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Effect of different treatments to bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*), bacterial speck (*Pseudomonas syringae* pv. *tomato*) in tomato, and bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) in pepper

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Summary: In ecological farming systems farmers can't use chemicals against pests. In ecological plant protection the aim is to prevent diseases; if it is not possible the use of allowed materials are permitted. Until now there haven't been enough effective and environmental friendly materials for seed treatment in organic farming. Seed borne diseases of tomato and pepper can cause serious losses in yield, so finding appropriate inhibitors has a great importance. Different materials were tested against these bacterial strains for seed treatment in this study. *In vitro* trials have shown that vinegar, cider vinegar, red wine vinegar and white wine vinegar have inhibiting effect against the causative agent of bacterial canker *(Clavibacter michiganensis* subsp. *michiganensis),* bacterial speck *(Pseudomonas syringae* pv. *tomato)* of tomato. These materials also have inhibiting impact on the causative agent of bacterial spot of pepper *(Xanthomonas campestris* pv. *vesicatoria).* Seed treatment with (natural alkaline material) sodium hydrogen carbonate ($NaHCO_3$) had no effect on the examined bacterial strains. Among examined essential oils cinnamon oil seemed to be the most effective, but all oils decreased the germination ability. Thyme and savory teas were effective against *Pseudomonas syringae* pv. *tomato.* Other examined materials had insufficient bactericide impact (sucrose, NaCl, ethanol, valerian extract, peppermint tea). The germination test has shown that examined vinegar types don't decrease germination ability.

Key words: bacteria, cinnamon oil, seed treatment, seed borne disease, thyme oil, vinegar, tomato seed, 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.

The application of synthetic dressing powders for protecting propagation materials is not permitted, however [illegible]lity healthy seeds are essential for successful organic

[illegible] this reason it is very important to find such substances which can provide a good alternative for seed protection. The aim of our study is to find new, environmental- friendly, permitted materials against seed borne bacterial strains of tomato and pepper, which do not ruin germination capacity.

In Hungary bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis)* of tomato cause high yield losses in tomato (*Lycopersicon esculentum*) production since 1960, while bacterial speck (*Pseudomonas syringae* pv. *tomato)* is just sparse problem.

Bacterial canker causes wide range of symptoms on tomatoes. The gradual wilting and parching is the most characteristics symptoms of the disease. Wilting of branches or entire parts of young and old plants develops with the disease progresses. Entire leaves and branches eventually die, become brown, and fall down from the plant. On the fruit there are 2–3 mm wide round patches without thrum and with necrosis on the centre. *Clavibacter michiganensis subsp. michiganensis* can survive in infected residues and seed is often introduced on infected transplants. Hard or driving rains and pruning are favourable for the natural spreading of bacteria to neighbouring plants only. The bacterium can spread with pruning, by tools, people, and equipments.

Pseudomonas syringae pv. *tomato*, infects all above ground parts of tomatoes. Bacterial speck causes black specks on leaves, stems, and fruits. It causes flower abortion and fruit spotting. The symptoms of bacterial speck on fruits are small, black spots on the surface. The bacteria over winter in infected plant residues and seeds. It can easily spread in rainy weather and it can also spread by tools, equipments, and people.

Bacterial spot (*Xanthomonas campestris pv. vesicatoria)* of pepper is one of the most important diseases of field produced pepper (mainly in moist weather). The bacteria can over winter 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. It is not able to survive alone in soil for a long time, but can survive on every part of pepper plant up to 6 months in infected crop debris in soil. It can occur on every part of pepper. Most important symptoms are small light brown patches on the leaves; afterwards it grows bigger and darker and finally the whole leaf turns yellow and dropped off from the plant. Infected seeds are the primary infection sources, which are located under the cotyledons, as well as the major means of long distance spread of the pathogen. Secondary infection sources are infected transplants. High temperature (30 C°) and 90% relative moisture content is favourable for the development of bacteria (*Glits* et al., 2001).

