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CHANGES IN SOIL MICROBIAL POPULATIONS AFTER FUMIGATION AND ALTERNATIVE METHODS TO CONTROL SOIL-BORNE DISEASES

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Abstract

The study was carried out in 2010 – 2012 on three farms. Soils were disinfested by chemical fumigation, biofumigation and active steam. Chemical fumigation was carried out in autumn of 2010 and 2011 with two different rates of either dazomet, metam sodium or chloropicrin + 1,3D. Steam disinfection with addition of CaO, maintaining soil temperature of about 70 ºC for 2 h, or biofumigation with *Brassica carinata* seeds meal were performed in spring of 2011 and 2012 at two or one locations, respectively. Soil bacteria and fungi populations were assessed 4 weeks after the treatments. All chemical treatments and steam disinfection caused a significant decrease of the total number of fungi in comparison to control, while biofumigation induced an increase of total soil fungi. The total number of bacteria generally increased by the chemical fumigation with all tested products in all soils. The bacteria population was not changed or increased, depending on the season, by active steam treatment, whereas it was 3-fold decreased by biofumigation, in comparison to not disinfected soil. In conclusion, the applied disinfection techniques modified the populations of soil microorganisms irrespective of the characteristics of the soil.

Key words: active steam, biofumigation, chloropicrin, dazomet, metam sodium, 1,3 dichloropropene

Soil fumigation is a common and important phytosanitary practice in horticultural plant cultivation. However, soil disinfection is still not widely practiced in Poland. Several soil plant pathogens (*Fusarium* spp., *Phytophthora cactorum*, *Phytophthora fragariae* and species of the genera *Pythium*, *Leptosphaeria*, *Cylindricarpon*, *Rhizoctonia*, and *Phoma*) and nematodes (*Pratylenchus* spp., *Ditylenchus dipsaci*, *Longidorus* spp. and *Xiphinema* spp.) can cause significant losses in nurseries and other plantations. It can also restrict the export potential of plants in case of presence of regulated quarantine pathogen species (Mumford, 2002).

The phase out of methyl bromide (MB) under the Montreal Protocol has stimulated research on chemical fumigants as well as alternative methods for the control of soil-borne pathogens (Colla et al., 2012). Biofumigation with *Brassica* species and steaming can substitute chemical fumigation (Nederpel, 1979), but their practical application is still limited. We have thus evaluated the effect of both chemical fumigants and alternative methods on the fungal and bacterial populations in soils characterized by different chemical-physical characteristics.

MATERIAL AND METHODS

Soil disinfection

The disinfection of soil was performed in three locations characterized by soils with very different chemical and physical characteristics: Przytyk, having a light podsol, Lisowola, having a clay sandy soil, and Kozienice, having a heavy alluvial loamy clay soil. The soil fumigation was carried out in the months of October 2010 and November 2011 using the following products and doses:

a – Basamid 97 GR (97% dazomet) at a dose of 30 or 40 g/m^2;

b – Nemasol 510 SL (510 g/l metam sodium) at a dose of 60 or 90 ml/m^2;

c – Chloropicrin (99%) at the dose of 30 ml/m^2;

d – Telopic C-35 (a mixture of 35% chloropicrin and 65% 1,3 Dichloropropene) at the dose of 35 or 50 ml/m^2.

The disinfection with active steam or biofumigation was applied in April 2011 and 2012 only in Przytyk. Basamid 97 GR was applied to the soil with a machine formed by a dosing system mounted over a rotating mechanical hoe which was ploughing the soil and mixing the product up to 35 cm of depth. The liquid products (Nemasol 510 SL, Chloropicrin and Telopic C-35) were applied to 30 – 35 cm depth by means of injection. In both cases, the soil was then compacted with a roller and covered with virtually impermeable polyethylene film (VIF) that was removed 10 days after the application of the fumigants. The treatment with active steam was performed with the self-propelled tractor Eco Star SC 600 (Bioflash System™; Tesi et al., 2007). The machine distributed CaO on the soil (400 g/m^2) and mixed it while injecting water steam

Table 1. The size of the fungi population present in three locations after soil disinfection with chemical fumigation

Treatment		Average number of CFU/g dry weight of soil x 10^4					
		Przytyk		Kozienice		Lisowola	
		2010	*2011*	*2010*	*2011*	*2010*	*2011*
Control		35.5	19.2	14.2	18.9	13.8	11.8
Basamid 97 GR	30 g	-	-	-	-	0.9	2.1
Basamid 97 GR	40 g	1.7	1.3	5.1	6.9	1.2	0.5
Nemasol 510 SL	60 ml	-	0.4	0.9	14.0	0.9	2.3
Nemasol 510 SL	90 ml	-	-	1.8	14.1	0.1	0.5
Telopic C-35	35 ml	-	-	2.3	9.5	0.05	3.1
Telopic C-35	50 ml	-	-	0.6	8.3	0.004	4.2
Chloropicrin	30 ml	-	-	-	-	0.6	2.1

(-) Not tested.

