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Examinations of potential environmental friendly materials against tomato and pepper patogens

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Summary: In organic farming systems the focus is on prevention with regards to plant protection. To follow the rules of Good Agricultural Practice one is able to avoid serious yield losses; if it is not possible the use of allowed materials are permitted. Organic farmers have less material to protect their plants so it is necessary to find effective potential materials. Bacterial and fungal diseases of tomato and pepper can cause serious losses in yield. Different materials were tested against some plant pathogen bacterial (*Clavibacter michiganensis* subsp. *michiganensis, Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria* and fungal (*Phytoptora infestans, Rhizoctonia solani, Sclerotinia sclerotium*) strains in order to find potential materials in the field of organic seed treatment. *In vitro* trials have shown that vinegar, cider vinegar, red wine vinegar, white wine vinegar, cinnamon and thyme oil have inhibiting effect against the causative agent of bacteria and fungi. Germination test has shown that examined vinegar types do not decrease germination ability if the concentration is low, but in higher (more than 5%) concentration it ruins the germination ability. Even in 0,5% concentration of red – and white vine vinegar have good effect on germination capacity.

Key words: fungi, in vitro examinations, vinegar, pH, germination ability

Introduction

EU decree No. 2092/91 deals especially with ecological plant cultivation, regulates reproduction and usage of seed and propagation material (EC Council Regulation on Organic Agriculture article 6, No. 2092/91). According to EU decree No. 1452/2003 (14 August, 2003) the use of ecological propagation material is obligatory in organic farming. High quality healthy seeds are essential for successful organic farming, but in this field there are fewer tools than in conventional farming. Our aims were to find potential materials to avoid or decrease the pathogens of tomato and pepper. We try to find materials which are effective with a spectrum as broad as possible, so these have effect not only on the recently investigated bacteria, but on plant pathogen fungi, too. Optimal materials have cid effect both against bacteria and fungi; collectively these ones can be called germicide materials. Besides this, it is important for these materials having no or as less as possible negative effect on germination ability of seeds. In our examination we tried to augment the range of effective materials in seed treatment. In the course of experiments the following plant pathogen fungi have been investigated.

Bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis)* of tomato causes high yield losses in tomato production since 1960, while bacterial speck (*Pseudomonas syringae* pv. *tomato)* is just sparse problem. *Clavibacter michiganensis* subsp. *michiganensis* can survive in infested residues and seed is often introduced on infected transplants. The bacterium can spread with pruning, by tools, people, and equipments (*Glits* et al., 2001).

Pseudomonas syringae pv. *tomato*, infect all above ground parts of tomatoes. The bacteria overwinter in infected plant residues and seeds. It can easily spread in rainy weather and it can also spread by tools, equipments, and people (*Glits* et al., 2001).

Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria)* of tomato and pepper is one of the most important diseases of field produced pepper (mainly in moist weather). The bacteria can overwinter on the surface, or inside the seeds and remains viable for up to 10 years in crop residue in soil, or left on the surface, and on wild host plants (*Glits* et al., 2001).

In conventional farming kasugamycin agent chemicals were generally usedagainst examined bacterial pathogens for seed dressing (*Glits* et al., 2001). In ecological farming just 1,5% Sodium-hydroxide is allowed to use against these diseases which is actually a disinfectant.

Rhizoctonia solani occur everywhere where vegetable plants are grown, because it has several suitable host plants. The fungi primarily attacks underground plant parts, seeds, hypocotyls, and roots, but is also infecting above ground

plant parts. "Damping-off" is the most common symptom of *Rhizoctonia* disease it is characterized by non- germination of severely infected seed whereas infected seedlings can be killed either before or after they emerge from the soil. Infection source is the soil where it can survive the bad conditions for a long time in the form of pseudo-sclerotium. The pathogen can survive for many years by producing small (1 to 3-mm diameter), irregular-shaped, brown to black sclerotia on plant tissue and in soil. The pH optimum of the pathogen is between: 5,8-8,1 but it can wear the conditions from pH 4,5 to 10,4 (*Webster & Weber*, 2007). Control of *Rhizoctonia solani* is made by the treatment of the soil. In convetional farming steaming or the injection of fungicides to the soil is widespread.