In Hungarian conventional farming kasugamycin agent chemicals are generally used (for 100 kg 2L Kasumin) against these pathogens for seed dressing (*Glits* et al., 2001). In ecological farming only maximum 1,5% Sodium-hydroxide (NaOH) is allowed to use against these diseases; however it is a disinfectant, so currently there is no seed dressing material is allowed in ecological farming in Hungary.

Acetic acid is still examined in other countries as seed treating material, but it was applied only against fungi in arable cultures. Other examinations are also developed new adequate methods for seed treatment in ecological vegetable production, for example: natural compounds (*Tinivella* et al., 2004) (plant extracts (*Hartman* et al., 1995), essential oils, and natural acids), antagonistic micro organisms, physical treatments (aerated steam treatment, hot water treatment (*Roberts* et al., 2006). According to literature thyme oil was the most effective among natural compounds (*Smith* et al., 2004). Seed dressing with alkaline materials is used in agriculture; the aim of it is to change the pH on the seed surface. Vinegar also changes pH, so the new environment is not suitable for bacteria. Acetic acid is a natural substance with low oral toxicity to humans, birds, and others who have contact with it (*Borgen*, 2003).

Materials and methods

Bacteriological examinations were made by cup plate method. It is a horizontal (radial) diffusion method, in which the zone of the inhibition is dependent upon concentrations (*Gavin*, 1956). First, 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 into the double Nutrient agar plate. Cups were completely filled with the examined materials (*Table 1*).

After 4 hours long diffusion period in the refrigerator plates were placed into a thermostat at 26 C° for 24–48 hours. After this incubation period the inhibition zones around the holes was measured. The size of the inhibition zones showed the inhibition effect. These examinations were made in four replicates.

Data have been analyzed by SPSS 14.0 program and with Tukey b, Duncan or Games-Howell test (SD 5%).

Table 1 Examined materials in bacteriological assays

Examined materials:
vinegar 10%, 5%, 2,5%, 0,5%;
cider vinegar 6%, 5%, 2,5%, 0,5%
red wine vinegar 6%, 5%, 2,5%, 0,5%
white wine vinegar 6%, 5%, 2,5%, 0,5%
thyme oil 100%, 50%, 25%, 1%, 0,5%, 0,1%
cinnamon oil 100%, 50%, 25%, 0,5%, 0,1%
peppermint oil 100%, 50%, 25%
caraway oil 100%, 50%, 25%;
valerian plant extract 1%, 0,5%
sodium hydrogen carbonate ($NaHCO_3$) 1%, 0,5%
saccharose 10%
NaCl 10%
ethanol 15%, 10%, 5%
peppermint tea 5g, 3,5g
thyme tea 5g 3,5g
savory tea 5g
Controls:
Stretomycin sulphate 50 ppm
Kasumin 2L
sodium-hydroxide (NaOH) 1,5%,
water

Examined materials were chosen according to literature (*Issekutz* et al., 1968). All vinegar is biologically fermented. Stretomycin sulphate antibiotics are not allowed in Hungary for plant protection.

Examined bacterial strains originated from NCAIM, Hungary. These were *Pseudomonas sypringae pv. tomato* B.01277, B.0 1682, B.01538; *Xanthomonas campestris pv. vesicatoria* B.01771, B. 01226; *Clavibacter michiganensis subsp. michiganensis* B. 01778, B. 01779.

Table 2 Examined materials in germination ability test

Examined materials:
vinegar 5%, 0,5%;
cider vinegar 5%, 0,5%
red wine vinegar 5%, 0,5%
white wine vinegar 5%, 0,5%
thyme oil 25%
cinnamon oil 25%
Controls:
Stretomycin sulphate 50 ppm
Kasumin 2L
sodium-hydroxide (NaOH) 1,5%,
water

Germination ability was examined with an ISTA standard (MSZ 6354-3: 1991) in the National Institute for Agricultural Quality Control in Hungary. The germination ability test was made with 50 seeds in four replicates. The emergent numbers of seedlings were counted on day 8 and on day 14. Before the test the seeds were soaked in materials for 10 minutes and then they were dried (*Table 2*).

