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Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extracts

J.M. VAN DER WOLF, Y. BIRNBAUM, P.S. VAN DER ZOUWEN AND S.P.C. GROOT

Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands
(E-mail: Jan.vanderWolf@wur.nl)

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Summary

Various essential oils, organic acids, Biosept, (grapefruit extract), Tillecur and extracts of stinging nettle and golden rod were tested for their antimicrobial properties in order to disinfect vegetable seed. In *in vitro* assays, thyme oil, oregano oil, cinnamon oil, clove oil and Biosept had the highest activity against the seed borne pathogens *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *Alternaria dauci* and *Botrytis aclada*. Low antimicrobial activity was found for the organic acids against the fungal pathogens.

Seed treatment for 0.5 h with selected essential oils or Biosept in concentrations between 0.1 and 1% eliminated > 99% of total bacteria associated with cabbage seed. It also reduced the percentage of seeds contaminated with fungi in blotter tests from 70% to less than 10%. Extended periods of treatment did not significantly improve results. Cinnamon oil and Biosept used in concentrations exceeding 1% had a negative effect on seed germination. Antimicrobial effects with organic acids were variable, but in general, the organic acids ascorbic acids, propionic acid, acetic acid and lactic acid at concentrations of 2.5% or higher, reduced seed-associated bacteria. Of the organic acids, only propionic acid reduced seed germination at a concentration higher than 1%. Thyme oil was considered the most promising natural compound for reducing seed borne pathogens in seed.

Introduction

Seed-borne diseases can seriously affect crop yield and quality. The most effective means of control is by exclusion and reduction of the inoculum during seed production. Unfortunately, infection and contamination cannot always be avoided and thus various seed treatments are used to reduce the amount of seedborne inoculum. Seed treatments are strongly favoured over field sprays, as relatively low amounts of the active compounds are required and applications may be done in contained areas which reduce health risks and avoid drift of crop protection sprays.

In organic agriculture, the options for reducing seedborne pathogens by seed treatment are limited, as no synthetic crop protection agent may be used. Currently, mainly physical treatments, such as hot water, electron beam treatments and hot (humid) air treatments are commercially used for seed disinfection. Physical treatments may reduce the inoculum load but are often not able to fully eliminate seedborne inoculum without affecting seed vitality (Forsberg *et al.*, 2002). The use of hot water treatment for a bulk product such as cereal is expensive and introduces the additional risk of reduced seed vigour. As heat treatment can be detrimental to seed vitality, seed lots should be tested for their tolerance to elevated temperatures (Forsberg *et al.*, 2003).

Natural antimicrobial compounds can be used for seed disinfection as an alternative to, or in combination with physical treatments. Several groups of natural compounds have been already described for seed disinfection, including organic acids, essential oils, crude plant extracts and dairy products. For control of common bunt in wheat, mustard flour, skimmed milk powder, acetic acid and lactic acid were effective, reducing infection levels by 64% to 96% (Borgen, 2004; Borgen and Kristensen, 2001; El-Naimi *et al.*, 2000; Saidi *et al.*, 2001). Singh *et al.* (1979) found plant extracts of *Cannabis sativa*, *Eucalyptus globules*, *Thuja sinensis* and *Datura stramonium* effective, but these results could not be reproduced by others (Borgen, 2004). The oils of cassia and clove reduced seedborne inoculum of *Aspergillus flavus, Curvularia pallescens* and *Chaetomium indicum* in maize (Chatterjee, 1990). Aqueous extracts of *Strychnos nux-vomica*, garlic bulb, ginger rhizome, basil leaf and *Azadirachta indica* fruit were used to control *Alternaria padwickii* in rice seed (Shetty *et al.*, 1989). *Filix mas* and *Blatta orientalis* extracts suppressed the population of *Fusarium oxysporum* in the seed mycoflora of wheat (Rake *et al.,* 1989). Soybean oil, applied at a rate similar to that used to suppress grain dust, reduced storage fungi growth in maize and soybean during 12 months in field storage bins in Iowa (McGee *et al.*, 1989; White and Toman, 1994). Thyme oil significantly inhibited the growth of storage fungi *Aspergillus flavus, Penicillium chrysogenum, Fusarium oxysporum* and *Fusarium equiseti* isolated from naturally infected cowpea in South Africa (Kritzinger *et al.*, 2002).

