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NON-CHEMICAL SEED TREATMENT METHODS FOR THE CONTROL OF *SEPTORIA PETROSELINI* ON PARSLEY SEEDS

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ABSTRACT

The aim of the present study was to identify seed treatment methods for eradicating S*eptoria petroselini* from parsley`s seeds in organic production. Septoria leaf blight caused by the fungus S*eptoria petroselini* is one of the major and important diseases in parsley occurring in many countries. The use of high quality seeds is one of the conditions for an efficient crop production. As the fungus is a seedborne, use of clean and certified seeds is important for disease control especially for use in organic farming. Resistance inducers, commercially formulated and non-formulated selected bacterial strains were applied as seed treatments on naturally infested seeds and were tested under controlled and field conditions. Most of these treatments had positive effect on seed germination. Among the seven resistance inducers tested in greenhouse experiments, Jasmonic acid had the best result and increased seed germination by 25.6%. All the three commercial products and the experimental strain *Bacillus subtilis* K3 increased the number of plants, the yield and decreased the disease incident significantly in field experiments. The experimental bacteria reduced *Septoria* infection by 70% and increased the yield by 24%. Results indicate that several options for non-chemical control of this pathogen exist and can be recommended for better quality and quantity of the parsley crop production.

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INTRODUCTION

Parsley [*Petroselium crispum* (Mill.)] is a biennial herb that is attacked by Septoria leaf spot, also called *Septoria* blight caused by the fungus *S. petroselini*, a common, important disease, of parsley worldwide. The pathogen can survive on crop debris for at least 3 years and on overwintered plants and volunteers, in addition to seed-borne inoculum. Under favorable environmental conditions, warm and wet weather, the disease can spread rapidly and cause serious loss, affecting yield and quality. Yield losses of up to 70% have been reported (Kurt and Tok, 2006). Disease development is dependent on presence of water for pycnidia to release spores and infection to occur. Temperature around 20-25°C is optimum for infection development (Green and Roberts, 2010).

The host range for each *Septoria* species is limited. *Septoria. petroselini* exclusively infects *P. crispum*, whereas wild growing herbs do not show leaf-symptoms, indicating the specialization on its host plant (Hagner-Holler, 2002).

Control of leaf blight is important since leaves are the marketable portions of the plants. The critical feature of disease epidemics worldwide is transmission of *S. petroselini* by infected seed; use of clean seed is important for disease avoidance. This disease is

generally controlled by using uninfected or treated seed, and by using fungicide sprays on foliage (Smith et al., 1988). A number of fungicides have been reported to control *S. petroselini* specially when used before disease appearance (Green and Roberts, 2010).

For organic vegetable production, seed-borne pathogens pose a particular hurdle, since the use of synthetic chemicals during the seed propagation phase or for seed sanitation is prohibited, therefore alternative methods for seed sanitization are needed. A total of 101 Microbial Biological Control Agents (MBCAs) have been registered in 2017 in Australia, Brazil, Canada, Europe, Japan, New Zealand, and the United States for disease control (van Lenteren *et al.*, 2018).

Plant Resistance Inducers (chemical compounds and microbial or plant extracts) as alternative to chemicals have been applied to foliage to control foliar pathogens where seed had been treated mostly against soilborne pathogens (Alexandersson *et al.*, 2016; Rocha *et al.*, 2019).

This study was undertaken to evaluate, and compare, the efficacy of agents of natural origin as seed treatments and also to compare their efficacy with the chemical fungicide Thiram which is used as standard to control many seedborne pathogens in vegetables for control of *S. petroselini* on parsley seed.

MATERIAL AND METHODS

Parsley seed (cv. Gigante d'Italia/Hilmar, seed lot (U446a) naturally infested by the fungus *S. petroselini* was used in filter paper, pot tests in the greenhouse and field trials. The pathogen was identified based on conidial morphology under a binocular microscope and by light microscopy. The fungi *Alternaria* spp. and *Fusarium* spp. were also isolated.

