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Examination of materials and methods potential for organic seed treatment

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BACKGROUND OF RESEARCH AND OBJECTIVES

Everywhere in the world, thus in Hungary, as well, species and propagation material have significant role in successful agricultural production.

The maintenance of the favourable qualities which have been improved through the past centuries and their handing down for further generations in appropriate quality, play a significant role from food purposes. The higher and higher market price of seeds represents the appreciation of these valuable properties. From the beginning of the 21th century not only the quantity but also the quality of food has become significant, especially for developed societies. Beside traditional production this need has led to the evolution of integrated and organic farming systems. Higher quality requirements define all the moments of production, for it is important to assure and preserve quality from propagation material to products.

Healthy and high quality propagation material plays a significant role from economic aspect, which places the propagation material production industry into the focus of improvement and research. In organic farming, propagation materials have to originate from organic production from 1. January 2004, which emerged the need of their protection parallelly.

Seed treatment, as one of the most important protective procedure, ensures the protection of seeds against pathogenic agents and insects. Organic farming is a closed system, to be in harmony with nature, therefore a demand for organic seeds emerged and its necessity was declared by European Seed Association unanimously in 2005. In world trade, besides the legislation of the European Union - Codex Alimentarius Guidelines for Organically Produced Food 1999/2001 and IFOAM Basic Standards 2000 – international regulations are concerned with organic agriculture and the issue of organic seed production.

High quality propagation material is one of the keys of successful production. The maximum yield of propagation material can only be reached with accurate work and care within appropriate conditions. In the case of seeds, seed treatment is part of this process, which is gaining ground slowly not only in traditional but organic farming, as well. More and more researches are engaged with developing organic seed treatment procedures and methodology, which may represent economic advantage for Hungarian agriculture in the future.

In traditional agriculture, numerous methods are known and used for seed treatment during the production process. However, organic seed treatment is so recent, that only insufficient number of methods for the examination of seeds and materials for treatment are

available which can be applied in practice successfully. This might be an excellent means for the improvement of the quality of production, which might as well help prevention together with biological control. In organic pest control prevention plays a key role and can protect our crop most efficiently. In Hungary, according to the leading organic certification agency, no seed treating material is licensed. In practice, farmers can use maximum 1,5% concentration of natrium hydroxide for disinfection. For filling this market and technological gap, appropriately tested and efficient materials are necessary, if we are to implement organic agriculture seriously and increasingly in Hungary.

Objectives

During my work the main aim was to find environmentally friendly, cheap and easily applicable materials for organic seed treatment. To be able to fulfil this objective I approached through the following elements:

- Selecting appropriate materials for organic seed treatment according to scientific literature and former examinations.
- *In vitro* microbiological examinations of perspective materials against previously chosen pathogenic microorganisms.
- Defining the lowest, yet effective concentration of the materials which have been proven to be microbiologically effective to inhibit the reproduction of microorganisms safely.
- Comparing *in vitro* methods appropriate for the examination of the inhibiting effect of the chosen materials.
- Examining the effect on germination ability of the chosen materials via *in vitro* tests on germination ability and *in vivo* tests on seed emergence.
- Examining the effect on seed vigour in the case of materials which had not shown a negative effect on germination ability.
- Examining the effect on seedlings in the case of materials which had not shown a negative effect on germination ability.
- Summarising the results of my own research and defining directions for further examinations according to recent scientific literature.

MATERIAL AND METHOD

The materials for examination have been chosen on the grounds of scientific literature: materials applied in food conservation and of natural origin.

Microbiological tests were implemented at Corvinus University of Budapest, Faculty of Food Science, Natural Collection of Agricultural an Industrial Microorganisms, where the following microorganisms were provided:

Clavibacter michiganensis subsp. *michiganensis* NCAIM B001778

Clavibacter michiganensis subsp. *michiganensis* NCAIM B001779

Pseudomonas syringae pv. *tomato* NCAIM B001277

Pseudomonas syringae pv. *tomato* NCAIM B001682

Pseudomonas syringae pv. *tomato* NCAIM B001538

Xanthomonas campestris pv. *vesicatoria* NCAIM B001771

Xanthomonas campestris pv. *vesicatoria* NCAIM B001226

Xanthomonas campestris pv. *vesicatoria* NCAIM B001807

Xanthomonas campestris pv. *vesicatoria* NCAIM B001533

Sclerotinia sclerotium F00738

The following microorganisms were provided by the Plant Protection Institute of the Hungarian Academy of Sciences:

Rhizoctonia solani 268

Phytophtora infestans K39

Natrium-hydroxide, Kasumin 2L, Streptomycin-sulfate and destillated water were applied as controlls.