In this study we examined the potential of a number of natural compounds for seed disinfection. The activity of natural compounds was first determined via *in vitro* assays on two important seedborne bacteria, *Clavibacter michiganensis* subsp. *michiganensis,* the causal agent of bacterial canker in tomato and *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot in *Brassica* species. Additionally, the *in vitro* activity of the natural compounds was determined against two seed-borne fungi, *Alternaria dauci*, causal agent of Alternaria blight in carrot and *Botrytis aclada*, causal agent of neck rot in onion. Secondly, the effect of seed treatments on the total populations of bacteria and fungi in the spermasphere of cabbage and tomato seeds was determined, as was the effect on seed germination.

Our studies focused on the use of essential oils and organic acids. From studies on medical and plant pathogens, it is known that various essential oils contain terpenes with strong antimicrobial properties (Hammer *et al.*, 1999; Scortichini and Rossi, 1991). In many countries within the European Union, essential oils that are used as flavours and fragrances are allowed as crop protection agents for dipping and drenching of plant material. In the European Union, essential oils are described in Annex IIB of EU Regulation 2092/91, which list crop protection agents accepted in organic agriculture (Anonymous, 1991). Therefore essential oils are of particular interest in organic agriculture if the potential for seed disinfection can be proven.

Various organic acids exhibit antimicrobial activity and are used as preservatives in the food industry and for the disinfection of surfaces (Bloukas *et al.*, 1997). Some of them, such as lactic acid and acetic acid, have been used for seed disinfection. Organic acids, such as acetic acid and propionic acid, are currently used in organic agriculture as additives in cattle feed and it is therefore likely that this sector will accept them as crop protection agents. Although the eco-toxicological risks are known and considered to be low, organic acids are not yet registered as crop protection agents.

Materials and methods

Bacterial and fungal strains and growth conditions

Xanthomonas campestris pv. *campestris* strain PRI nr. 3811 and *Clavibacter michiganensis* subsp. *michiganensis* strain PRI nr. 542 were grown on Trypticase Soy Agar (BBL) for 24 h at 25°C prior to use. *Alternaria dauci* PRI nr. 337 and *Botrytis aclada* PRI nr. 390 were maintained on PDA at 4°C and grown on PDA for one week at 20°C prior to use.

Test compounds

The following essential oils were tested: basil (Chi, Breda, NL), cinnamon (Roth, Karlsruhe, DE), clove (Chi 2953), manuka, (Tairawhiti Pharmaceuticals, Te araroa, NZ), oregano (Ropapharm, Zaandam, NL), peppermint (Chi 3442) and thyme (Chi 4223). The following organic acids were tested: vinegar (EKO, Albert Heijn, NL), containing ca. 6% acetic acid, L^+ ascorbic acid (Merck, Haarlem, NL, art. 127), citric acid (Agros, Seattle, USA), lactic acid (90%, Merck, art. nr. 366), propionic acid (Sigma, Zwijndrecht, NL, P1386) and Molkosan (A. Vogel, Bioforce AG, SU), containing 9% lactic acid.

The following plant extracts were tested: Biosept, Tillecur and extracts of stinging nettle and golden rod. Biosept (Cintamani, Piaseczo, PL) is an extract of grapefruit containing essential oils and organic acids. Tillecur (Dr Schaette AG, Bad Waldsee, Germany) is a mixture of dry local plant extracts supplemented with coating products such as starch, which are allowed in organic agriculture. Extracts of stinging nettle and golden rod were prepared as follows. Twenty grams of ground dry leaf material was extracted with 80% (v/v) ethanol, by adding 10 times the volume of leaf material. The mixture was vigorously shaken and centrifuged for 5 min at 5000 g. The supernatant was concentrated for 1.5 h in a rotary evaporator (Rota Vapor, Buchi, Zürich, Germany). The concentrate was diluted with 20% (v/v) ethanol in water to the starting volume and centrifuged for 5 min at 10,000 g to remove the debris.

