THE EFFECT OF SOIL DISINFECTION WITH CHEMICAL AND ALTERNATIVE METHODS ON FUNGAL AND BACTERIAL POPULATIONS

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Aim

- Determination of total number of bacteria and fungi population 4 weeks after soil disinfection with chemical and alternative methods

- Determination of fungal pathogens population number 4 weeks after soil disinfection

- Identification of fungal pathogens and bacteria present in soil

Methods

Serial dillution and plating on Tryptic Soil Agar, King B, Martin's medium

- 10g soil sample was taken from 1kg sample collected with soil stick and suspended in 90 ml of sterile water
- After 30 minutes of shaking on reciprocal shaker (180rpm) the suspension was serially dilluted and plated on agar media
- After 3-5-7 days of incubation the number of bacterial and fungal colonies were counted




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Identification:

Bacteria and fungi – conventional methods

Verticillium dahliae – conventional and molecular

Chemical fumigants:

- Basamid 97GR: *dazomet* (*97%*)
- Nemasol 510 SL: *metam sodium* (*51%*)
- Telopic C-35EC: *chloropicrin* (*35%*) *+1.3 D* (*61%*)
- Chloropicrin

Alternative methods:

- Active steam
- Biofumigants: seed meal of *Brassica carinata* or *Sinapis alba*

Setting up of experiments in 4 farms

Experiments 2010/2011

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Farm - Treatments - dose/m^2

A	B	C	D
1/ Dazomet 30 g 2/ Active steam 3/Biofumigation (*B. carinata*)	1/ Dazomet 30 g 2/ Dazomet 40 g	1/ Dazomet 40 g 2/ Metam sodium 90 ml 3/ Metam sodium 60 ml 4/ Chloropicrin+1.3 D 35 ml 5/ Chloropicrin+1.3 D 50 ml	1) Dazomet 30 g 2) Dazomet 40 g 2) Metam sodium 90 ml 3) Metam sodium 60 ml 4) Chloropicrin + 1.3 D 35 ml 5) Chloropicrin+1.3 D 50 ml 6) chloropicrin 40 g 7/ Active steam

Chemical fumigation: autumn 2010
Alternative methods: spring 2011

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Chemical fumigation

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Total number of fungi present in soil after fumigation

Treatment	Farm / cfu x 10^4			
	A	B	C	D
Check (untreated soil)	39.1	35.5	14.2	13.8
dazomet 30 g/m^2	28.2	-	-	0.9
dazomet 40 g/m^2	9.3	1.7	5.1	1.2
metam sodium 60 ml/m^2	-	-	0.9	0.9
metam sodium 90 ml/m^2	-	-	1.8	0.1
chloropicrin+ 1,3 D 35 ml/m^2	-	-	2.3	0.05
chloropicrin + 1,3 D 50	-	-	0.6	0.004
ml/m^2	-	-	-	0.6
chloropicrin 40 g/m^2				

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Effect of fumigation on soil pathogens

Farm A

(cfu x 10^4)

Fungi	Check	Dazomet 30 g/m^2	Dazomet 40 g/m^2
Total	39.1	28.2	9.3
F. culmorum	0.01	0	0
F. oxysporum	0.8	0.2	0.08
F. solani	0.4	0.04	*0.8*
Fusarium sp.	0.1	0	0
Rhizoctonia spp.	0	0	0.08
Verticillium dahliae	0.0004	0	0

Farm B

Fungi	Check	Dazomet 40 g/m^2
Total	35.5	1.7
Fusarium oxysporum	2.7	0
Fusarium solani	0.2	0
Rhizoctonia spp.	0.2	0.004
Verticillium dahliae	0.0005	0

Farm C

(cfu x 10^4)