Materials examined in the experiments:

savory essential oil (*Aetheroleum saturejae)*
peppermint essential oil (*Aetheroleum piperitae)*
cinnamon essential oil (*Aetheroleum cinnamomi)*
thyme essential oil (*Aetheroleum thymi)*
caraway essential oil (*Aetheroleum carvi)*
vinegar
red wine vinegar
white wine vinegar
cider vinegar
baking soda (*Natrium-hydrogencarbonicum*)
70% diluted alcohol
paraffine oil
Hydrogen peroxide

ioded salt
propolis
valerian extract (*Valeriana officinalis*)
peppermint tea
savory tea
thyme tea

I have chosen two species for the examination: tomato and pepper, on the basis of their nutritional and economic importance in Hungary.

For testing the seeds *in vitro* I needed specially equipped laboratory, this was provided by the Seed Testing Official Department of Central Agricultural Office in Budapest, Hungary. For pre-testing two tomato- (ACE 55 VF és Mano) and one pepper seed items (Pritavit F1) were used. Further experiments were implemented with Austrian Pusztagold organic pepper seeds, Heros organic tomato seeds and Heros conventional tomato seeds.

Emergence tests were implemented at the Experimental and Research Farm of Corvinus University of Budapest, Department of Ecological and Sustainable Production Systems. The experiments took place in propagation trays in plastic tunnel to ensure the highest level of precision, repeatability and comparability. During emergence tests Pusztagold organic pepper seeds, Heros organic tomato seeds and Heros conventional tomato seeds were used, as well.

The seeds were steeped in the solutions at 20 °C permanent temperature for five or ten minutes, then dried on filter paper at room temperature for 24 hours. The treated seeds were examined right after this period.

During the experiment *in vitro* and *in vivo* examinations were implemented.

The exact composition of the tested essential oils were revealed through gas chromatography tests at Corvinus University of Budapest, Department of Medicinal and Aromatic Plants.

Microbiological efficiency was tested by the following *in vitro* methods:

Cup plate method

Disk diffusion test

Poison agar assay

Inhibition by volatile component on Clavibacter michiganensis subsp. *michiganensis B001778 bacterium*

Germination ability was tested in *in vitro* and then *in vivo* conditions. Vigour of the seeds were examined by the following methods:

Cold test

Measuring electronic conductivity

Examining seedling growth

Germination ability and the characteristics of seedlings were tested by the following examinations:

Emergence tests

Tests on tomato plantlets

The following parameteres of plantlets were measured, from which further characteristic rates were calculated.

- Germination
- Height of plant
- Diameter of stem
- Fresh gross of one plantlet
- Dry matter content of green parts
- Fresh gross of one root
- Dry matter content of one root
- Chlorophyll content /SPAD measurement

Handling and primary processing of the data during the experiments was executed with the help of MicrosoftExcel 2003 programme. The statistic analysis of the data was done by SPSS for Windows 14.0 statistic softver and Ropstat programme package.

RESULTS

The results gained from the experiments are presented in **Table 1-3**., summarised according to the types of tests. First I was examining the inhibitant effect against bacteria at laboratory, the results are presented in Table 1.

Table 1.: Materials performing well in bacteriological tests

Strains	*Pseudomonas syringae* pv. *tomato* B.01277	*Pseudomonas syringae* pv. *tomato* B.01682		*Xanthomonas campestris* pv. *vesicatoria* B.01807	*Xanthomonas campestris* pv. *vesicatoria* B.01771	*Xanthomonas campestris* pv. *vesicatoria* B.01226		*Clavibacter michiganensis* subsp. *michiganensis* B.01778		*Clavibacter michiganensis* subsp. *michiganensis* B.01779
Methods	*	*	**	*	*	*	**	*	**	*
vinegar 10%	+!	++	+	++	+!	++	+	++	+	+!
vinegar 5%	+!	+	+	+	+!	+	=	+	+	+!
vinegar 2,5%	+!	+	+	+	+!	+	=	+	=	+!
vinegar 0,5%	+!	+	+	=	+!	+	=	+	=	+!
cider vinegar 6%	+!	+=S	+	++	+!	++	+	++	+	+!
cider vinegar 5%	+!	+	+	+	+!	+	+	++	+	+!
cider vinegar 2,5%	+!	+	+	+	+!	+	+	+	+	+!
cider vinegar 0,5%	+!	+	=	=	+!	=	=	+	=	+!
red wine vinegar 6%	+!	0	+	0	+!	++	+	+!	+	+!
red wine vinegar 5%	+!	0	+	0	+!	++	+	+!	+	+!
red wine vinegar 2,5%	+!	0	=	0	+!	++	=	+!	=	+!
red wine vinegar 0,5%	+!	0	=	0	+!	=	=	+!	=	=
white wine vinegar 6%	+!	0	+	0	+!	++	+	+	+	+!
white wine vinegar 5%	+!	0	+	0	+!	++	+	+	+	+!
white wine vinegar 2,5%	+!	0	+	0	+!	+=S	=	+	+	+!
white wine vinegar 0,5%	+!	0	=	0	+!	=	=	=	=	=
cinnamon oil 100%	+!	+	+	0	+!	+	++	+	+	+
cinnamon oil 50%	+	+	+	0	+!	+	++	+	+	+
cinnamon oil 25%	+	+	+	0	+!	+	+=S	+	+	+
thyme oil 100%	=	+	+	0	0	+	+	+	+	+
thyme oil 50%	-	+	+	0	0	=	+	+	+	+
thyme oil 25%	-	+	+	0	0	=	+	+	+	+