All dilutions were made in sterile demineralized water. For seed treatments, essential oils were emulsified in water by sonication (Vibra Cell™, Danbury, USA) for 5 sec at maximum capacity (50 W), prior to use.

Microdilution assay for bacteria

The microdilution assay was performed as described by Mann and Markham (1998) with few modifications. Bacterial inoculum densities were adjusted to 10^8 cfu ml $^{-1}$ in soft agar. Soft agar contained Trypticase Soy Broth (Oxoid) with Bacto agar (Difco) added to a final concentration of 0.15% (w/v). To each well of a sterile 96-well microtiter plate the following compounds were added: 150 µl of bacterial suspension in soft agar, 10 µl of a 0.01% (w/v) resazurin (Sigma) solution and 20 µl of essential oil diluted in soft agar. Negative controls did not contain bacterial cells, resazurin or essential oil. All wells were filled with demineralized water to adjust the final volume to 200 µl. Samples were tested in four replicates. The plates were incubated in the dark for 48 hours at 27°C.

Due to bacterial growth the blue compound resazurin is irreversibly reduced to pink resorufin (Frandsen, 1958). Results were recorded by visual observation. In each experiment, some of the blue coloured wells in which resazurin was not converted due to the antimicrobial activity of the test compound were checked for bacterial growth by plating on Trypticase Soy Agar (TSA) (BBL). No bacterial growth was observed in any of the blue coloured wells. The minimum inhibitory capacity (MIC) value was defined as the lowest concentration of the test compound which resulted in complete inhibition of bacterial growth.

Radial growth assay for fungi

Compounds were tested for fungicidal activity using a mycelium radial growth assay. Natural compounds were diluted in 1.5 ml of soft potato dextrose agar (PDA) (4% malt extract (Difco, Sparks, USA), 0.15% PDA (Difco, Sparks, USA)) and mixed with 13.5 ml of molten PDA at a temperature of 50°C, prior to pouring. One ml of each dilution was poured into a well in a sterile 24-well plate (Greiner). One punch of fungal mycelium (diameter 3 mm) was taken from a full-grown PDA plate and inverted into the centre of a well in the tissue culture plate, with the mycelium in contact with the PDA-containing test compounds. All dilutions were tested in three replicates. Plates were incubated for 1 week at 20°C. The MIC value was defined as the lowest concentration of the test compound which resulted in complete inhibition of fungal growth.

Seed treatment

The effect of seed treatment with different natural compounds on bacteria and fungi present on cabbage seeds (*Brassica oleracea*) was assessed. Two subsamples of 1000 seeds were soaked in 7 ml of the compound of various concentrations and shaken using an electric shaker for 0.5 h at room temperature. After treatment, the seeds were placed in a sieve and excess compound was removed with running tap water. The seeds were placed in Petri dishes to dry overnight at 20°C and 32% RH.

Detection of seed associated bacteria

Four seed extracts of one thousand Brassica seeds each were prepared by incubating seeds in an equal weight of 0.85% NaCl with 0.2% Tween 20 for 0.5 h while shaking. Extracts were pour-plated in TSA supplemented with 100 µg ml^{-1} cycloheximide according to the following procedure. TSA was melted and cooled to 45°C. Filter-sterilized cycloheximide in a stock solution of 100 mg ml^{-1} was added to a final concentration of 1 mg ml^{-1}. From undiluted and 10x diluted seed extracts, 100 µl was added to a well in a sterile 24-well tissue culture plate. Thereafter, 300 µl of the TSA medium was added while shaking. Plates with the solidified medium were incubated for 48 h at 25°C and the number of cfu per well was counted using a dissecting microscope.

