIDGE J., 1985. Parasitism of eggs, females and juveniles of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. *Fitopatologia Brasileira, 10*: 577-596.

FREUND R. J. and LITTELL R. C., 1981. SAS for linear Models: *A guide to the ANOVA and GLM procedures*. SAS Institute Inc., Cary, 231 pp.

KANWAR R. S., JAIN R. K., PARUTH I. J. and GUPTA D. C., 1991. Effect of sodium hypochlorite on egg liberation from egg masses, hatching and infectivity of *Meloidogyne javanica* juveniles. *Nematologica, 37*: 237-240.

KERRY B. R., 1987. Biological control, pp. 233-263. *In: Principles and Practice of Nematode Control in Crops* (R. H. Brown and B. R. Kerry eds). Academic Press, Sydney.

KERRY B. R., 1990. Selection of exploitable biological control agents for plant parasitic nematodes. *Aspects of Applied Biology, 24*: 1-9.

KERRY B. R. and CRUMP D. H., 1977. Observations on fungal parasites of females and eggs of the cereal cyst nematode, *Heterodera avenae*. *Nematologica, 23*: 93-201.

MORGAN-JONES G. and RODRIGUEZ-KABANA R., 1985. Phytonematode pathology: Fungal mode of action. A perspective. *Nematropica, 15*: 107-114.

ODUOR-OWINO P., 1993. Effects of aldicarb, *Datura stramonium, Datura metel* and *Tagetes minuta* on the pathogenicity of root-knot nematodes in Kenya. *Crop Protection, 12*: 315-317.

ODUOR-OWINO P. and WAUDO S. W., 1994. Comparative efficacy of nematicides and nematicidal plants on root-knot nematodes. *Tropical Agriculture, 71*: 272-274.

ODUOR-OWINO P., WAUDO S. W. and MAKHATSA W. L., 1993. Effect of organic amendments on fungal parasitism of *Meloidogyne incognita* eggs and growth of tomato (*Lycopersicon esculentum* Mill) c.v. Money maker. *International Journal of Pest Management, 39*: 459-461.

ORION D. and KRITZMAN G., 1991. Antimicrobial activity of *Meloidogyne javanica* gelatinous matrix. *Revue de Nematologie, 14*: 481-483.

POPE D. D. and HILL N. S., 1991. Effects of various culture media, antibiotics and carbon sources on growth parameters of *Acremonium coenophialum*, the fugal endoparasite of tall fescue. *Mycologia, 83*: 110-115.

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