Fungi	Check	DA 40 g	MS 60 ml	MS 90 ml	CP+1.3 D 35 ml	CP+1,3 D 50 ml
Total	14.2	5.1	0.9	1.8	3.1	0.6
F. culmorum	1.2	0	0	0	0	0
F. oxysporum	0.4	0.08	0	0	0	0.4
F. solani	0.8	0	0	0.2	0	0
Fusarium spp.	0.1	0	0	0	0.8	0
Rhizoctonia spp.	0.8	0.08	0.06	0.4	0.5	0.05

Farm D

(cfu x 10^4)

Fungi	Check	DA 30 g/m²	DA 40 g/m²	MS 60 ml/m²	MS 90 ml/m²	CP+1,3 D 35 ml/m²	CP+1,3 D 50 ml/m²	CP 40 g/m²
Total	13.8	0.9	1.2	0.9	0.12	0.05	0.004	0.7
F. culmorum	0.4	0.02	0.04	0	0	0	0	0
F. oxysporum	0.7	0	0.04	0.01	0	0	0	0.008
F. solani	0.4	0	0	0	0	0	0	0
B.cinerea	1.5	0	0	0	0	0	0	0
P.cactorum	1.0%	0	0	0	0	0	0	0
V.dahliae	0.0002	0	0	0	0	0	0	0

Number of bacteria population in soil after fumigation

(cfu x 10^7/g)

Location/ Treatment	Total	Fluorescent *Pseudomonas* spp.	*Bacillus* spp.
FARM A. Check	6.9	0.1	0.2
Dazomet – 30 g/m²	7.1	0.7	0.2
FARM B. Check	8.1	0.8	0.3
Dazomet 40 g/m²	15.1	1.1	0.2
Dazomet 30 g/m²	13.3	8.0	0.3
FARM C. Check	5.3	0.2	1.1
Dazomet 40 g/m²	**3.5**	0.2	0.6
Metam sodium 90 ml/ m²	7.0	2.4	0.7
Metam sodium 60 ml/ m²	7.2	2.0	1.2
Chloropicrin + 1,3 D 35 l/m²	7.6	**0.1**	0.7
Chloropicrin + 1,3 D 50 l/m²	8.1	1.2	0.8
Farm D. Check	3.4	0.05	0.3
Dazomet 30 g/ m²	7.3	0.5	**0.2**
Dazomet 40 g/m²	5.5	1.2	**0.1**
Metam sodium 90 ml/ m²	5.2	0.2	**0.1**
Metam sodium 60 ml/ m²	4.7	0.1	**0.1**
Chloropicrin + 1,3 D 35 l/m²	6.7	1.7	**0.1**
Chloropicrin + 1,3 D 50 l/m²	**3.4**	1.0	**0.1**
Chloropicrin 40 g/ m²	5.6	1.5	**0.2**

Summary

- The total number of bacteria increased after application of all fumigants on farms A, B and D. Only in farm C the number of bacteria after fumigation with dazomet decreased of 30%.

- On all treated plots the number of fluorescent Pseudomonads increased from 2- to 100-fold in comparison with non treated plots **great increase of soil biological potential**, presumably including its suppressiveness

- The number of *Bacillus* spp. (also playing an important role in soil suppressiveness) remained unchanged on plots treated with dazomet on farms A and B, but on farm D decreased twice after using this product at 40 g/m^2 dose.

- Chloropicrin and metam sodium reduced number of *Bacillus* spp. up to about 80% in relation to control
  The exception was the farm D, where chloropicrin didn't decrease population of this group of bacteria.

Alternative methods

1/ Biofumigation

The seed meal of *Brassica carinata* (and *Sinapsis alba* in 2012*)* was hand spread at a rate of 150 g/m^2 on the soil surface and then incorporated to a depth of about 17 cm with a tractor-driven rotary tiller, 7 days prior to planting.