Detection of seed associated fungi
Two sets of one hundred cabbage seeds were placed in 14×20 cm plastic boxes in which two filter papers were placed, wetted with 60 ml of tap water. The boxes were closed with a transparent lid and incubated for 1 d at 20°C, 1 d at -20°C (both in the dark) and 5 days at 20° under NUV-light. Thereafter, the percentage of seeds covered with fungi and bacteria was determined.

Percentage of normal seedlings
The percentage of normal seedlings was determined by incubating 2 or 4 replicates of 50 seeds on a Copenhagen table at 20°C (8 h light and16 h dark). Seeds were placed on round filter papers (diameter 10 cm) wetted with tap water. Filters were covered with a bell jar. Seedlings were examined according to the Rules from the International Seed Testing Association (ISTA).

Statistics
Results were analyzed using the programme GenStat, (Rothamsted Experimental Station, UK, release PL16). To analyse the effect on bacterial densities, pair wise differences were calculated after $\log_{10}$ transformation using the Student's T test. To assess germination effects, pairwise differences were calculated on the number of normal seedlings.

Results

In vitro assays
Among the seven essential oils originally tested, thyme and oregano were the most active. These oils had relatively low MIC-values (< 0.03%) for bacterial and fungal pathogens (table 1). Biosept was active against fungi and, in particular, against the tested bacteria with relative low MIC-values of 0.07-0.15%. Tillecur, golden rod and stinging nettle extract showed no activity. Propionic acid was the most active organic acid when tested against fungi. Acetic acid and lactic acid showed a slight activity against *Alternaria dauci* only. Ascorbic acid and citric acid showed no *in vitro* activity, even at the highest concentration tested of 10%. Xcc and Cmm were comparable in their sensitivity to the essential oils and plant extracts. Manuka and thyme oil were more active against *Alternaria dauci* than against *Botrytis aclada*, but the opposite was found for peppermint oil.

Effect of seed treatments on total microbial populations of seeds
Thyme oil applied in different concentrations for 0.5 or 4 h reduced the total bacterial population from 10^4 cells per ml for the untreated seeds and 10^5 cells per ml for the water-treated seeds to less than 10^3 cells per ml, irrespective of the concentration or the treatment period (figure 1).

The effect of different concentrations of thyme, oregano, cinnamon and clove oil and the two organic acids, propionic acid and ascorbic acid, were compared (table 2). All treatments except Biosept at a concentration of 0.33% and Oregano oil at 0.1 and 0.33% reduced the bacterial counts significantly compared to the untreated seeds and the water

Table 1. Minimum inhibitory concentrations of natural compounds (MIC-values in percentage) for control of *Xanthomonas campestris* pv. *campestris* (Xcc), *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Alternaria dauci* and *Botrytis aclada.*

Compound	Xcc	Cmm	*A. dauci*	*B. aclada*
Basil oil	5[a]	2.5	5	5
Cinnamon oil	0.6	1.25	0.3	0.3
Clove oil	2.5	2.5	0.3	0.6
Manuka oil	>10	>10	1.25	>10
Oregano oil	0.15	0.15	0.3	0.3
Peppermint oil	1.25	2.5	2.5	0.3
Thyme oil	0.3	0.3	0.07	0.3
Biosept	0.15	0.07	0.6	0.6
Tillecur	>10	>10	>10	>10
Golden Rod extract	>10	>10	>10	>10
Stinging Nettle extract	>10	>10	>10	>10
Acetic acid	ND[b]	ND	5	>10
Ascorbic acid	ND	ND	>10	>10
Citric acid	ND	ND	>10	>10
Lactic acid	ND	ND	5	>10
Propionic acid	ND	ND	0.3	1.25

[a] MIC values were determined in an *in vitro* assay and indicate the minimium percentage compound at which no bacterial growth was found according to an indicator for bacterial growth (resazurin)
[b] ND = not determined