The control agents were assigned to 1 of 3 groups, the first being resistance inducers/plant-derived products: Bion 50 WG, 1 mg·L^{-1}; Chitoplant, 0.5% (w/v); salicylic acid 10 mg L^{-1}; jasmonic acid, 1 mg L^{-1}; Comcat 0,5 mg L^{-1}; Milsana flüssig 1% (v/v); and Kendal 1% (v/v (Tinivella *et al.*, 2009). Seed were placed in the different solutions/emulsions (usually in 100 mL beakers) for 4 h with continuous stirring. Untreated controls were seed submerged in distilled water and stirred for 4 h. The microbial products in group 2 were applied by shaking with seed for about 60 sec in a flask. In the case of the dry/powder formulations rates used per 10 g of seed were 100 mg for FZB 24 (*Bacillus subtilis*), MBI 600 (*Bacillus subtilis* strain MBI 600), and Serenade (*Bacillus subtilis* strain QST 713), 50 mg for Mycostop Mix (*Streptomyces griseoviridis* strain K61) and 300 mg for F251/2 (non-pathogenic strain *Fusarium oxysporum* 251/2). The liquid formulation of BA 2552 (*Pseudomonas chlororaphis* strain MA 342) was applied at 300 µL/10 g of seed. The experimental microorganisms (group 3) used were: strain Z 17 (*Burkholderia* sp.), strain MF 416 (*Pseudomonas* sp.), strain Ki 353 (*Pseudomonas* sp.), strain K 3 (*Bacillus subtilis*) and strain L 18 (*P. fluorescens)*. Their cultivation on laboratory media has been described (Schmitt *et al.*, 2009). The seed were immersed for 15 min in microbial cultures or spore suspensions, respectively, and used immediately, or allowed to dry overnight, and sown the following day. Seed immersed for 15 min in distilled water served as controls.

As the chemical standard, Aatiram (Stähler, Stade, Germany; 670 g×kg^{-1} Thiram®) was included in all experiments. This was applied by shaking an excess amount of the product together with seed in a flask. After individual treatment seed were sown in pots (20 cm dia and 15 cm tall) filled with a commercial peat mix substrate (Weibull AB enhetsjord (K-jord, Sweden) and covered with a layer (approx. 5 mm thick) of vermiculite. In each experiment 4 pots with 50 seed/pot were used, and experiments were repeated 3 times (unless stated otherwise). The pots were watered, covered loosely with lids for 3 days and placed in a randomized complete block design on a growth room bench at 20-24°C (day) and 16-18°C (night) for 4-8 weeks. Extra light (Philips HP1-T mercury lamps, 400 W, Amsterdam, the Netherlands) was supplied to give a light period of 16 h. The effectiveness of each treatment in disease suppression was evaluated by recording the number of emerged, diseased and healthy plants.

Field tests were performed on conventional agricultural land. In first year at Borgeby (Southern Sweden) using seed from the same treated batch as those used in the growth chamber tests. Seed were sown in completely randomized blocks. Each treatment was sown in 3 replicates and with a total number of 21 blocks. The number of emerged parsley plants was counted twice - first count after 4 weeks from sowing and the second after 6 weeks. On each occasion, the number of plants was counted in four 1 m lengths of row per block.

At the end of the season plants were harvested and yield recorded. Plants from 0.5 m^2 (2 × 0.5 m) of each block

were cut about 1 cm above the soil surface and weighed. At the same time, numbers of plants infected by *Septoria* from 100 plants was recorded.

In second year, the experiment was performed as in year 1 except a few changes. The field was in another area but also in Southern Sweden on conventional agricultural land at Bjuv. Number of blocks and replicates were the same as in first year. Numbers of parsley plants grown were counted twice as in first year, after 4 and 6 weeks from sowing. Numbers of plants were counted in 3, 0.5 m lengths of row per block. At harvest yield data were collected. Plants from 15 m^2 (10 × 1, 5 m) of each block were cut about 1 cm above soil surface and weighted. At the same time numbers of plants infected by *S. petroselini* from an area of (2 × 0.5 m)/block were counted.