Key to symbols:
*: Cup plate method;
**: Disk diffusion
+!: significantly more efficient, than control (1,5% NaOH), not tested with Streptomycin-sulfate 50 ppm
+: significantly more efficient, than control (1,5% NaOH)
++: significantly more efficient, than control (1,5% NaOH) and Streptomycin-sulfate 50 ppm
-: significantly not more efficient, than control (1,5% NaOH)

-!: significantly not more efficient, than control (1,5% NaOH), not tested with Streptomycin-sulfate 50 ppm
0: not tested
=: its effect is equal with 1,5%-os concentration of NaOH-val (with control)
+=S: its inhibitant effect is the same as Streptomycin-sulfate 50 ppm and significantly better, than NaOH 1,5%

I have tested numerous other materials on the chosen bacteria strains, but these have proven to be inefficient or of little efficiency against the tested strains. During my experiments my objective was to choose materials with the widest spectrum of activity, thus in further tests (against fungi, germination ability, vigour- and emergence tests) only the bacteriologically efficient materials were examined. The results of the tested materials on fungi is presented in **Table 2.**

Table 2: Results of tests on fungi

Strains	***Rhizoctonia solani* R268**			***Sclerotinia sclerotium* F 00738**			***Phytophtora infestans* K39**
Methods	a	b	c	a	b	c	a
Tested agents, concentrations							
control	-	-	-	-	-	-	-
NaOH 1,5%	+		+	-		+	++
material with kasugamycin	=			-		-	
vinegar 10%	++	++		++			++
vinegar 5%	*	++	+	+!	++	+	-
vinegar 4%			+			+	
vinegar 2,5%	+		+	-		=	-
vinegar 0,5%	=		=	-			
red wine vinegar 6%	+		+	-	++	+	-
white wine vinegar 6%	+		+	+!	++	+	
cider vinegar 6%	+		+	*	++	+	
cinnamon oil 100%	-			++			+
thyme oil 100%	+!			+!			-
propolis 100%	*			-			

Key to symbols:
a: poison agar assay
b: further test on *cid* effect
c: direct contact method
++ : total inhibition: fungi stop growing
+ : significantly slower growth of fungi in comparison with the control
+! : significantly slower growth of fungi in comparison with the control and 1,5% NaOH
- : significantly faster growth of fungi in comparison with the control
=: growth of fungi equal to control
* : slower growth of fungi in comparison with the control

The effect of the chosen materials on germination ability and seed vigour is presented in **Table 3.**

Table 3.: Effect of tested materials on germination ability and vigour

	Heros organic tomato seed				**Heros tomato seed**				**Pusztagold organic tomato seed**			
Methods	I	II	III	IIII	I	II	III	IIII	I	II	III	IIII
Tested agents, concentrations												
NaOH 1,5%	=	-	-	=	=		-		+	+	-	=
watery control	=	=	++	=	*		++		+	+	++	-
agent with kasugamycin	-	=		=					-	+		=
Streptomycin sulfate 50 ppm	=			=					=			=
vinegar 5%	-			=					-			-
vinegar 2,5%				=								
vinegar 0,5%	=	-	++	=	=		++		-	-		
cider vinegar 5%	=			=					-			-
cider vinegar 2,5%				=								
cider vinegar 0,5%	=	=	++	=	=		++		+			
red wine vinegar 5%	=			=					-			-
red wine vinegar 2,5%	=	=	+	=	=		++					
red wine vinegar 0,5%	*	=	+	=	=		++		+	++	++	
white wine vinegar 5%	=			=					-			-
white wine vinegar 2,5%	=	=	+	=	=		++					
white wine vinegar 0,5%	*	=	++	=	*		++		*	+	++	
propolis	*	=	+	=	=		++		-	+		
0,24 Mm H2O2	*			-					=			
0,12 Mm H2O2	*			=					+			
thyme oil 50%	-			=					-			-
thyme oil 25%	-								-			
cinnamon oil 50%	-			-					-			-
cinnamon oil 25%	-								-			