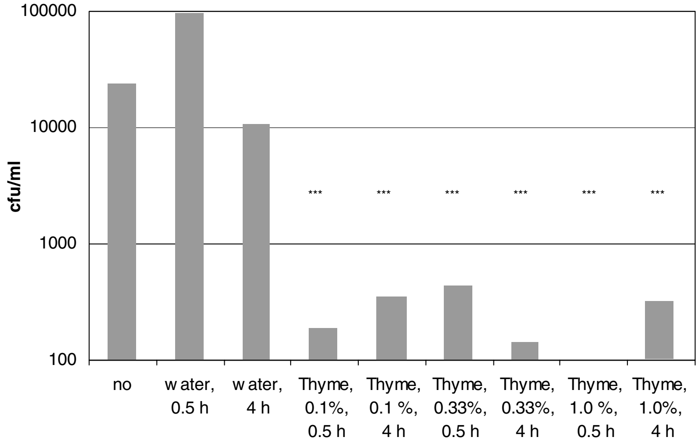

Figure 1. Effect of treatment with thyme oil concentrations for 0.5 h or 4 h on seed-associated bacteria present on cabbage seed. The effect of treatments indicated with an asterisk are highly significant compared to the untreated control (P< 0.001%)

Table 2. Effect of treatment with essential oils, Biosept and organic acids on seed-associated bacteria present on cabbage seed.

Treatment	Average log cfu/ml ± standard deviation[a]				
% Compound	0	0.1	0.33	1	3.3
No	6.1±0.1				
Water	5.4±0				
Thyme		4.0±0.2	3.7±0.0	3.4±0.6	2.9±0
Oregano		4.9±0.1	4.9±1.0	3.8±0.3	0
Cinnamon		3.2±0.4	4.2±0.3	3.5±0.1	3.5±0.5
Clove		4.1±0.1	4.3±0.2	2.7±0.0	4.0±0.2
Biosept		4.3±0.2	5.0±0.6	0	0
Ascorbic acid		4.2±0.3	3.9±0.5	3.2±0.4	0
Propionic acid		3.4±0.0	3.9±0.5	0	0

[a] All treatments except treatments with Oregano oil in a concentration of 0.1 and 0.33% and Biosept in a 0.33% concentration were highly significantly different from the untreated and water control (P< 0.05%)

control (P< 0.05%). A dose-response effect was found for thyme oil and ascorbic acid,. Oregano oil, propionic acid and ascorbic acid at a concentration of 3.3% and Biosept at a concentration of 1 and 3.3% eliminated completely the populations of culturable seed-associated bacteria.

The effect of different concentrations of acetic acid (vinegar), ascorbic acid, lactic acid, the organic food product Molkosan, containing 9% lactic acid and thyme oil was studied on seed-associated bacteria. Treatments conducted with thyme oil (0.33%), acetic acid (2.5%), Molkosan (5%), lactic or ascrobic acid (5%) reduced significantly bacterial counts on seed by more than 99% (figure 2). A concentration of 0.5% had no significant effect on bacterial counts, for either of the organic acids.

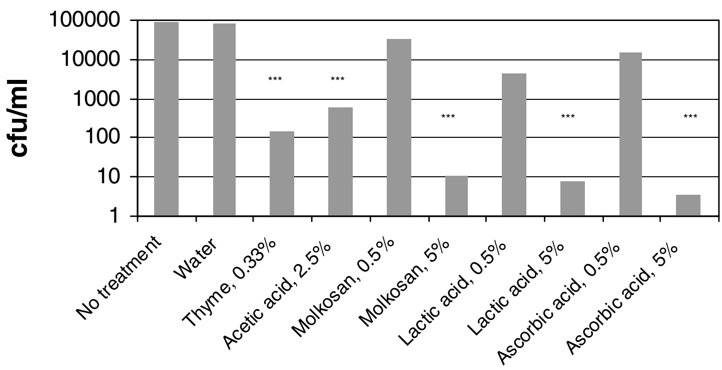

Figure 2. Effect of treatment with thyme oil and organic acids on seed-associated bacteria present on cabbage seed. The effect of treatments indicated with an asterisk are highly significant compared to the untreated control (P< 0.001%)