Data have been analyzed by SPSS 14.0 program with Duncan test (SD 5%).

GC-MS chromatography was used to identify active ingredients of examined essential oils. The examination was made by Corvinus University of Budapest, Department of Medical and Aromatic Plants.

Results

Bacteriological assays

In the cup plate method 1.5% NaOH and 50 ppm Streptomycin sulphate were the control.

In our preliminary experiments 10% sucrose and 10% NaCl were not effective. So we didn't use them further. The essential oils examined in 100% concentration had inhibition effect. Among oils the cinnamon was the most effective it had significantly bigger inhibition zone than the 1.5% NaOH control. In all examined materials the 10% of vinegar caused the highest inhibition (*Figure 1*). It was significantly better than the examined other materials, and obviously than the control (1.5% NaOH).

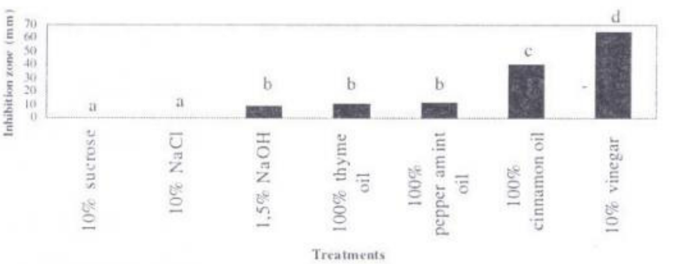

Figure 1 Zones of inhibiton in different treatments against *Pseudomonas syringae* pv. *tomato* B. 01277 strain. (Letters show the significant differences in 95% significance level).

Vinegar and cider vinegar were examined against *Pseudomonas syringae* pv. *tomato* B. 01277, B. 01682 and both of them showed good inhibition effects (*Figure 2*).

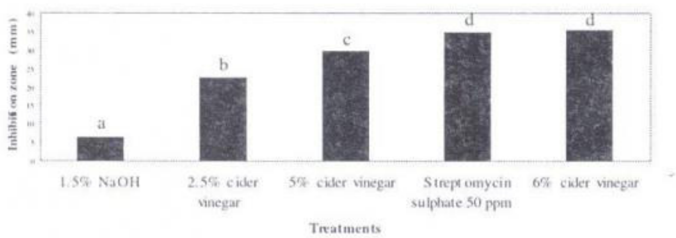

Figure 2 Zones of inhibition with use of different concentrations of cider vinegar in *Pseudomonas syringae* pv. *tomato* B. 01682 strain. (Letters show the significant differences in 95% significance level).

The highest concentration was the most effective, though the lowest 0.5% vinegar and 2.5% cider vinegar had also impact. Comparing with the Streptomycin sulphate 50 ppm control only 6% cider vinegar and 10% vinegar had stronger bactericide effect.

The white wine vinegar was examined only against B. 01277 strain and it showed strong bactericide effect in all concentrations (5%, 2.5%, 0.5%) compared with 1.5% NaOH control. The 5g/2dl savory and thyme tea were as effective as 1.5% NaOH, but the peppermint tea in 5g/2dl concentration did not produce inhibition zone.

Concerning the use of teas the assay showed that 3,5g/2dl teas were not as effective as essential oils. Peppermint tea caused the lowest inhibition zone, thyme tea and 1.5% NaOH caused approximately the same size of inhibition zones, but all treatments were less effective than streptomycin 50 ppm (*Figure 3*).