Key to symbols:
I : Germination ability tests
II : Cold test
III : Electronic conductivity test
IIII : Emergence test on field
++ : significantly better germination ability than control and 1,5% NaOH
+ : significantly better germination ability than control
- : significantly worse germination ability than control
= : germination ability is equal to untreated control
* : better germination ability than control

NEW SCIENTIFIC RESULTS

According to my experiments the following are to be stated:

- Vinegars in 10% concentration have *cid* effect on tested phytopathogenic bacteria and fungi.
- Cider vinegar, red and white wine vinegar have fungistatic effect up to 2,5% concentration, in 0,5% concentration they slightly decelerate the growth of the examined fungi strains *(Sclerotinia sclerotium, Rhizoctonia solani, Phytophtora infestans).*

- 0,5 % red and white wine vinegar treatment has positive effect on germination ability and vigour of Heros tomato seeds.

- 0,5 % red wine vinegar treatment improves the vigour of Pusztagold pepper seeds.

- Comparing cup plate method with disk diffusion is more sensitive due the its higher material content, thus I recommend this for testing materials for seed treatments in the case of *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria*.

- The tested natural acids (vinegar, cider vinegar, red and white wine vinegar) are all appropriate in 0,5 % concentration for organic seed treatment due to their wide spectrum of activity: they all have bacteriostatic and fungistatic effect.

CONCLUSIONS AND RECOMMENDATIONS

The objectives of my experiments were to find materials and methodology suitable for seed treatment for organic farmers. These materials have to fullfil numerous fundamental criteria, for they have to be easily applicable, should have a positive effect on seeds and the reasonability of the price is also a significant factor. To consider an effect positive these materials are to inhibit the utmost phytopathogenic microorganisms spread either by seeds or soil, furthermore they must not effect germination ability negatively. If all the requirements are fulfilled, the agent can be considered promising for seed treatment in organic farming, as well. If a material presents antimicrobial effect but worsens germination ability, can be treated as promising agent at other fields of plant protection. However, if a material has a positive effect on germination ability and seed vigour but not antimicrobially, might worth to be considered useful as a seed or plant conditioner.

The tested bacteria play an important role in growing vegetables in Hungary. During my experiments all concentrations of the selected vinegars inhibited the growth of the examined strains of bacteria, for which the pH value, that is the potential of hydrogen of the medium is responsible. For the pH sensitivity of bacteria these materials successfully inhibit their germination. These pathogens require the optimal pH value = 7,2 for their growth according to scientific literature, thus the medium made acid by vinegars is not suitable for their development.

Vinegars in 0,5% concentration inhibit reproduction and increasing concentration this effect can be multiplied. The inhibiting effect of vinegar in 10% concentration exceeds that of 50 ppm Streptomycin-sulfate. Materials, proven to be efficient in *in vitro* experiments should also be tested for other means of plant protection (E.g.: crop treatment) which is also an important part of farming. Hydrogen ion pH 3-6 has bacteriostatic, while pH <3 bactericid effect, which was proven in the case of the tested bacteria strains. Alkalis, however, have a lot less effect on the propagation of bacteria. Alkaline medium does not present such an extent of inhibition on the propagation of bacteria as acids. Baking soda did not have any effect, while 1,5% NaOH solution, the pH value of which is 13, only showed little effect on the growth of the strains.

The effect of 0,5% vinegars had the same effect as 1,5% NaOH, while in more than 2,5% concentration it has proved to be more efficient. (**Table 1.**).

Cinnamon and thyme essential oils needed to be applied in at least 25% concentration to perform inhibition on the propagation of bacteria. 25% concentration compared to 50%

showed efficiency and did not present significant difference, however from ecological point of view the less concentration is reasonable. In the case of cinnamon essential oil, the effect is due to its main active component, cinnamon aldehyde which was also shown in gas chromatography test. In the case of thyme essential oil, thymol is represented in the greatest proportion and might be responsible for its efficiency. These oils inhibited the growth of all three bacteria from 25% concentration.