The effect of the organic acids acetic acid, ascorbic acid, lactic acid and Molkosan on seed-associated bacteria was studied in four concentrations. Disinfection with lactic acid at 0.5% resulted in a reduction of the bacterial count by more than 99% (figure 3). Treatment with lactic acid of up to 10% provided no further reduction. The other organic acids reduced the bacterial count by more than 99% at concentrations of 2.5% and higher, but not at 0.5%.

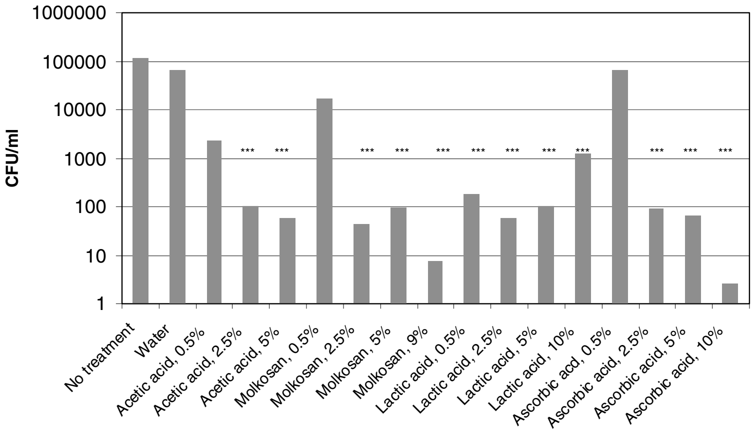

Figure 3. Effect of treatment with different concentrations of organic acids on seed-associated bacteria present on cabbage seed. The effect of treatments indicated with asterisks are highly significant compared to the untreated control (P< 0.001%).

To reduce the fungal inoculum on seed, thyme oil, ascorbic acid, soap, Biosept and the use of thyme oil were studied. Also combinations of different treatments were tested: thyme oil followed by ascorbic acid and Biosept followed by thyme oil.

All treatments, except for a single treatment of ascorbic acid, reduced significantly the percentage of fungal contaminants on seeds from ca. 70% to less than 10% infected seeds (figure 4). A single treatment with 2.5% ascorbic acid was less effective and reduced the frequency of fungal infected seeds from 70% to only ca. 30%. Combinations were slightly more efficient than single treatments, although the effects were not significant. Most fungi were identified as *Penicillium*; although small numbers of *Alternaria alternata* and *Phoma lingam* were found.

Seed germination

The effect of thyme, oregano, cinnamon and clove oil and of Biosept, ascorbic and propionic acid on seed germination was studied with a low vigour seed lot (P4037). Untreated seed from the low vigour seed lot produced about 27% normal seedlings (table 3). Seeds were damaged by cinnamon oil and propionic acid at concentrations of 1 and 3.3% and by Biosept at a concentration of 3.3%, resulting in a significant reduction in the percentage of normal seedlings. For the other treatments no significant effect was found on seedling quality.