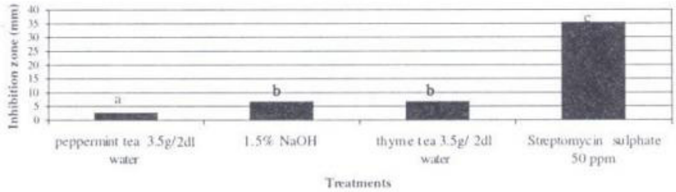

Figure 3 Inhibition zones of peppermint and thyme tea used against *Pseudomonas syringiae* pv. *tomato* B 01682. (Letters show the significant differences in 95% significance level)

In case of *Clavibacter michiganensis* subsp. *michiganensis* all examined strains (B 01778, B 01779) were significantly inhibited by white wine vinegar and red wine vinegar in concentrations 2.5% and 5%. The 0.5% concentration was not effective enough against B 01779 strain comparing with 1.5% NaOH control (*Figure 4*).

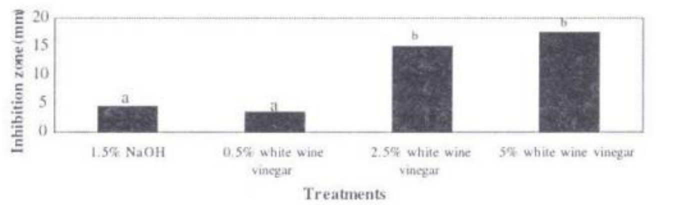

Figure 4 Inhibition zones in case of treatment with different concentrations of white wine vinegar against *Clavibacter michiganensis* subsp. *michiganensis* B 01779 (Letters show the significant differences in 95% significance level).

The effect of vinegar was the same in all strains (B 01778, B 01779). The examined highest concentration (10%) was the most effective and it was better than Streptomycin sulphate 50 ppm; 2.5%, 5%, 10% concentrations were significantly better than the other control 1.5% (NaOH) (*Figure 5*). Cider vinegar had also effect; it inhibited the development of *Clavibacter michiganensis* subsp. *michiganensis* (B 01778, B 01779), in concentrations of 2.5%, 5%, 6% to a higher extent compared to 1.5% NaOH and streptomycin 50 ppm.

Cinnamon oil in concentrations of 0.1% and 0.5% were ineffective, only higher dose (50%, 25%) showed bigger

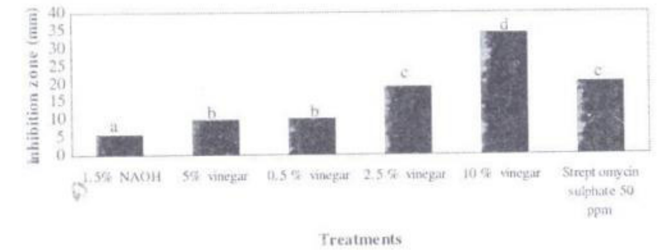

Figure 5 Zones of inhibition in different concentrations of vinegar against *Clavibacter michiganensis* subsp. *michiganensis* B 01778 strain. (Letters show the significant differences in 95% significance level).

inhibition zones compared to 1.5% NaOH, but both of them had lower zone, than Streptomycin sulphate 50 ppm (*Figure 6*). Thyme oil was as effective as cinnamon oil.

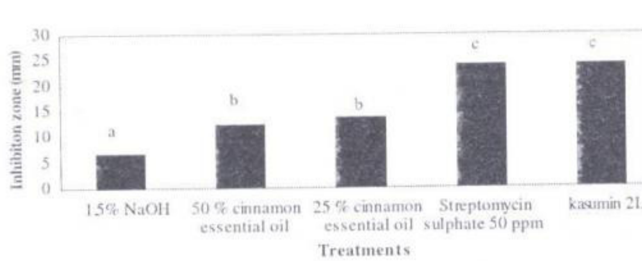

Figure 6 Zones of inhibition in *Clavibacter michiganensis* subsp. *michiganensis* B 01778 with use of cinnamon essential oils 50%, 25%. (Letters show the significant differences in 95% significance level).

Against *Xanthomonas campestris* pv. *vesicatoria* B. 01771 and B. 01226 2.5%, 5%, 10% concentrations were better than the control 1.5% NaOH, but only the 10% concentration had better effect than Streptomyces sulphate 50 ppm. White and red wine vinegars had also significant bactericide effect in case of B. 01771 strain (*Figure 7*) according to 1.5% NaOH in every examined concentrations (0.5%, 2.5%, 5%). Correlation was observed between concentrations and inhibition zones. All types of vinegars showed correlation. Results on cinnamon oil and thyme oil are presented in *Figures 8* and *9*.