2/ Active steam

Steam + CaO (KOH)

$CaO + H_2O \leftrightarrow Ca(OH)_2$ ($\Delta H_r = -63.7$ kJ/mol CaO)

$KOH \leftrightarrow K^+ + OH^-$ ($\Delta H_r = -57.6$ kJ/mol KOH)

Steam + CaO

↓

70 - 90°C

soil

after 1 h **60 - 70°C**

after 2 h **40 - 60°C**

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Active steam

Growth of pepper plants in the soil treated with the active steam; on the right - check

Effect of disinfection with alternative methods on soil pathogens

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Farm A

Total number of fungi populations inhabiting soil after disinifestation

(cfu x 10^4/g soil)

Check	Active steam
55.8	12.1

Check	Biofumigation
41.2	74.1 (+ *Mucorales*)

Farm A:

Active steam (cfu x 10^4)

Fungi	Check	AS	%
Total	55.8	12.1	- 78.4
F. oxysporum	0.4	0.04	- 89.5
F. equiseti	1.4	0.0	- 100.0
Rhizoctonia spp.	0.1	0	- 100.0

Biofumigation (*B. carrinata*)

Fungi	Check	B	%
Total	41.2	74.1	**+ 79.8**
F.equiseti	0.2	0	- 100.0
Rhizoctonia spp.	0.2	0	- 100.0

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Farm A

Number of bacteria population in soil after active steam treatment and biofumigation

cfu x 10^7/ g soil

Treatment	Total	Fluorescent Pseudomonands	*Bacillus* sp.
Check	6.7 a	0.09 b	0.3 a
Active steam	5.7 a	0.02 a	0.4 b
Check	6.3 b	0.1 a	0.2 a
Biofumigation (B.c)	2.3 a	0.1 a	0.2 a

Summary

- Total number of bacteria did not change significantly after active steam treatment, but changed the population structure. Number of a Pseudomonads decreased nearly 5-fold but the number of *Bacillus* spp. increased by half.
- Active steam treatment caused nearly 5-fold reduction of soil fungi and completely eliminated the harmful for peppers pathogenic *Fusarium* and *Rhizoctonia*).
- Application of biofumigation increased the number of Penicillium, and eliminated other fungi, except those of Mucorales. Simultaneusly, this treatment decreased the total number of bacteria nearly 3-fold , while the number of Pseudomonads and *Bacillus* sp. did not change.

