The effect of bromoxynil and profenfos on the populations of osmophilic fungi in soil

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Omar, S. A. (1994). The effect of bromoxynil and profenfos on the populations of osmophilic fungi in soil. – Sydowia 46(1): 185–190.

The herbicide Brominal (bromoxynil: 3,5-dibromo-4-hydroxybenzonitrile) caused a significant decrease in populations of osmophilic fungi when added in concentrations of 0.6 and 3 ppm to soil. The incorporation of the herbicide into the agar medium significantly decreased the frequencies of fungi recovered on plates. The total counts of osmophilic fungi and total Aspergilli in soil treated with the insecticide Selecron [profenfos: O-(4-bromo-2-chlorophenyl) O-ethyl S-n-propyl phosphorothioate] were significantly decreased after most incubation periods at concentrations of 0.9 and 4.5 ppm. In agar medium mixed with Selecron, the inhibitory effect of the insecticide on soil fungi was very similar to that of Brominal.

Osmophilic fungi represent a large physiological group of soil fungi not yet extensively studied in relation to pesticide applications. The present investigation aimed to assess the effect of two pesticides either mixed with soil or incorporated into the agar medium on osmophilic fungi.

Material and methods

The soil used in this investigation was a clay soil collected from a layer at a depth of 0-20 cm in the Botanical Garden of the Assiut University. The pH was 7.2, the total soluble salts 0.32 % and the organic matter 1.8 %.

The herbicide Brominal (bromoxynil: 3,5-dibromo-4-hydroxybenzonitrile) and the insecticide Selecron [profenfos: O-(4-bromo-2-chlorophenyl) O-ethyl S-n-propyl phosphorothioate] were used in this investigation.

The soil was air dried, passed through a 2-mm sieve and placed in polyethylene bags (500 g in each, on oven dry basis). Pesticides were diluted with distilled water to give concentrations equal to amounts recommended for field application and five-fold field doses, and applied to the soil as a part of the moisture required to adjust the soil to 40% of its water holding capcity. Control soil were treated with

water without pesticides. Application rates were calculated as active ingredients but commercial formulations were used. Brominal was used at 0.6 and 3 ppm and Selecron at 0.9 and 4.5 ppm. Conversion of field application rates to mg pesticide per kg dry soil was calculated assuming an even distribution of the pesticide in the plough layer (2 million kg soil/ha). Control and treatments were set up in triplicates and incubated at 28° C. Water loss by evaporation was compensated weekly to avoid dryness.

After 1, 2, 6 and 8 weeks incubation, soil subsamples were aseptically taken for counting of osmophilic soil fungi using the dilution plate method (Johnson & al., 1959). Czapek's agar supplemented with 40% sucrose, to which rose bengal (65 ppm) was added as a bacteriostatic agent, was used as isolation medium (Smith & Dawson, 1944). Plates were incubated at 28° C for 7 days and the developing fungi were identified and counted.

Pesticides were added to the molten warm Czapek's-sucrose agar at the two rates used for soil treatment just before pouring one ml of the appropriate soil dilution in each plate. Plates were incubated and the fungi recovered were identified and counted as mentioned above.

Results and Discussion

When added to soil Brominal decreased significantly the counts of total osmophilic fungi (Tab. 1). In addition, the frequency of occurrence of other fungi (*Aspergillus* spp., *A. niger* van Tieghem, *A. terreus* Thom, *Eurotium* spp.) was also decreased and the inhibition in most cases was effective with both doses. The effect of the herbicide on *A. fumigatus* Fres., *Emericella nidulans* (Eidam) Vuill., *Eurotium repens* De Bary and *Trichoderma viride* Pers. ex Gray was not consistent. The other fungi listed in Tab. 1 were only slightly influenced by the Brominal application. Fungi infrequently isolated during the study are not presented in Tab. 1 and include *Aspergillus alutaceus* Berk. & Curt., *A. candidus* Link, *A. flavus* Link, *A. japonicus* Saito, *A. melleus* Yukawa, *Botroyotrichum atrogriseum* Beyma, *Cladosporium cladosporioides* (Fres.) de Vries and *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes.

When added to soil, Selecron significantly decreased the total count of osmophilic fungi (Tab. 2). The frequency of occurrence of *A. flavus* was significantly increased over the incubation periods. Several fungi only slightly influenced by the insecticide application included *A. oryzae* (Ahlb.) Cohn, *E. amstelodami* Mangin, *Penicillium aurantiogriseum* Dierck. On the other hand, some fungi recovered in plates showed an inconstant behaviour (Tab. 2). Rare fungal species were *A. sydowi* (Bain. & Sart.) Thom & Church, *A. ustus* (Bain) Thom &

Tab. 1. – Effect of soil treatment with Brominal on soil osmophilic fungi recovered on 40% Czapek's-sucrose agar plates at different periods after treatment (count per mg dry soil).