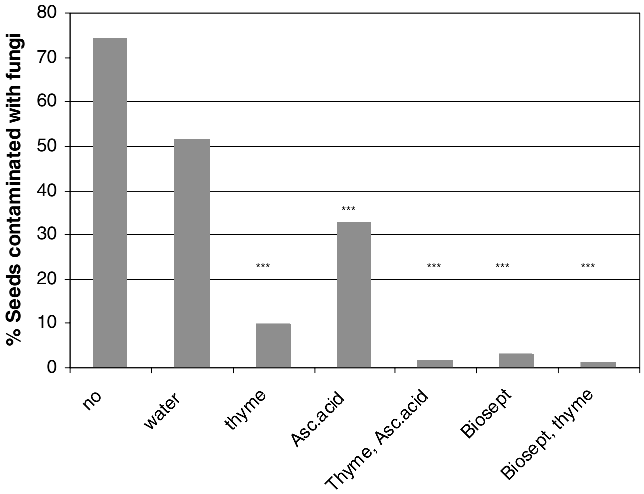

Figure 4. Effect of combinations of treatments on seed-associated fungi, expressed as the percentage of seeds contaminated with fungi. Thyme oil: 0.33%, Ascorbic acid: 2.5%, Biosept, 1%. The effect of treatments indicated with an asterisks are highly significant compared to the untreated control (P< 0.001%).

Table 3. Effect of treatment with essential oils, Biosept and organic acids on germination expressed as the percentage normal seedlings.

Treatment	Nr. of normal seedlings + standard deviation[a]				
% Compound	0	0.1	0.33	1	3.3
No	27±7.1				
Water	33±1.4				
Thyme		33±4.2	29±4.2	27±1.4	27±9.9
Oregano		23±1.4	30±11.3	26±2.8	20±5.7
Cinnamon		33±1.4	21±4.2	3±4.2[b]	0[b]
Clove		35±4.2	21±4.2	26±0	20±5.7
Biosept		37±7.1	21±7.1	29±7.1	11±1.4[a]
Ascorbic acid		29±1.4	26±0	31±1.4	24±0
Propionic acid		31±9.9	40±8.5	17±1.4[b]	0[b]

[a] Two times fifty seeds were tested
[b] Treatments significantly different from the untreated and the water control (P< 0.1%)

The effect on seed germination of Biosept, thyme oil and ascorbic acid, used in different concentrations, was further evaluated with seed lot P4037 and with a more vigorous seed lot (P4097) giving 84% of normal seedlings. For both seed lots, only the treatment with Biosept at a concentration of 3.3% resulted in a significantly lower percentage of normal seedlings (data not shown).

Discussion

The various essential oils and organic acids that were tested in this study possessed antimicrobial properties and were consistently able to drastically reduce seed-associated micro-organisms. The *in vitro* assays showed that several essential oils and in particular thyme, oregano and cinnamon oil were highly active against seed-borne bacteria and fungi. A high antimicrobial activity was also found for a citrus extract, Biosept, which contains both essential oils and organic acids. The organic acids tested were relatively inactive, possibly because the pH was buffered in the in vitro assay by the growth medium (results not shown). It is known that at least part of the activity of the organic acids is due to the low pH.

The broad-spectrum antimicrobial activity of essential oils has been demonstrated in other studies on plant pathogenic bacteria and fungi and on micro-organisms that play a role in the food industry or in the medical and veterinary field (Hammer *et al.*, 1999; Harpaz *et al.*, 2003; Kritzinger *et al.*, 2002, Pradhanang *et al.*, 2003; Scortichini and Rossi, 1991). The essential oils were also able to reduce seed-borne bacteria (Nguefack *et al.*, 2005). In this study, thyme and basil oil applied at a concentration of 4% reduced the transmission of *Acidovorax avenae* subsp. *avenae* in rice from seed to seedling and had a positive effect on the seed germination of infected seeds.

Relatively high concentrations of at least 0.1% were required to obtain a bactericidal effect. In contrast, many antibiotics are active in concentrations between 0.001 – 0.01% (Burr and Norelli, 1990). The relatively high MIC-values observed in this study may be partly due to the poor solubility of the oils in water, as it has been demonstrated that the microbial activity of essential oils is related to water solubility, as well as the hydrogen bonding capacity (Griffin *et al.*, 1999). To enhance the contact between oil and micro-organism in a water-based system, in this study a sonication procedure was developed to obtain a stable emulsion. The activity may possibly be enhanced by the addition of chelating divalent cations which stabilizes the anionic lipopolysaccharide layer in the outer membrane of Gram-negative bacteria (Skandamis *et al.*, 2001).