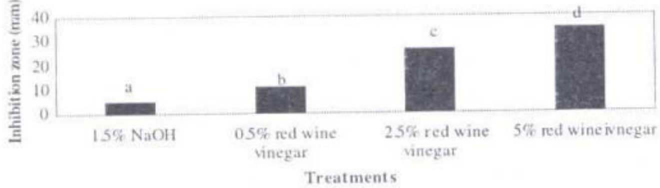

Figure 7 Zones of inhibition in different concentrations of red wine vinegar against *Xanthomonas campestris* pv. *vesicatoria* B. 01771 strain. (Letters show the significant differences in 95% significance level).

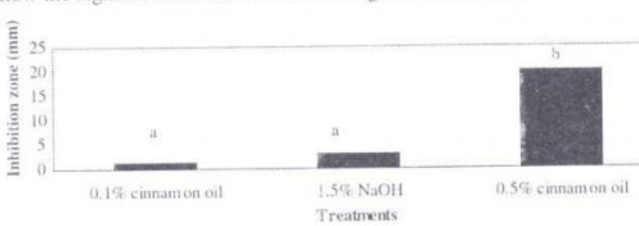

Figure 8 Inhibition zones of 0,1% and 0,5% cinnamon oil against *Xanthomonas campestris pv. vesicatoria* B. 01771 strain. (Letters show the significant differences in 95% significance level).

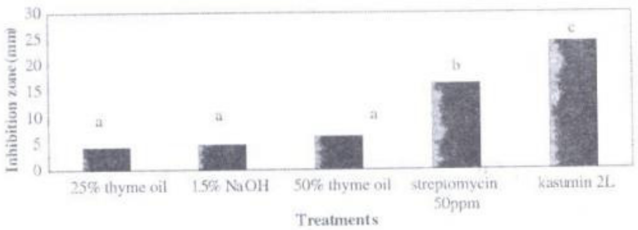

Figure 9 Inhibition zones of treatment with thyme oil against *Xanthomonas campestris* pv. *vesicatoria* B 01226. (Letters show the significant differences in 95% significance level).

The following materials had no inhibition effect against all examined strains:

Peppermint essential oil in 25% and 50% concentration, ethanol (10%, 15%), NaHCO3 and valerian extract had not antimicrobial effect. Peppermint, thyme and savory tea (3.5g, 5g) were effective only against *Pseudomonas syringiae pv. tomato* test strains.

The starter pH values of the examined materials are shown in *Table 3*.

Table 3 Starter pH of examined materials was measured with wtw pH 340 i type digital pH measurement tool

Examined materials	Measured pH
Vinegar 10%	2.4
Vinegar 5%	2.7
Vinegar 2.5%	2.9
Vinegar 0.5%	3.2
Cider vinegar 6%	3.2
Red wine vinegar 6%	2.9
White wine vinegar 6%	2.9
Savory tea 3.5g/ 2dl	6.8
Water	7.2
NaOH 1.5%	13
$NaHCO_3$ 5%	9.23

Germination ability tests on tomato seeds (on the 14th day)

According to germination ability test the examined vinegars did not show negative effect on seed germination ability except the vinegar in 5% concentration. On the other hand, they have not had significantly positive effect on