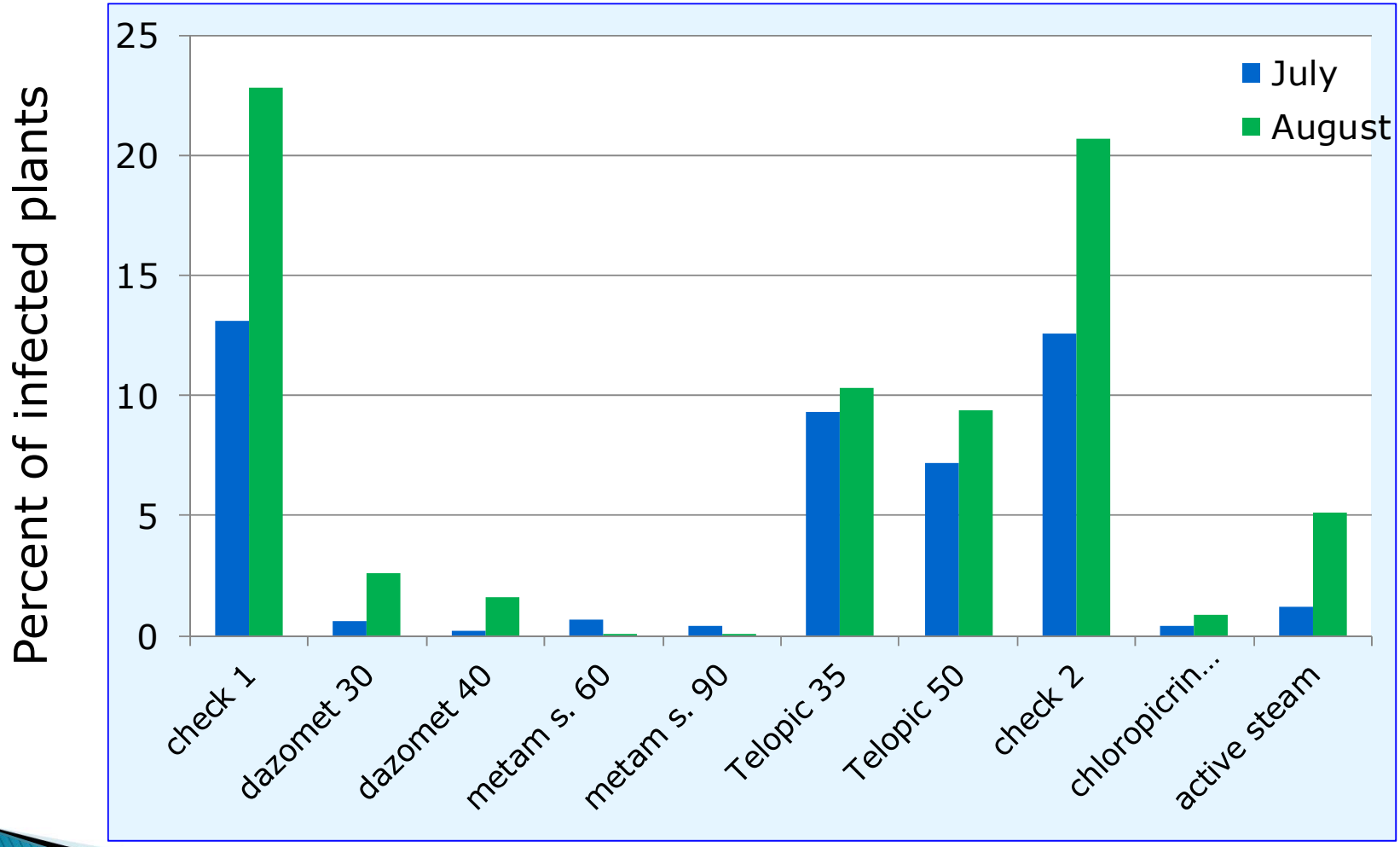
Plants infested by *Verticillium dahliae* – *8 months after fumigation*