Testing volatile components clearly revealed that not only essential oils but vinegars have great amount of volatile components, which might influence their effect on microorganisms and mean new perspectives for the use of these materials for distinct purposes (e.g.: in storage). Because of volatile components, it is useful to define the optimal duration of the seed treatment technology. If the seeds are treated right before sowing, the material might inhibit the growth of microorganisms in the soil, due to its volatile character. Strongly volatile materials (vinegars above 5% concentration) have *static* or *cid* effect on *Clavibacter michiganensis* subsp. *michiganensis* B001778, however this compound is not recommended for seed treatment since it worsens germination ability. These materials are worth to be tested at other fields (e.g.: crop treatment) or via other means (e.g.: vaporization) of plant protection.

In the case of the tested strains of fungi it is considered to be a good result if the material decelerates their growth, thus providing vantage for the germination of seeds. The chosen materials generally decelerate the growth of fungi (*static* effect), however not many of them could have *cid* effect. In the case of *Rhizoctonia solani* and *Sclerotinia sclerotium* the majority of the tested agents in higher concentrations (> 6%) had *cid* effect, while in the case of *Phytophtora infestans* only the 1,5% NaOH, vinegar in 10% concentration and undiluted cinnamon essential oil inhibited growth. These materials also inhibited the growth of the other strains of fungi. In the case of mildews, natural acids under pH 3 did not have any inhibition effect. The results of the methodological comparative examinations revealed, that from the methods used cup plate method is considered to be more sensitive due to the higher amount of materials, i.e. in these cases the amount of the tested agents is eleven times more, than in the case of disk diffusion. Germination ability and emergence tests verified that essential oils worsen germination ability. The negative effect on germination ability of vinegars is in inverse ratio to concentration, however from 2,5% concentration they do not have negative effect on germination ability compared to the control, what is more, in the case of pepper germination ability was enhanced. White wine vinegar in very low condition (0,5%) stimulated mainly the germination ability and vigour of tomato seeds, while red wine vinegar

had the same effect on the vigour of pepper seeds. On the basis of my experiments the antimicrobial effect of vinegar, cider vinegar, white and red wine vinegar can be established.

The above mentioned compounds in higher concentration have *cid* effect on bacteria, while stronger acids have the same effect on fungi, as well. Microbiological efficiency of vinegars is directly proportional to their concentration, however enhancing concentration might deplete germination ability of seeds. The tested vinegars are efficient in lower dose, therefore their application is to be implemented in such, for they decelerate the speed of the growth of microbes, which might be a key factor of prevention and plant protection in organic farming. With their better performance, plants are able to reach a level in their development for the unfavourable period, so that pathogenic microbes cannot infect them in healthier and better condition. In 0,5% or lower condition no negative effect can be excepted, what is more, as my vigour tests have confirmed, some vinegars have expressly positive effect on seed vigour. (red and white wine vinegars, cider vinegars).

Vinegars, red and white wine vinegars, cider vinegars in 0,5% concentration have presented complex spectrum of activity, for they have been efficient against both the tested bacteria and mildews, furthermore they have slightly ameliorated (or at least not worsened) seed vigour. As for expenses, NaOH 1,5% is the cheapest, followed by vinegar 0,5%, cider vinegar 0,5%, red wine vinegar 0,5% white vine vinegar 0,5%, and the agent with kasugamycin content. The expense of vinegars can be reduced further, for it is possible to produce them at the farm on the spot or purchase by the gross. According to my examinations these agents can be recommended for organic seed treatment. The tested agents can be placed in organic plant protection, where other means of application have to be considered, too. In such use, concentration does not have limiting role, for the surface of plants is less sensitive than seeds, thus treatments cannot be so harmful. Developing agents with appropriate effect at fields beyond seed treatment is to be researched further. Results are to be applied and their use in practice is to be tested widely in the future.

During my searching scientific resources I have found numerous agents, which are worth being examined in further experiments and their technology of application should be elaborated. Many compounds among fitoncids can be used not just in plant protection or seed treatment but also for conservation of food either in conventional or organic agriculture. The significance of further research lies in the fact, that the tools of plant protection are to be broadened not just in organic but also in conventional farming, and as long as it is possible to provide more environmentally friendly and cheaper agents, than presently available ones, all

members of the consumer chain might benefit both from the aspect of environmental protection and economy.

PUBLICATIONS IN THEME OF DISSERTATION

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