All data were statistically analysed with SAS System for Windows (ver. 9.1, SAS Institute, Cary, NC) using the Generalised Linear Model. For separation of means arcsine transformed data were compared with the Student-Newman Keuls (SNK) test. Experiments with replicates within repetitions were analysed by a block model.

RESULTS

Early symptoms were brown leaf spots on leaves and cotyledons. Spots were usually small and variable in shape. As leaf spots age, black fungal pycnidia were often visible. Presence of pycnidia on leaves is a key diagnostic feature for this disease. Infested seed could be identified by the presence of the fungal flask-shaped pycnidia on the seed surface. Conidia protruding from the pycnidia were long, multiseptated (0 to 4), and hyaline (Figure 1).

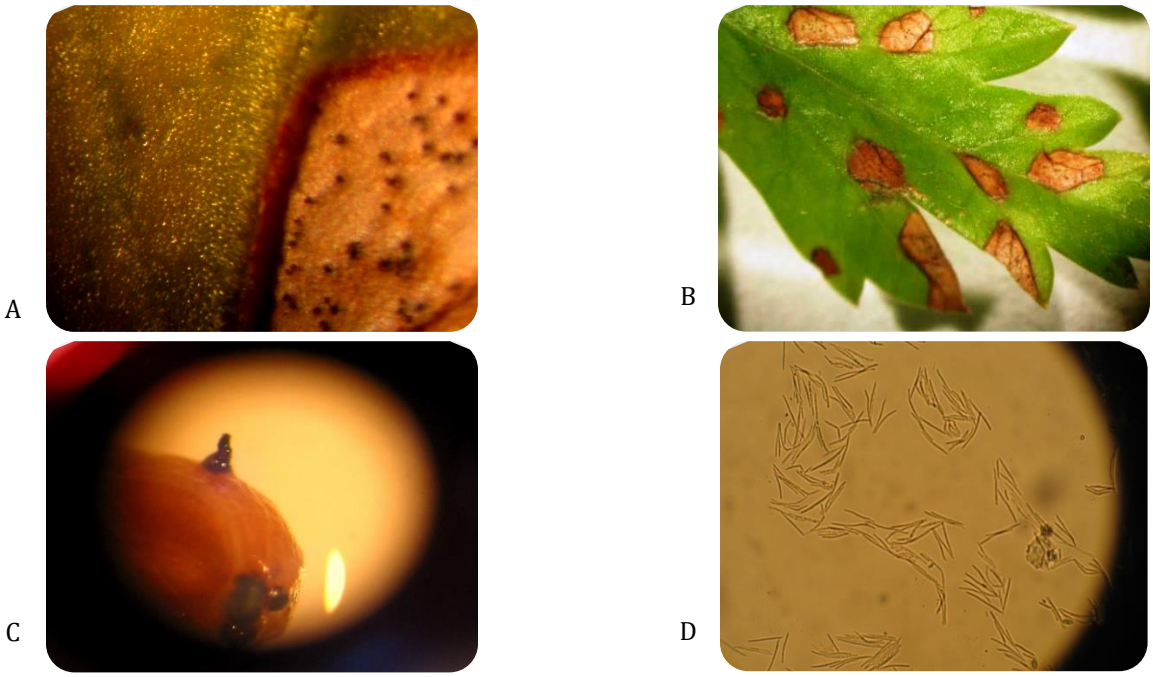

Figure 1. Symptoms of parsley infection with *Septoria petroselini.* A & B. brown irregular leaf spot on leaves with visible tiny black fungal pycnidia. C. fungal flask-shaped pycnidia on the seed. D. fungal conidia.

For tests performed with resistance inducers, beside the chemical, Jasmonic acid. Com Cat, salicylic acid and Milsana exhibited significant effect increasing the germination rate compared to the untreated control (Figure 2).

Compared to the untreated control, all commercialized products (formulated microbial products) increased germination with *B. subtilis* MBI 600 as the one with the highest effect (Figure 3).

In the case of experimental microorganisms, the significant and best effect had the bacterial strains *B. subtilis* (K 3) and (Ki 353) which increased numbers of germinated seed by 16 and 14% respectively. The remaining microorganisms had no effect on the germination rate (Figure 4).