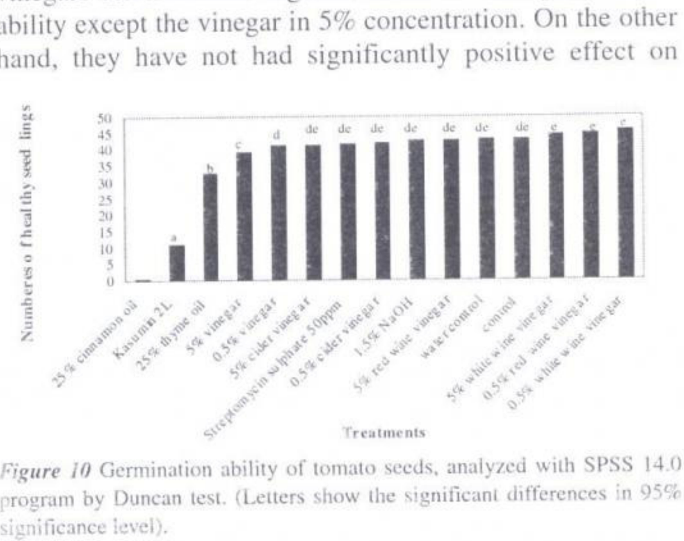

Figure 10 Germination ability of tomato seeds, analyzed with SPSS 14.0 program by Duncan test. (Letters show the significant differences in 95% significance level).

Table 4 Proportion of components (%) in cinnamon essential oil

p-cimen	1,8-cineol	linalool	**cinnamic aldehid**	eugenol	metil-cinnamat	beta-kariofillen	cinnamil-acetat
2.3	2.14	2.42	**86.72**	1.11	1.41	1.4	2.5

Table 5 Proportion of components (%) in thyme essential oil

alfa-pinen	camfen	beta-mircén	alfa-terpinen	p-cimen	limonen	1,8-cineol	gamma-terpinen	terpinolen
0.49	1.09	0.9	1.18	25.53	0.41	3.51	5.14	0.17

linalool	borneol	terpinen-4-ol	alfa-terpineol	**thymol**	carvacrol	beta-kariofillen	alfa-humulen
4.08	0.38	0.58	0.32	**50.21**	3.15	1.6	0.16

germination ability. Low concentration (0.5%) of red and white wine vinegar mend the germination capacity, but this effect is not significant. Essential oils and Kasumin 2L debased germination capacity, according to the control (*Figure 10*).

Our experience was the same as that of Borgen (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 and 5% of white wine vinegar have simulative effect on germination ability.

The proportions of components in cinnamon and thyme essential oils are presented in *Tables 4* and *5*.

Discussion

Results showed that examined bacterial strains were more sensitive to acidic than alkaline circumstances. The lowest examined concentration (0.5%) of vinegars had also bactericide impact. In alkaline materials it is necessary to use higher concentration of at least pH 13 (1.5% NaOH), but it had not impact in all case. The highest soluble concentration of $NaHCO_3$ was 10% (pH 9). We can conclude that all examined vinegar types could be useful in biological plant protection systems against bacterial diseases of tomato and pepper.

In low concentrations (0.1%, 0.5%, 1%) the examined volatile oils had no inhibition effect except in case of *Xanthomonas campestris* pv. *vesicatoria* B. 01771 with use of cinnamon oil. Peppermint essential oil was not effective except in 100% concentration against *Pseudomonas syringae* pv. *tomato* B 01277 strain. The caraway oil 100% concentration made damage in plastic Petri dishes, but it had not impact in 10% and 25% concentrations. 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 cinnamon was effective because of cinnamon aldehide content, our GC-MS examinations (*Table 4*) showed the same result. Thyme oil was effective because of thymol content (*Table 5*); the menthol content of peppermint oil was not as high so maybe this is the explanation of low effectivity. Teas had effect only against *Pseudomonas syringae* pv. *tomato*, but this impact was not stabile enough so in the future optimization will be the exercise.

Vinegar types seem to be environmental friendly, cheap, and perspective dressing materials in ecological seed treatment.

We plan to test further materials and we will carry out *in vivo* experiments with the most effective ones on field conditions, too. Different combinations of materials and treatments could give better effects against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*), bacterial speck (*Pseudomonas syringae* pv. *tomato*) of tomato and bacterial spot of pepper (*Xanthomonas campestris* pv. *vesicatoria*). Focused on economical aspects and germination ability view we will try to use the lowest effective concentrations.

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