Phytophthora infestans infects a wide range of solanaceous species. Economically important hosts are potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), eggplant and some others. It is distributed worldwide. *Phytophthora infestans* causes serious losses of potato crops worldwide and is probably the most important pathogen of potato and tomato today. In Hungary the first publication about it was in 1957, but in North-America and West-Europe cause epidemic around 1830. It causes great, yellowish spots initiating from the edges of the leaves, even overcoming the diameter of 10–20 mm, which will become brown and die off. After few days the tomato plant loose its whole foliage, looks like as if it would have been infused. Infection sources are the dead parts of tomato, and potato and the tuber where the fungi overwinter, with mycelium. Besides this, the infection can survive in the soil by saprophytic lifestyle. It can spread by air-flow or wind. It requires wet plant surface enduring several hours for infection. The prevention is the most important aspect of the protection against *Phytophtora infestans*. It is a very dangerous pathogen so not just for conventional but for in organic farmers too allowed to apply copper-containing materials (*Glits* et al., 2001).

Sclerotinia sclerotiorum is a poliphagous pathogen. It is distributed worldwide in Hungary till 1960 causing serious problems (mainly in greenhouses). The typical initial symptom of the disease is the presence of a cottony, white, dense mat of mycelial growth on the surface of the host and on the soil surface. The fungus colonizes host tissues and finally it cause brown or gray-brown lesion *Webster & Weber* (2007). The sclerotia provide the fungus to survive for several years in the soil. *S. sclerotiorum* produces large (2-10 mm in diameter) rounded sclerotia. *S. sclerotiorum* survive between crops as sclerotia in soil or as mycelium in infected plant debris. If the weather is favorable, sclerotia can probably survive up to 3 years in soil if it has not host plant. It saves their viability for more than 10 years under dry conditions. There is a very effective biological control against this pathogen called *Coniothyrium minitans*. It is a natural enemy of this pathogen; it is allowed to use in all type of farming systems (*Glits* et al., 2001).

Materials and methods

Bacteriological examinations were made by disc diffusion- and cup plate method, in which the zone of inhibition is dependent upon concentrations (*Gavin*, 1956). In case of cup plate method 15 ml from Nutrient agar with 2% agar-agar content was poured into Petri-dishes, after solidification 4 ml Nutrient agar with 1% agar-agar content was poured on it which included the examined bacterial strain. After solidification depressions were made by a sterile cork borer in the double Nutrient agar plate. Cups were completely filled with the examined materials. After 4 hours long diffusion period in the refrigerator plates were placed into a thermostat at 26 °C for 24-48 hours. In case of disc diffusion method we made the same double layer Nutrient agar and we put a sterile 5 mm diameter disc on it. Discs contained the examined materials. After this incubation period the inhibition zones around the holes were measured. The size of the zones showed the inhibition effect.

Examined bacterial strains originated from National Collection of Agricultural and Industrial Microorganisms (NCAIM) Budapest, Hungary. These were *Pseudomonas sypringae* pv. *tomato* B.01277, B.01682. *Xanthomonas campestris* pv. *vesicatoria* B.01771, B. 01226; *Clavibacter michiganensis* subsp. *michiganensis* B. 01778, B. 01779.

The *in vitro* examination of plant pathogen fungi were made by toxic agar test. First we made Potato Dextrose Agar (PDA) or Pea Broth Agar (PBA) for *Phytophtora infestans* strain. The prepared substrate has been cooled down to about 50 °C, in water bath after autoclaving, then the investigated materials have been mixed to 1000 ml PDA agar media in all cases with the following doses: 10 µl unsophisticated cinnamon oil; 10 µl unsophisticated thyme oil; 10 µl unsophisticated propolis; 30 ml 10% vinegar; 30 ml 6% red vine vinegar; 30 ml 6% white vine vinegar; 30 ml 6% cider vinegar.