	Weeks after treatment											
	1			2			6			8		
Species	0[a]	1	5	0	1	5	0	1	5	0	1	5
Aspergillus spp.	34.6	34.4	19.2*	38.2	24.8*	25.4*	21.6	8.1*	3.3*	23.6	14.5*	12.2*
A. fumigatus Fres.	0.3	1.6*	2.3*	2.0	4.0*	0.6*	3.3	0.6*	0.0*	3.3	2.6	0.0*
A. niger V. Tieghem	16.5	23.0*	10.0*	7.3	1.6*	6.6	5.0	2.6*	1.0*	9.0	4.3*	5.0*
A. oryzae (Ahlb.) Cohn	0.3	0.0	0.0	18.3	10.0*	8.6*	0.3	2.6*	2.3*	0.3	0.0	0.0
A. terreus Thom	15.5	7.6*	5.6*	10.3	3.6*	6.0*	12.0	2.3*	0.0*	11.0	6.6*	6.6*
A. ustus (Bain.) Thom & Church	0.5	0.6	0.0	0.3	0.3	2.6*	1.0	0.0	0.0	0.0	1.0	0.6
A. versicolor (Vuill.) Tirab.	1.5	1.6	1.3	0.0	5.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Emericellla nidulans (Eidam) Vuill.	1.0	3.3*	1.0	3.3	4.3	8.0*	5.6	1.3*	0.0*	3.3	3.6	5.0
Eurotium spp.	1.3	4.6*	1.3	0.0	0.0	1.3	1.3	1.3	0.0*	2.6	3.2	1.0*
E. amstelodami Mangin	1.3	4.6*	1.3	0.0	0.0	0.0	0.0	1.0	0.0	1.6	0.6	1.0
E. repens De Bary	0.0	0.0	0.0	0.0	0.0	1.3	1.3	0.3*	0.0*	1.0	2.6*	0.0
Penicillium spp.	3.2	1.3*	1.9*	1.9	0.6*	2.6	1.9	0.9	0.0*	0.6	1.0	1.0
P. aurantiogriseum Dierckx	0.8	0.3*	0.0*	0.0	0.0	1.0	0.0	0.0	0.0	0.6	1.0	1.0
P. chrysogenum Thom	1.6	1.0	1.3	1.3	0.6	1.0	0.6	0.6	0.0*	0.0	0.0	0.0
P. funiculosum Thom	0.8	0.0*	0.6	0.6	0.0*	0.6	1.3	0.3*	0.0*	0.0	0.0	0.0
Trichoderma viride Pers. ex Gray	3.3	0.3*	1.0*	0.3	0.0	0.6	0.3	3.0	7.3*	1.6	0.3*	0.0*
Total count	43.4	43.9	24.4*	43.7	29.7*	37.9*	30.4	14.6*	10.6*	31.7	22.6*	19.2*

* Significant difference from control P = 0.05.

[a] Dose 0 = control; 1 = field dose (0.6 ppm); 5 = 5-fold field dose (3 ppm).

Tab. 2. – Effect of soil treatment with Selecron on soil osmophilic fungi recovered on 40% Czapek's-sucrose agar plates at different periods after treatment (count per mg dry soil).

	Weeks after treatment											
	1			2			6			8		
Species	0[a]	1	5	0	1	5	0	1	5	0	1	5
Aspergillus spp.	33.6	41.9	28.3	38.5	29.5*	23.2*	21.3	4.0*	8.9*	23.9	11.5*	10.8*
A. flavus	1.0	0.3	1.0	0.3	0.6*	0.0	1.0	1.0	8.3*	0.3	1.3*	0.0
A. fumigatus	0.3	2.0*	3.0*	2.0	2.3	2.3	3.3	0.0*	0.0*	3.3	1.3*	2.3
A. niger	16.5	28.3*	11.3*	7.3	6.6	12.3*	5.0	1.0*	0.6*	9.0	5.6*	5.6*
A. oryzae	0.3	0.3	0.0	18.3	17.3	7.6*	0.0	0.0	0.0	0.3	0.0	0.3
A. terreus	15.5	11.0*	13.0	10.6	3.3*	1.0*	12.0	2.0*	0.0*	11.0	3.3*	2.6*
Emericellla nidulans	1.0	2.0*	0.6	3.3	3.3	3.7	5.6	1.0*	0.0*	3.3	1.0*	1.0*
Eurotium amstelodami	1.3	0.3	1.0	0.0	0.0	0.0	0.0	1.6	1.3	1.6	0.3*	0.6*
Penicillium spp.	2.9	5.6*	1.9	1.9	0.6*	0.9	1.9	1.0*	0.3*	1.2	0.3*	0.0*
P. aurantiogriseum	0.8	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0*	0.0*
P. chrysogenum	1.3	2.0	0.3*	1.3	0.3*	0.3*	0.6	0.0*	0.0*	0.0	0.0	0.0
P. funiculosum	0.8	2.6*	1.0	0.6	0.3	0.6	1.3	1.0	0.3*	0.6	0.3	0.0*
Trichoderma viride	3.3	0.3*	0.3*	0.3	0.0	0.0	0.3	5.0*	5.6*	1.6	0.0	1.0
Total count	42.1	50.1	32.1*	44.0	33.4*	27.8*	29.1	12.7*	16.1*	31.6	13.1*	13.4*