In field trials the commercial product BA 5252 exhibited the highest response in terms of emerged seedlings compared with the untreated control (Table 1). The experimental strain *B. subtilis* (K3) and the commercial products Serenade® also increased seed germination

significantly. All treatments suppressed numbers of diseased parsley plants infected by *S. petroselini* with best result for bacterial strains *B. subtilis* (K 3 (Table 1). This strain had also best yield increase.

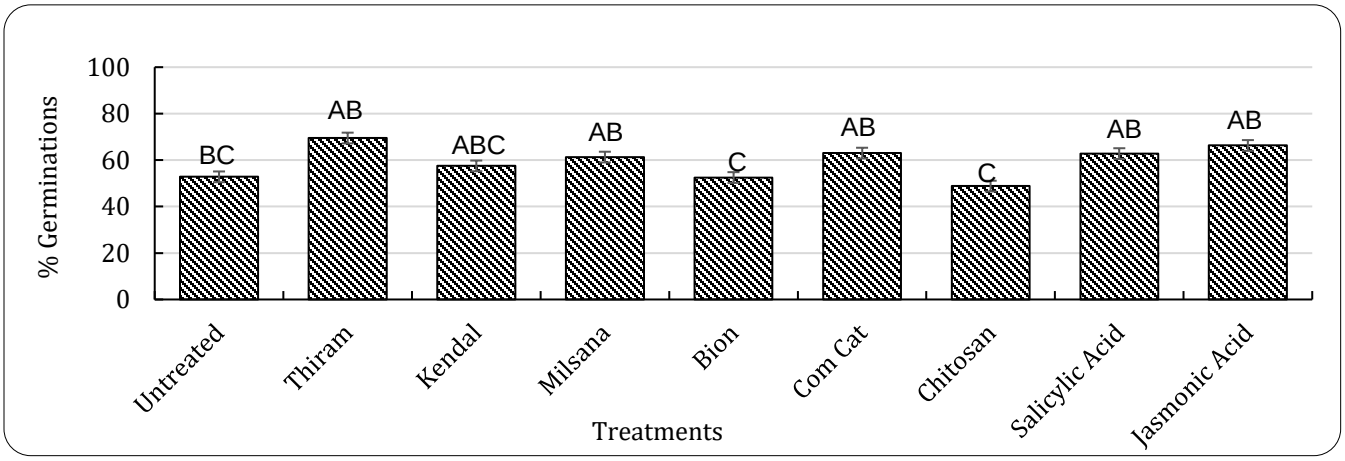

Figure 2. Effect of parsley seed treatments with resistance inducing agents on plant germination. Parsley, cv. Gigante d'Italia/Hilmar, seed lot U446a; naturally infested with *Septoria petroselini* was used. Means of 2 greenhouse experiments each with 4 pots per treatment (50 seed per pot). Different letters above bars indicate statistically significant (P=0, 05) differences.