very poor infection –
metam sodium, chloropicrin

single infected plants -
dazomet

check -
strong infection

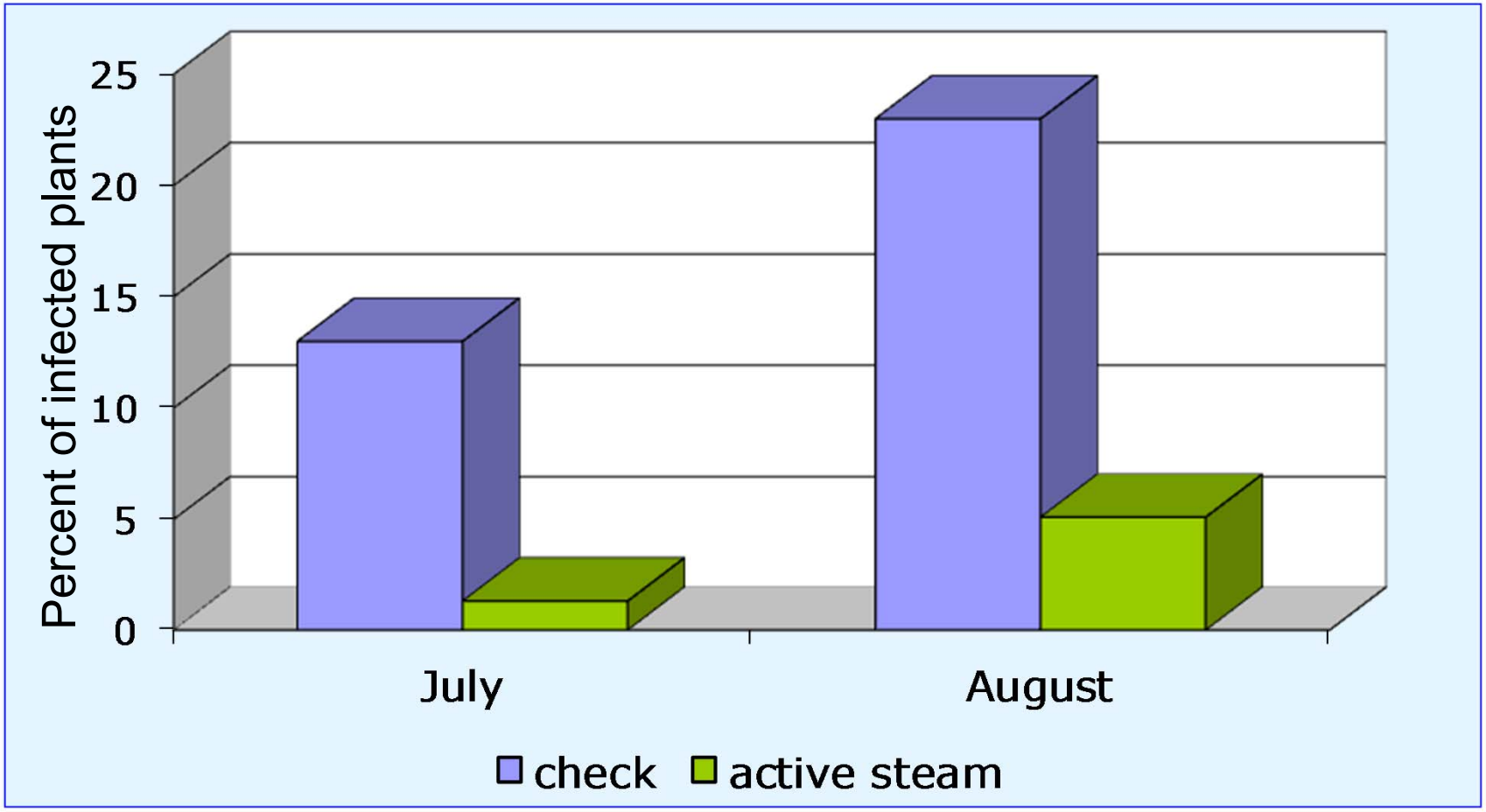
Effect of chemical fumigation on *Verticilium* wilt severity on strawberry plants



Evaluation 8 and 9 months after fumigation

Effect of active steam application on *Verticilium* wilt severity on strawberry plants

(3 and 4 months after treatment with active steam)



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FARM D

Verticilium dahliae presence in soil after chemical and alternative disinfection

Treatment	Number of propagules (cfu/g)
Check	**39**
Dazomet 30 g*	6.5
Dazomet 40 g*	3.5
Metam sodium 60 ml*	3.5
Metam sodium 90 ml*	1.5
Chloropicrin + 1,3 D 35 ml*	20
Chloropicrin + 1,3 D 50 ml*	19
Chloropicrin 40 g*	0
Active steam**	3

Evaluation after: *8 months, **3 months

Experiments 2011/2012

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Farm/treatments /dose/m^2

A	B	C	D
1/ Dazomet 40 g 2/Metam sodium 70 ml 3/ Active steam	1/ Dazomet 30 g 2/ Dazomet 40 g	1/ Dazomet 40 g 2/ Metam sodium 90 ml 3/ Metam sodium 60 ml 4/ Chloropicrin+1.3 D 35 ml 5/ Chloropicrin+1.3 D 50 ml 6/Biofumigation (*B. carinata*) 7/Biofumigation (*S. alba*)	1) Dazomet 30 g 2) Dazomet 40 g 2) Metam sodium 90 ml 3) Metam sodium 60 ml 4) Chloropicrin+1.3 D 35 ml 5) Chloropicrin+1.3 D 50 ml 6) chloropicrin 40 g 7) Active steam