* Significant difference from control P = 0.05.

[a] Dose 0 = control; 1 = field dose (0.9 ppm); 5 = 5-fold field dose (4.5 ppm).

Tab. 3. – Effect of incorporation of pesticides into the agar medium on osmophilic fungi calculated as count per mg dry soil.

		Brominal		Selecron	
Species	0[a]	1	5	1	5
Aspergillus spp.	114.0	63.5*	32.1*	51.5*	65.5*
A. aculeatus Lizuka	0.3	1.6*	0.6*	0.3	0.0
A. candidus Link	0.6	0.0*	0.0*	0.0*	0.0*
A. flavus Link	4.6	9.6*	4.6	1.3*	2.6*
A. fumigatus Fres.	27.6	14.0*	6.0*	5.6*	9.6*
A. niger V. Tiegh	32.0	23.3*	12.6*	14.3*	23.0*
A. terreus Thom	47.6	15.0*	8.3*	30.0*	30.3*
A. ustus (Bain.) Thom & Church	0.6	0.0*	0.0*	0.0*	0.0*
A. versicolor (Vuill.) Tirab.	1.0	0.0*	0.0*	0.0*	0.0*
Cunninghamella echinulata (Thaxt.) Thax. ex Blak.	0.6	0.3	0.0*	0.0*	0.0*
Emericellla nidulans (Eidam) Vuill.	15.3	0.0*	0.6*	0.0*	0.0*
Eurotium amstelodami Mangin	5.3	2.3*	3.6*	5.3	4.6
Fennellia flavipes Wiley & Simmons	1.3	0.0*	0.0*	0.0*	0.0*
Humicola grisea Traaen	0.6	0.0*	0.0*	0.0*	0.0*
Neosartorya fischeri (Wehmer) Malloch & Cain	2.0	0.0*	1.0*	14.0*	10.0*
Total count	139.4	66.1*	37.3*	70.8*	80.4*

* Significant difference from control P = 0.05.
[a] Dose 0 = control; 1 = field dose; 5 = 5-fold field dose.

Church, *A. versicolor* (Vuill.) Tirab, *Myrothecium verrucaria* (Alb. & Schweiz.) Ditm. & Fres., *Nectria haematococca* Berk. & Brown, *Rhizopus oryzae* Went. & Geerling and were omitted from Tab. 2.

The two pesticides mixed to the agar media significantly suppressed the total counts of osmophilic fungi as well as those of *Aspergillus* spp., *A. fumigatus*, *A. terreus* and *E. nidulans*. Several fungal species were also completely eliminated by both doses of the two pesticides (Tab. 3). *Botryotrichum atrogriseum*, *Cladosporium cladosporioides* and *Nectria haematococca* were rare and thus not included in Tab. 3.

The effect of pesticides on osmophilic fungi and on their biological activity in soil has not yet been studied in detail. Only few investigations have been published that deal with the effect of pesticides on soil fungi (Ou & al., 1978; Sinha & al., 1979; Banerjee & Banerjee, 1987).

When incorporated into the agar medium, both Brominal and Selecron significantly decreased the total counts of soil fungi and of most genera and species. The effect of the pesticides was more pronounced than in the soil. This difference may be attributed to the adsorption of pesticides to soil particles and the uneven distribution

of pesticides within soil (Jeater & McIlvenny, 1965; Anantha-cumaraswamy & al., 1987). It seems likely that osmophilic fungi are affected either directly or indirectly by pesticide application. This study emphasize the necessity for preliminary tests of pesticides for persistence and side-effects on soil microorganisms and their biological activities, especially on those connected with soil fertility.

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(Manuscript accepted 1st November 1993)