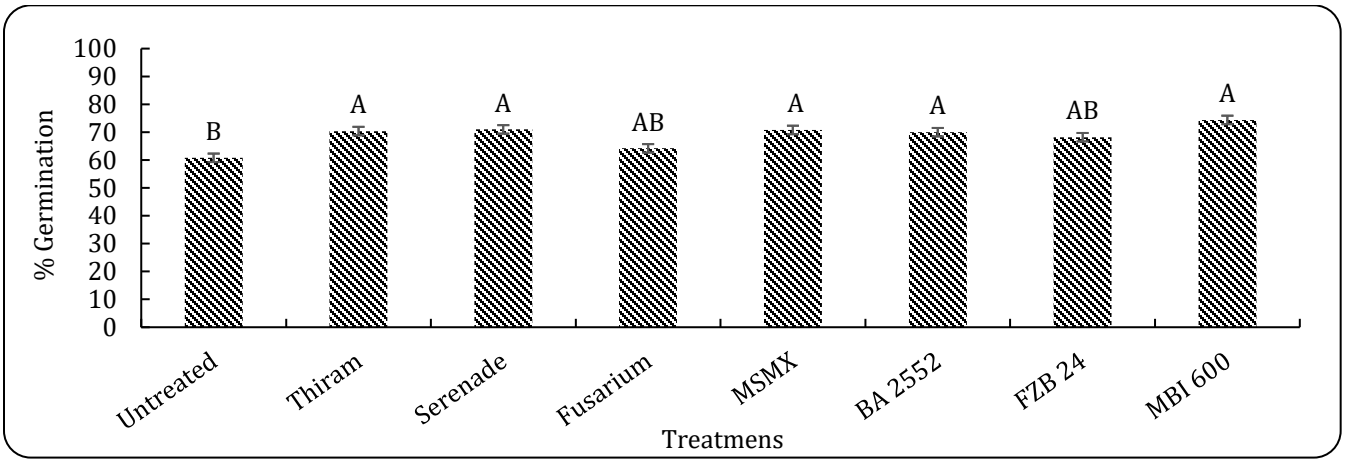

Figure 3. Effect of parsley seed treatments with commercial microorganism preparations on plant germination. Parsley, cv. Gigante d'Italia/Hilmar, seed lot U446a; naturally infested with *Septoria petroselini* was used. Means of 2 greenhouse experiments each with 4 pots per treatment (50 seed per pot). Different letters above bars indicate statistically significant (P=0, 05) differences.

Table 1. Effect of selected biological seed treatment on parsley [cv. Gigante d'Italia / Hilmar, seed lot (U446a)] gemination, *Septoria* disease incidence and yield in two field trails.

Treatments	Nr. Plants/ m^2	% Septoria	Yield gm/m^2
Untreated (Cont)	96 c	9.0 a	1520 a
Aatiram	87 d	7.7 a	1576 ab
Mycostop Mix *(S.Griseoviridis)*	89 d	5.3 b	1680 c
Serenade (*B. subtilis)* strain QST 713)	124 b	5.0 b	1818 d
P. chlororaphis strain (BA2552)	133 a	4.3 bc	1830 d
Bacillus subtilis strain (K3)	124 b	2.7 c	1898 e

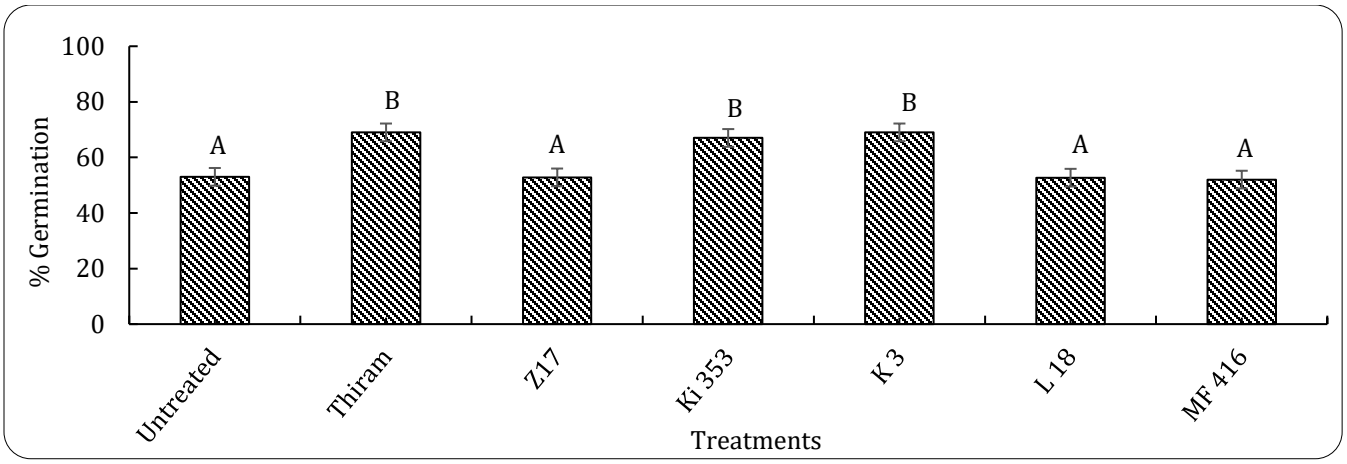

Figure 4. Effect of parsley seed treatments with experimental microorganism preparations on plant germination. Parsley, cv. Gigante d'Italia/Hilmar, seed lot U446a; naturally infested with *Septoria petroselini* was used. Means of 2 greenhouse experiments each with 4 pots per treatment (50 seed per pot). Different letters above bars indicate statistically significant (P=0, 05) differences.