For the examinations the strains have been reproduced and inoculated to PDA or PBA discs to grow uniformly and make clear vegetation. Discs of 4mm diameter were cutted from the clean fungal vegetation and we put it onto the surface of solid agar.

The examined *Sclerotia sclerotium* strain (NCAIM F.00738) originated from NCAIM Budapest, Hungary. *Phytophtora infestans* (K39) *and Rhizoctonia solani* (R268) originated from Plant Protection Institute Department of Plant Pathology.

Examined materials were chosen according to published data. All vinegar is biologically fermented. Controls were: Stretomycin-sulphate antibiotics (not allowed in Hungary for plant protection), Kasgamycin-laden material, and 1,5% sodium-hydroxide (NaOH).

All the examinations were made in four replicates. Data have been analyzed by SPSS 14.0- or Ropstat program with Tukey b, Duncan or Games-Howell test (SD 5%).

Germination ability was examined with an ISTA standard (MSZ 6354-3: 1991) in the National Institute for Agricultural Quality Control in Hungary. Germination ability test was made with 100 seeds in four replicates. The emergent

numbers of tomato seedlings were counted on day 8. and on day 14. day and those of the pepper were counted on day 12. Before the test the seeds were soaked in materials for 10 minutes and then they were dried for 24 hours. Data have been analyzed by SPSS 14.0 program with Tukey, Duncan test or with Games-Howell (SD 5%).

Results

Reasults of bacteriological assays

Table 1: The results of *in vitro* bacteriological examinations

Strains		B.01277	B.01682		B.01807	B.01771	B.01226		B.01778		B.01779
Methods		*	*	**	*	*	*	**	*	**	*
Examined materials concentrations, pH and theireffect											
vinegar 10%	2,4	+!	++	+	++	+!	++	+	++	+	+!
vinegar 5%	2,7	+!	+	+	+	+!	+	=	+	+	+!
vinegar 2,5%	2,9	+!	+	+	+	+!	+	=	+	=	+!
vinegar 0,5%	3,2	+!	+	0	0	0	+	0	+	0	0
cider vinegar 6%	3,01	+!	+=S	+	++	+!	++	+	++	+	+!
cider vinegar 5%	3,05	+!	+	+	+	+!	+	+	++	+	+!
cider vinegar 2,5%	3,1	+!	+	+	+	+!	+	+	+	+	+!
cider vinegar 0,5%	3,6	+!	0	0	0	0	=	0	0	0	0
red vine vinegar 6%	2,73	0	0	+	0	+!	++	+	+!	+	+!
red vine vinegar 5%	2,77	0	0	+	0	+!	++	+	+!	+	+!
red vine vinegar 2,5%	2,83	0	0	=	0	+!	++	=	+!	=	+!
red vine vinegar 0,5%	3,05	0	0	0	0	+!	=	0	+!	0	=
white vine vinegar 6%	2,65	+!	0	+	0	0	++	+	+	+	+!
white vine vinegar 5%	2,73	+!	0	+	0	+!	++	+	+	+	+!
white vine vinegar 2,5%	2,8	+!	0	+	0	+!	+=S	=	+	+	+!
white vine vinegar 0,5%	3,03	+!	0	0	0	+!	=	0	0	0	-!
cinnamon oil 100%		+!	+	+	0	+!	+	++	+	+	+
cinnamon oil 50%		+	+	+	0	+!	+	++	+	+	+
cinnamon oil 25%		+	+	+	0	+!	+	+=S	+	+	+
cinnamon oil 0,5%		-	-	0	0	+!	0	0	-	0	-
cinnamon oil 0,25%		-	-	0	0	=	0	0	-	0	-
cinnamon oil 0,1%		-	-	0	0	=	0	0	-	0	-
thyme oil 100%		=!	+	+	0	0	+	+	+	+	+
thyme oil 50%		-	+	+	0	0	=	+	+	+	+
thyme oil 25%		-	+	+	0	0	=	+	+	+	+
thyme oil 1%		-	-	0	0	-	-	0	-	0	-
thyme oil 0,25%		-	-	0	0	-	-	0	-	0	-
thyme oil 0,5%		-	-	0	0	-	-	0	-	0	-