The risks for development of resistance against these multiple target compounds are considered to be low (Ohno *et al.*, 2003). Terpenes from essential oils interfere with different metabolic functions of micro-organisms, including respiration (Walsh *et al.*, 2003). Terpenes also attack the cytoplasmic membrane of Gram-negative bacteria, thereby destroying its integrity and causing a malfunctioning of electron and nutrient uptake and of ATPase activity (Cox *et al.*, 1998; Tassou *et al.*, 2000). Even the multiple resistant *Staphylococcus aureus* bacterium that causes clinical problems is sensitive to relatively low concentrations of tea tree oil (*Melaleuca alternifolia*) (Raman *et al.*, 1995).

Results of the seed treatments showed that the essential oils also effectively eliminated bacteria and fungi associated with seeds. Oregano oil had a relatively low activity, but thyme, cinnamon and clove oil reduced the CFU of seed-associated bacteria by more than 99% at the relatively low concentration of 0.1%. Under the test conditions used, significant seed damage was found only for cinnamon oil and only when used at a concentration of 3.3%. However, it was found that increased periods of seed treatment with thyme oil for 1 h or more could result in seed damage (results not shown). The phytotoxicity of some essential oils was already known from studies on carvone (*Carum carvi)*, which was used to inhibit ware potatoes from sprouting (Beveridge *et al.*, 1981). Studies on peppermint oil revealed that a relatively low concentration of 0.05% inhibited the respiration of cucumber plants by 50% (Mucciarelli *et al.*, 2001). In contrast, application of essential oils may also stimulate germination, as has been found for rice seed treated with oils extracted from leaves of *Callicarpa japonica* (Leth, 2002). Optimization of the concentration is therefore essential.

The organic acids acetic acid, propionic acid, ascorbic acid and lactic acid showed a high antibacterial effect in the seed treatments, although in most experiments relatively high concentrations (≥ 2%) were required. Only propionic acid was active in the *in vitro* assays, but was also phytotoxic at high concentrations of 3.3%. The antifungal properties of ascorbic acid, even when used at a high concentration of 2.5%, were less than that of the essential oils. Organic acids are used as preservatives and disinfectants in the food industry (Ricke, 2003). Some were also found to be effective for the control of seed-borne pathogens. Acetic acid was successfully applied for the control of common bunt (*Tilletia tritici*) in wheat (Borgen and Kristenson, 2001) and *Alternaria dauci* in carrot (Lizot *et al.*, 2002). The antimicrobial mechanism is not fully understood and the activity is dependent on the physiological status of the pathogen and the physicochemical properties of the environment (Ricke, 2003). In previous studies, synergistic effects were found between acetic acid with sodium chloride and various essential oil components (Kurita and Koike, 1982). The use of different active compounds, however, will complicate the regulatory permission for registration as a crop protection agent.

The activity of the essential oils and organic acids was not selective and seed treatment resulted in the elimination of pathogens as well as other seed-associated micro-organisms, resulting in a relatively sterile seed surface. The effect of seed disinfection on the susceptibility to infection by soil pathogens after sowing needs to be investigated further.

Tillecur, water extracts of stinging nettle (*Urtica dioica*) and golden rod (*Solidago speciosa*) had no effect in our studies on seed borne pathogens, although other studies indicated that these crude extracts might have antimicrobial properties. Tillecur contains mustard flour, which has antifungal properties and has been claimed to be effective against common bunt in wheat. It increased germination percentage up to 30%, although the results have never been published in a refereed journal (Borgen, 2004).

Because thyme oil had a relatively low phytotoxicity, was highly active against plant pathogens in the *in vitro* assays and also effective as a seed disinfectant, it is an interesting compound for further evaluation. In several countries within the EU, thyme oil is allowed as a crop protection agent for the drenching of seeds, as the eco-toxicological risks are considered low. Thyme oil is also acceptable as a crop protection agent in organic agriculture as described in the limited list of organic EU regulation (2092/91) at annex IIB (Anonymous, 1991).

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