DISCUSSION

Due to lack of researches in the subject of alternative control methods of Septoria leave blight caused by the fungus *Septoria petroselini* on Parsley seeds, it is difficult to discuss the obtained results. Improvement of planting material quality is a key element to increase agricultural productivity. Enhanced yields can come from more uniform and vigorous crop stands, clean seed free from pests and disease. Fungicides use as seed treatment are effective in reducing seedborne inoculum, however, toxic residue in the environment is always a problem with chemical usage.

Under controlled conditions, most seed treatments with putative resistance inducing agents did not affect numbers of germinated seedlings, except for Jasmonic acid- based treatment. Parsley seed treatments with these agents failed to control the seedborne pathogen *S. petroselini*. The same compounds failed to control *Alternaria brassicicola* on cabbage (*Brassica oleracea* var. *capitata*) (Persoon) (Amein *et al.*, 2011) and failed to protect peas (*Pisum sativum*) L. against the seedborne pathogen *Ascochyta spp*. (Tinivella *et al.*, 2009) and lamb´s lettuce (*Valerianella locusta*) against *Phoma valerianellae* (Schmitt *et al.*, 2009). Koch *et al.* (2010) reported that these compounds in general failed to protect carrot (*Daucus carota*) (Hoffm.) against *Alternaria dauci* and *A. radicina*. Therefor these agents were not included in field trails.

Most of the commercial products and a few experimental microorganisms, showed positive results under greenhouse conditions; therefor some of them were further tested in the field. In field trails, seed treatment with BA 2552 and Serenade provided an improvement in plant establishment and yield. Mycostop, despite not showing a positive effect on the number of plants, improved yield. BA 2552 and Mycostop were among the most effective products tested in greenhouse to control *A. brassicicola* on cabbage (Amein *et al.*, 2011). Also BA 2552 had some effects on cabbage yield and against bean anthracnose caused by seed-borne fungus *Colletotrichum lindemuthianum* and *Alternaria* on carrot seeds (Amein *et al.*, 2011; Tinivella *et al.*, 2009; Koch *et al.*, 2010) respectively.

Among the experimental, non-commercialized microorganisms, the best result was obtained with *B. subtilis* K 3 under controlled conditions. This strain also had a good effect on number of plants established, disease incidence reduction and yield increase in field trails. *Pseudomonas. fluorescens* L 18 also showed a positive effect on number of germinated seed in the field trail, but not under controlled conditions. Good effect was obtained by this strain and *Pseudomonas* sp. MF 416 against *Alternaria* diseases on carrot (Koch *et al.*, 2010). The same strain was the most effective strains against *A. brassicicola* on cabbage (Amein *et al.*, 2011). Some of the tested microorganisms have shown good effect against *P. valerianellae* on lamb´s lettuce, *C. lindemuthianum* on bean and *Ascochyta* spp. on pea (Schmitt *et al.*, 2009; Tinivella *et al.*, 2009). *Ampelomyces quisqualis*, strain (AQ10), effectively

restricted development of *S. petroselini* on the leaves of parsley and also *Bacillus amyloliquefaciens* (RhizoVital42) effectively protected against the disease (Nawrocki and Machura, 2016).

The different performances provided by some of these strains in different experiments and pathosystems may due to optimization conditions. Individual strain needs individual conditions such as growth medium, number of cell per seed (CFU/seed), storage period (shelf- live) and environmental conditions (temperature, light/darkness), etc. and this was not performed in our case.

In conclusion, the results presented in this research showed that many of these used methods are as effective as the chemical fungicide and could be used in organic and conventional farming.

AUTHOR CONTRIBUTIONS

Tahsein A. M. Amein completed the research trails and compiled the whole manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests.

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