Legend: * Cup plate method ;** Disc diffusion method

+!: significantly more effective than the control (1,5% NaOH), but the 50ppm Streptomycin-sulphate was not examined

+: significantly more effective than the control (1,5% NaOH),

++: significantly more effective than the control (50ppm Streptomycin-sulphate)

-: significantly less effective than the control (1,5% NaOH)

-!: significantly less effective than the control (1,5% NaOH), but the 50ppm Streptomycin-sulphate was not examined

0: not examined

=: its effect is equal with that of the 1,5% NaOH

+=S: its effect is equal with the 50ppm Streptomycin-sulphate and it is significantly more effective than 1,5% NaOH

Results of examinations on plant pathogen fungi

Phytoptora infestans

Throughout the poisoned agar disc investigations the vinegar, the 1,5% NaOH and 50% cinnamon oil/1L PDA was completely inhibited the growth of *Phytoptora infestans K39 strain.* The growth of the fungal vegetation was measured three times, 6, 12 and 21 days after the inoculation of the Petri-dishes. However, on the 6th day no growth was experienced. This can happen due to the fact, that the *Phytophtora infestans* K39 has slower growing characteristics, than that of the other investigated plant pathogen fungal strains. The 1,5% NaOH/1L PDA and the 10% vinegar/1L PDA could also totally inhibited the growing of the strains, while the 50% cinnamon oil/1L PDA significantly inhibited the growth, with respect to the control; however 2–4 mm of vegetation spreading could have been experienced (*Figure 1).*

In the case of K39 strain lower concentrations of vinegar and other materials have been tested, to see, whether these also influence the growth of the strains. The investigation showed at the 6th day, that 30ml 5% vinegar/1L PDA and 10 µl 50% thyme oil/1L PDA totally inhibited the growth of the strains, while 30ml 2,5% vinegar/1L PDA and 30ml 6% red vine vinegar/1L PDA only slowed down the growth significantly. However, at the 21st day we experienced, that the effect seems to be cid at the beginning was not perceptible, since all the treatments (30ml/2,5%–,5% vinegar, 6% red vine vinegar and 10µL/50% thyme oil/1L PDA) neither inhibited nor decelerated the growth of the strain *(Figure 2).*

Rhizoctonia solani

In case of the investigated *Rhizoctonia* strain the 10% vinegar totally inhibited the growth of the strain at the 6th day, while 1,5% NaOH delayed the growth in correlation with the control. The 50% cinnamon oil did not slowed down the propagation of the fungi. The tendency was similar in the 12th and 21st day, 10% vinegar totally inhibited the growth, while the 1,5% NaOH significantly decelerated the spreading; the 50% cinnamon oil had no effect on the growth according to the control.

Six days after beginning the *Rhizoctonia solani* grew significantly slower on the substrate poisoned with 50% cinnamon oil, than any other treatment and the control (*Figure 3*). The vinegar in 30 ml 5% vinegar/1L PDA concentration and the 30ml of 6% white vine vinegar/1L PDA also decelerated the growth significantly, though in a lower extent than that of thyme oil. 30 ml of 6% /1L PDA concentration of apple vinegar and white vine vinegar in slowed down the propagation of the strain, though the 10µl 100% propolis/1L PDA and 30 ml 2,5% /1L PDA vinegar did not impede according to the control. In the 21st day only the treatment with 10 µl 50% thyme oil/1L PDA could slow down significantly the growth of the strain in accordance with the control and the other treatments (*Figure 4*).