Chemical fumigation: Farms A, B, D: autumn 2011
Farm C: spring 2012
Alternative methods: spring 2012

Total number of fungi present in soil after chemical fumigation

(cfu x 10^4)

Treatment	Farm/cfu x 10^4			
	A	B	C	D
Check (untreated soil)	19.2	18.3	18.9	11.8
dazomet 30 g/m^2	-	8.1	-	2.1
dazomet 40 g/m^2	1.3	2.5	6.9	0.5
metam sodium 60 ml/m^2	0.4	-	**14.0**	2.3
metam sodium 90 ml/m^2	-	-	**14.1**	0.5
chloropicrin+ 1,3 D 35 ml/m^2	-	-	**9.5**	3.1
chloropicrin + 1,3 D 50 ml/m^2	-	-	**8.3**	4.2
chloropicrin 40 g/m^2	-	-	-	2.1

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Effect of fumigation on soil pathogens

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Farm A

(cfu x 10^4)

Fungus	Check	Metam sodium 70 ml/m^2	Dazomet 40 g/m^2
Total	19.2	0.4	1.3
F. culmorum	0.04	0	0
F. oxysporum	0.3	0	0
F. solani	0.1	0	0.04
Botrytis cinerea	0.1	0	0
Verticillium dahliae	0	0	0

Farm B

Fungus	Check	Dazomet 30 g/m^2	Dazomet 40 g/m^2
Total	18.3	8.1	2.5
F. oxysporum	0.4	0	0
Verticillium dahliae	0.0002	0	0

Farm C

cfu x 10^4

Fungi	Check	DA 40 g/m²	MS 60 ml/m²	MS 90 ml/m²	CP+1,3 D 35 ml/m²	CP+1,3 D 50 ml/m²
Total	18.9	6.9	14.0	14.1	9.5	8.3
F. culmorum	0.2	0.8	0.04	0.04	0	0
F. oxysporum	0.4	0	0.8	0.4	0	0
V. dahliae	+	0	+	0	0	0

Farm D

Fungi	Check	DA 30 g/m²	DA 40 g/m²	MS 60 ml/m²	MS 90 ml/m²	CP+1,3 D 35 ml/m²	CP+1,3 D 50 ml/m²	CP 40 g/m²
Total	11.8	2.1	0.5	2.3	0.5	3.1	4.2	2.1
F. culmorum	0.06	0	0	0	0	0	0	0
F.oxysporum	0.04	0.004	0	0.1	0	0.01	0.02	0
V. dahliae	0.0002	0	0	0	0	0	0	0

Effect of disinfection with alternative methods on soil pathogens

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Total number of fungi populations inhabiting soil after disinifestation (cfu x 10^4/g soil)

Farm A

Check	Active steam
22.2	10.9

Farm D

Check	Active steam
5.9	8.9

Farm A: active steam

(cfu x 10^4/g soil)

Fungi	Check	AS	%
Total	22.2	10.9	- 50.9
F. oxysporum	0	0.4	**+ 100.0**
F. solani	0.2	0.4	**+ 100.0**
V. dahliae	+	↓	