Table 2. The size of the bacteria population present in three locations after soil disinfection with chemical fumigation

Treatment		Average number of CFU/g dry weight of soil x 10^7					
		Przytyk		Kozienice		Lisowola	
		2010	*2011*	*2010*	*2011*	*2010*	*2011*
Control		6.9	3.1	5.3	5.9	3.4	0.4
Basamid 97 GR	30 g	-	-	-	-	7.3	2.4
Basamid 97 GR	40 g	7.1	9.4	3.5	14.5	5.5	2.2
Nemasol 510 SL	60 ml	-	10.1	7.2	5.1	4.7	2.7
Nemasol 510 SL	90 ml	-	-	7.0	6.6	5.2	2.7
Telopic C-35	35 ml	-	-	7.6	10.3	6.7	2.1
Telopic C-35	50 ml	-	-	8.1	14.8	3.4	2.1
Chloropicrin	30 ml	-	-	-	-	5.6	2.3

(-) Not tested.

Table 3. The size of fungi and bacteria populations present in soil after disinfection with active steam or biofumigation

Treatment	Fungi (CFU x 10^4)		Bacteria (CFU x 10^7)	
	2011	*2012*	*2011*	*2012*
	Przytyk			
Control	55.8	22.2	6.7	4.7
Active steam	12.1	10.9	5.7	10.7
	Kozienice			
Control	41.2	-	6.3	-
Biofumigation	74.1	-	2.3	-

(-) Not tested.

at 90 °C at a depth of 15 – 20 cm for a time of about 15 sec/m^2 to reach a soil temperature of 60 – 70 °C, which was maintained for about 2 hours; the soil was rolled and covered with VIF film for one day (Tesi et al., 2007). Biofumigation was performed applying a meal of *Brassica carinata* seeds that was incorporated into the soil at 15 – 20 cm depth, covering it with VIF film for seven days. The control plots were not disinfected.

Determination of fungi and bacteria populations size in soil

Soil samples from treated and control plots were collected from a depth of 20 cm with the use of a soil probe stick (diameter 20 mm), about 4 weeks after each treatment. The number of colony forming units (CFU) of fungi and bacteria in the soil samples was determined using plating methods on agar media. A subsample of 10 g was weighed from each sample and, after suspension into 90 ml of sterile distilled water, was shaken for 30 minutes. Three dilutions of the soil suspension (10^{-2}, 10^{-3}, 10^{-4}) were uniformly poured on Martin's (1950) medium (for fungal determination) and on King B and Tryptic Soy Agar media (for bacteria determination). The number of CFU for both microorganisms' groups was counted after 3-7 days of incubation at 24 °C and recalculated per gram of fresh soil weight.

RESULTS AND DISCUSSION

All chemical treatments provoked a significant decrease of the number of fungi (Table 1). Depending on the year, location and the active substance applied the fungi populations were decreased by 2.3 to 3500-fold in comparison to control. These findings are consistent with previous reports where metam sodium, dazomet and chloropicrin showed good efficacy in control of different soil fungal pathogens (Duniway, 2005; Ślusarski and Pietr, 2009; Tsror et al., 2006) and also showing that their efficacy could depend on various factors such as dose, soil incorporation depth, type of soil (Oloo et al., 2011).

The total number of bacteria was generally increased by the chemical fumigation with all tested products on all farms. The increase ranged from 0.5 to about 6.8-fold as compared to control. Only in one location, Basamid 97 GR at the higher dose and Nemasol 510 SL at the lower dose caused a reduction of about 30% and 20% of the bacteria populations, respectively (Table 2).

Active steam treatment caused a decrease of fungi, 5-fold the first season, 2-fold the second (Table 3). The size of the bacteria population was not changed by the active steam treatment in the first season, while it was doubled in the second (Table 3). The active steam machine was designed to achieve an effective reduction of weeds seed bank (Barberi et al., 2009). However, the effect of the treatment on soil microorganisms and the control of several soil-borne diseases has been demonstrated (Triolo et al., 2004) and is confirmed also by our data.

Opposite to chemical fumigation, biofumigation induced an almost 2-fold increase of total fungi (Table 3) and an almost 3-fold decrease of total bacteria population (Table 3). A soil similar from a physical point of view to the one of this study showed unique bacterial and fungal populations' profiles after biofumigation with a Brassica seeds meal in comparison to chemically fumigated and untreated soil (Mazzola and Strauss, 2013). However, the overall diversity of the microbiome was reduced in the biofumigated soil, suggesting that enhanced "microbial biodiversity" was not instrumental in achieving system resilience and/or pathogen suppression.

In conclusion, the applied soil disinfection techniques modified the populations of soil microorganisms irrespective of the physical characteristics of the soil. Steaming acted more similarly to chemical fumigation. Even though the active substances derived from *Brassica carinata* are similar to those of Basamid 97 GR and Nemasol 520 SL (isothiocyanates), their effect on the soil microorganisms' populations were opposite.

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