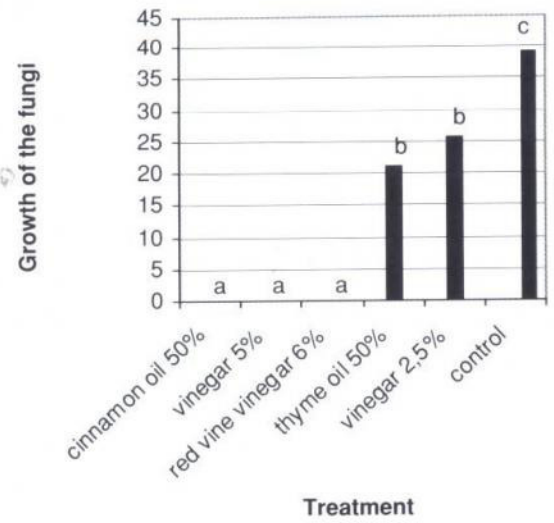

Figure 1: In vitro examinations of *Phytophtora infestans* K39 on the 12th days. Data were analised with Games-Howell test. (Letters show the significant differences in 95% significance level.)

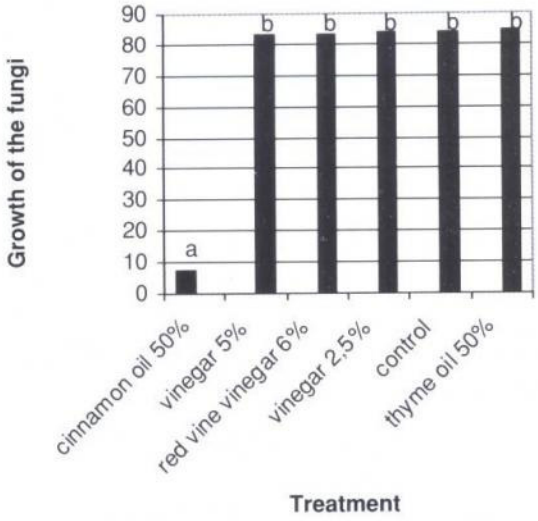

Figure 2: In vitro examinations of *Phytophtora infestans* K39 on the 21st days. Data were analised with Games-Howell test. (Letters show the significant differences in 95% significance level.)

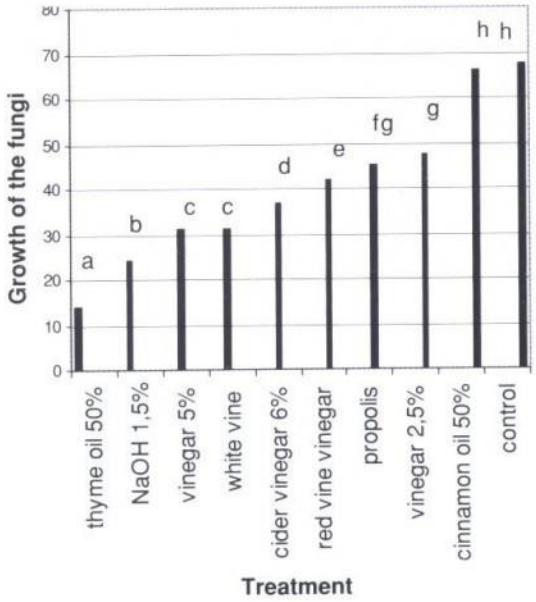

Figure 3: In vitro examinations of *Rhizoctonia solani* R268 on the 6th days. Data were analised by Tukey test. (Letters show the significant differences in 95% significance level.)