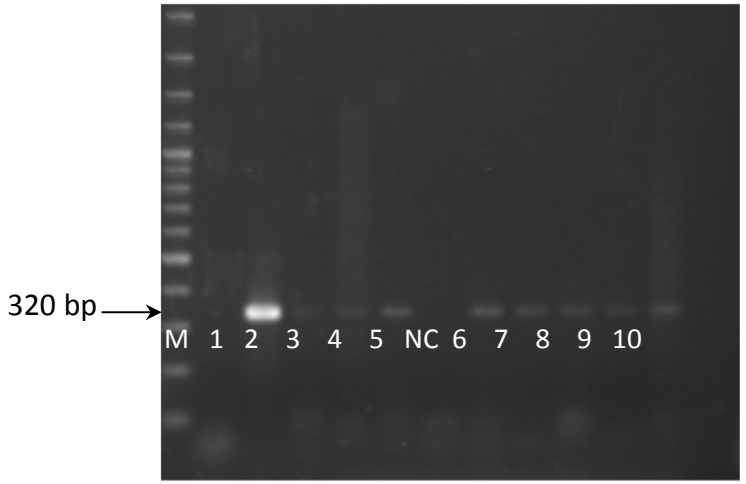
Farm D: active steam

Fungi	Check	AS	%
Total	5.9	8.9	+ 50.9
F. oxysporum	0.4	0.4	0
F. culmorum	0.04	0	- 100.0

Detection of *V. dahliae* using nested-PCR
(Kuchta et al., 2008)

FARM C
1 - *Sinapis alba*)
2 - *Brassica carinata*
3 – Dazomet 40 g/m2
4 – Metam sodium 50 ml/m2
5 - Metam sodium 35 ml/m2
6 - Check
7 - Nemasol 90 ml/m2
9 - Nemasol 60 g

FARM A
8 - Check
10 – Active steam



The 320 bp – product is specific for *V. dahliae*
NC – negative control in PCR reaction.

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Farm C

(cfu x 10^4/g)

Fungi	Check	*Brassica carinata*	*Sinapis alba*
Total	18.9	18.3	24.9↑
F.culmorum	0.2	0.4 ↑	0
F.oxysporum	0.4	0	0
V. dahliae	+	0	0

Number of bacteria population in soil after fumigation

(cfu x 10^7/g)

Location/ Treatment	Total	Fluorescent *Pseudomonas* spp.	*Bacillus* spp.
FARM A. Check	3.1	0.05	0.2
Dazomet – 40 g/m^2	9.4	2.7	0.1
Metam sodium 70 ml	10.1	0.6	0.1
FARM B. Check	3.1	0.04	0.3
Dazomet 30 g/m^2	14.9	1.1	0.3
Dazomet 40 g/m^2	23.6	6.6	0.3
FARM C. Check	6.0	0.009	0.6
Dazomet 40 g/m^2	14.5	0.2	0.9
Metam sodium 60 ml/m^2	5.2	0.009	0.5
Metam sodium 90 ml/m^2	6.5	0.01	0.5
Chloropicrin + 1.3 D 35 l/m^2	10.2	0.5	2.3
Chloropicrin + 1.3 D 50 l/m^2	14.8	0.7	2.9
Farm D. Check	4.3	0.04	0.4
Dazomet 30 g/ m^2	24.2	5.3	0.5
Dazomet 40 g/m^2	22.1	5.9	0.4
Metam sodium 60 ml/ m^2	27.1	1.9	0.6
Metam sodium 90 ml/ m^2	26.9	1.6	0.6
Chloropicrin + 1.3 D 35 l/m^2	21.1	1.1	0.7
Chloropicrin + 1.3 D 50 l/m^2	20.8	4.9	0.6
Chloropicrin 40 g/ m^2	23.1	6.1	0.3

Number of bacteria population in soil after active steam treatment (cfu x 10^7/ g soil)

Farm A

Treatment	Total	Fluorescent Pseudomonads	Bacillus sp.
Check	4.7 a	0.02 a	0.2 a
Active steam	10.7 b	0.07 b	1.5 b

Farm D

Check	1.2 a	0.04 a	0.05 a
Active steam	1.6 b	0.03 a	0.2 b

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Summary and Conclusion

- As regarding fungal pathogens there is a possibility to reduce doses of chemical fumigants
- After fumigations the populations of bacteria significantly increased
- Alternative methods reduced and in some cases totaly eliminated pathogens from soil (at detectable level)
- More research is needed

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