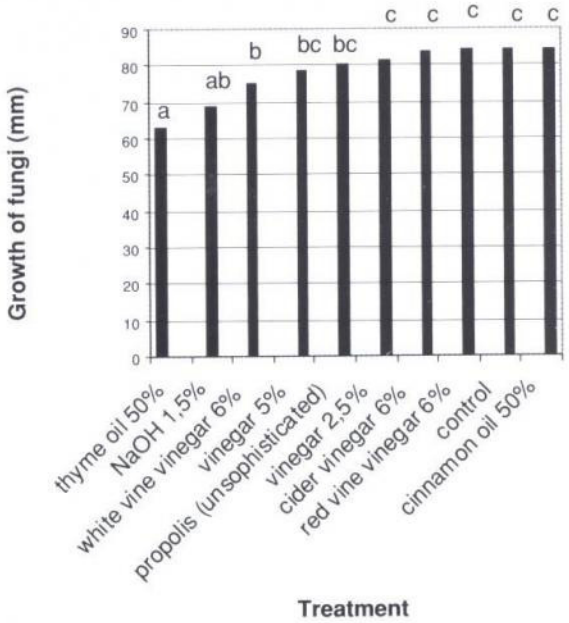

Figure 4: In vitro examinations of *Rhizoctonia solani* R268 on the 21st days. Data were analised by Games-Howell test. (Letters show the significant differences in 95% significance level.)

Sclerotinia sclerotium

The vinegar in 10% concentration and the cinnamon oil in 50% concentration were also inhibited the reproduction of *Sclerotinia sclerotium* F00738. Throughout the investigation the inhibition effect was checked three times, which showed clearly, that while the 1,5% NaOH treatment used as a control developed slower than the untreated strain and covered the total surface of the Petri dish until the third checking time; at the same time cinnamon oil and vinegar also inhibited the growth of the investigated strain (*Figure 6*).

Based on the promising results of the first investigation we tried to reduce the concentration of the vinegar and include new materials to the experiment so to find the lowest effective concentrations due to financial issues.

The 30 ml 5% vinegar/1L PDA slowed the growth of *Sclerotinia sclerotium* F00738 strain, however the 30 ml /1L PDA 2,5% concentration did not show inhibition effect yet. The 10 µl 50% thyme oil/1L PDA could also decelerate the extent of propagation, as the 30 ml 6%/1L PDA apple vinegar and same concentration of white vine vinegar did it so in case of Sclerotinia strain. Among investigated vinegars the red wine vinegar in 30ml 6%/1L PDA concentration had no inhibition effect.

Germination ability tests on tomato and pepper seeds

According to the germination ability test the examined vinegars show negative effect on seed germination ability

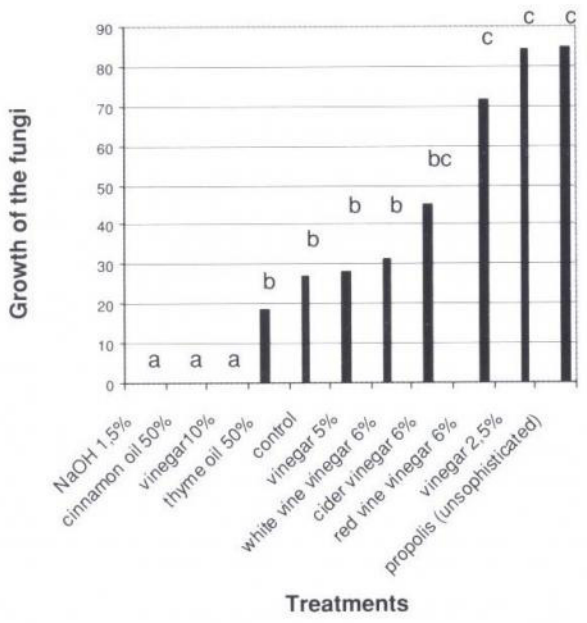

Figure 5: In vitro examinations on *Sclerotinia sclerotium* F00738 on the 6th day. Data were analised with Games-Howell test. (Letters show the significant differences in 95% significance level.)

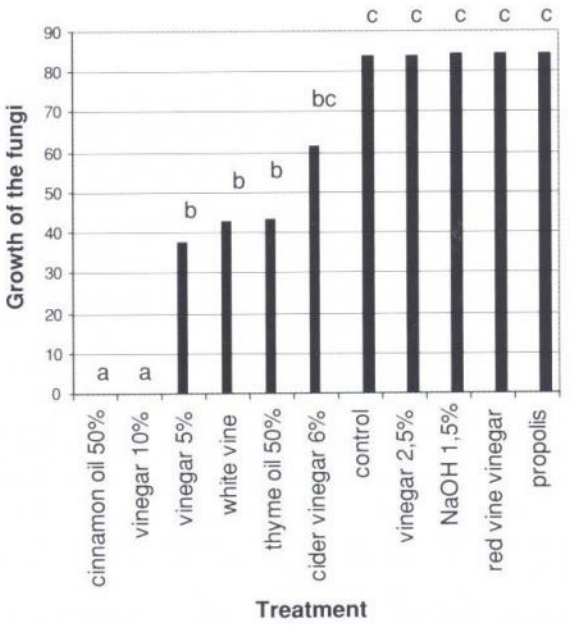

Figure 6: In vitro examinations on *Sclerotinia sclerotium* F00738 on the 21st day. Data were analised with Games-Howell test. (Letters show the significant differences in 95% significance level.)

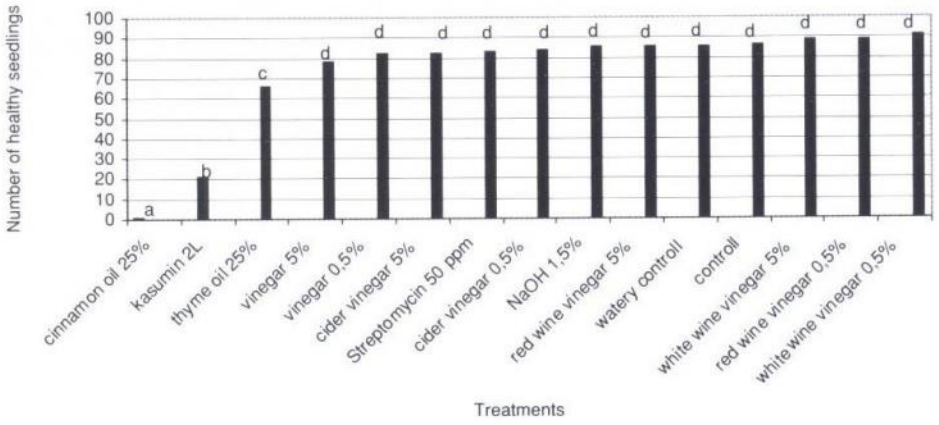

Figure 7: Germination ability of tomato seeds, analyzed with SPSS 14.0 program by Duncan test. (Letters show the significant differences in 95% significance level).

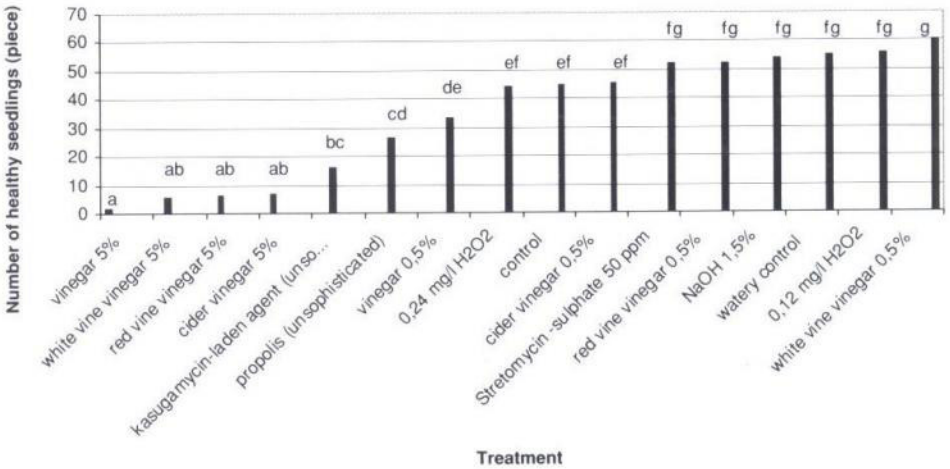

Figure 8: Germination ability of pepper seeds, analyzed with SPSS 14.0 program by Tukey test. (Letters show the significant differences in 95% significance level.)

except in low (0,5%) concentration. 0,5% concentration of red and white wine vinegar mend the germination capacity, but this effect is not significant on tomato seeds. Essential oils (thyme oil and cinnamon oil) ruined the germination capacity hardly in 100% and in 50% concentrations, too (Results were same with the use of different solvent.)

The 0,5% concentration of white vine-, red vine- and cider vinegar have positive effect on germination ability of pepper seeds. In 5% concentration, however all types of vinegar ruined the germination capacity.

Our experience was the same – on tomato and pepper seeds, too -as Borgen A. (2003) with vinegar in relation with concentrations' effect and germination ability. Negative effect on seed germination ability seems to be proportional to the applied dose. 0,5% of red and white wine vinegar had stimulative effect on germination ability. So we continued the tests with vigor examinations.

Discussion

Bacteriological assay showed that all vinegars, cider vinegar, red vine vinegar, and white vine vinegar had

also inhibition effect almost in every case if the concentration was at least 2,5%. In 0,5% concentration these have also inhibition effect generally but it was not as evident as in higher dose. With the increasing of the concentration the inhibition effect also increased, but the negative effect also grow with regards to germinating capacity. Examined bacterial strains were more sensitive to acidic than alkaline circumstances.

Among examined essential oils the most effective was cinnamon oil. Thyme oil was also effective but to a lesser extent, than cinnamon oil. According to literature and our GC-MS examinations the cinnamon was effective because of cinnamon-aldehide content, as thyme oil due to thymol content.

Against *Phytophtora* the 10% vinegar/1L PDA had significant inhibition effect, but in lower concentrations it just slowed down the growth of the strain; the 1,5% NaOH and cinnamon oil 50%/1L PDA had also significant inhibition effect.

Against Sclerotinia the 10% vinegar and the cinnamon oil have total inhibition effect but the control Sodium-hydroxide in 1,5% concentration just delayed the reproduction of the strain. Vinegar in 30 ml 5%/1L PDA, red vine–, white vine-, and cider vinegar in 30 ml 6%/1L PDA had inhibition effect.

The 10% vinegar/ 1L PDA had total inhibition effect against *Rhizoctonia,* but the 1,5% NaOH just slowed the reproduction; 50% cinnamon oil/ 1L PDA was ineffective. Vinegar in low concentration, red vine –, white vine and cider vinegar just delayed the growth, but thyme oil significantly inhibited the reproduction of the examined strain.

The microbiologically most effective material was the 10% vinegar/ 1L PDA followed by cinnamon oil in 50%. Other materials were also effective: 10µl 50%thyme oil /1L PDA, red vine-, white vine and cider vinegar in 30ml 6%/ 1L PDA, but propolis and H_2O_2 had no inhibition effect. The best if the examined material has cid effect but the delay of the reproduction can be also important because in this way seeds have extra time to overcome the microorganisms. It can be a competitive advantage against the pathogens which can be crucial at the time of emergence.

Germination ability test showed that essential oils and vinegars in high concentrations ruined germination ability. White vinegar and red vine vinegar in 0,5% concentration significantly enhanced the germination capacity.

We can conclude that all examined vinegar types seem to be useful in biological plant protection systems against examined pathogens of tomato and pepper. These materials are environmental friendly, cheap dressing materials with low oral toxicity to humans, birds, and others who have contact with it.

We plan to test the promising materials in other fields of plant protection which can be important for the organic farmers who have fewer tools than the conventional ones.

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