

CONTROL OF *ALTERNARIA* LEAF SPOT OF CORIANDER IN ORGANIC FARMING

E. Mangwende^{1,2}, Q. Kritzinger¹ and T. A. S. Aveling^{1,2*}

¹Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa.

²Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa.

*To whom all correspondence should be addressed: terry.aveling@fab.up.ac.za

Telephone: +27 124 203 264, Telefax: +27 124 204 588

Abstract

Increased consumer awareness on the means of production of food in recent decades has intensified pressure for growth of organic farming particularly for its benefit on the environment due to minimal use of synthetic chemicals. In this regard, non-chemical seed treatments, viz. biocontrol agents (*Trichoderma* and *Bacillus*), hot water treatments and plant extracts, were studied as alternatives to synthetic chemicals for the management of *Alternaria alternata* (Fr.) Keissl. causing *Alternaria* leaf spot affecting organically produced coriander (*Coriandrum sativum* L.). Antifungal activities of acetone, ethyl acetate and water extracts of *Allium sativum*, *Carica papaya*, *Datura stramonium*, *Lantana camara*, *Tagetes minuta* and *Zingiber officinale* were evaluated using the disc diffusion assay. Discs impregnated with acetone extracts of *Allium*, *Datura* and *Zingiber* at a concentration of 15 mg/mL completely inhibited growth of *A. alternata* whereas discs impregnated with *Tagetes* recorded the lowest antifungal activity. Ethyl acetate extracts of all plants except *Carica* and *Tagetes* at 15 mg/mL showed antifungal activity which was comparable to Celest[®] XL, a synthetic fungicide.

A comparison of water extracts showed that discs impregnated with *Lantana* extract at 15 mg/mL had the highest zones of inhibition (16.5 mm); however, discs impregnated with *Tagetes* at a concentration of 5 mg/mL yielded the lowest antifungal activity against *A. alternata* (0.3 mm). The greenhouse trial showed that all non-chemical seed treatments significantly improved percentage seedling emergence, except for seeds treated with *Lantana* extracts and hot water at 48°C for 60 min, when compared to untreated controls. The study showed that seeds treated with *Trichoderma* sp. yielded seedlings with the longest shoots, which were significantly higher than seedlings grown from seeds treated with Celest[®] XL. There was no incidence of *Alternaria* leaf spot disease on seedlings from seeds treated with *Bacillus* sp. and an extract of *Allium*, which compared well with seeds treated with Celest[®] XL. Since there are limited chemicals registered for management of diseases affecting herb production, the results of this study have shown that soaking coriander seeds in a hot water bath set at 54°C for 15 mins, and biocontrol agents (*Trichoderma* and *Bacillus*) and extracts of *Allium* and *Zingiber* are potential replacements of synthetic fungicides in controlling *Alternaria* leaf spot disease on coriander produced under organic farming.

Keywords: *Alternaria alternata*, biocontrol, *Coriandrum sativum*, hot water, plant extracts, seed treatment

Introduction

Coriander (*Coriandrum sativum* L.) is a member of the Apiaceae family and is native to the Near East region (Diederichsen, 1996). Today coriander is distributed and naturalised worldwide; however, commercial cultivation is mainly done in Bulgaria,

Central Europe, India, Morocco and Russia (Sriti et al. 2009). Cultivation of coriander is relatively new in South Africa (SADC Trade, 2014).

The market of herbs is increasing with the improvement of living standards worldwide (Sher, 2013). The Centre of Promotion of Imports of spices and herbs from developing countries reported a net import of 628 000 tons with a value of R27.786 billion into European Union member countries between the period of 2012 and 2016 (CBI, 2017). South Africa merely supplies 0.05% of the total exports of herbs and spices, which were valued at R4 million in 2015 (DAFF, 2016).

All parts of the coriander plant are edible, but dried fruits are the most valued product (Pathak et al. 2011). The fruits are a major component of pickling spices (Ramadan and Mörsel, 2002). Essential oil extracted from fruits is extensively used in perfumery, aromatherapy and production of soap, creams and lotions (Diederichsen, 1996). Despite the economic importance of coriander, current production trends are below the crops' genetic potential due to several biotic and abiotic stresses (Meena and Malhotra, 2006). Among biotic factors are some diseases caused by various fungi. Manoranjitham et al. (2003) reported a 60% loss of coriander yield by a wilting disease caused by *Fusarium oxysporum* f. sp. *coriandrii* (Fusacr). Although plants infected with *Alternaria* spp. and *Phoma multirostrata* (P.N. Mathur, S.K. Menon & Thirum) Dorenb & Boerema seldom die, the presence of lesions and other foliar blemishes may significantly reduce their market value (Hashmi and Ghaffar, 1991; Boedo et al. 2012; Mangwende et al. 2018).

Due to incidence of diseases, cultivation of herbs is frequently accompanied by application of synthetic fungicides throughout the growing season for production to be economically viable. In South Africa, management of diseases is complicated as there

are limited chemicals registered for use on herbs (Croplife, 2017). Considerable management of other foliar diseases of herbs has been achieved using unregistered chemical groups (Gilardi et al. 2013). However, a study conducted by McFrederick et al. (2008) has shown that other compounds of synthetic chemicals may react causing degradation of the natural constituents such as linalool of different plant parts responsible for the fragrance of aromatic herbs. It is imperative to ensure that all components of essential oils are naturally maintained from production in the field to final extraction or direct consumption without the use of synthetic chemicals as they may have an effect on the overall quality of the plant products (Anitescu et al. 1997).

Therefore, this study seeks to determine the efficacy of non-chemical methods, viz. plant extracts, biocontrol agents and hot water treatments as alternative means of managing *Alternaria* leaf spot disease of coriander cultivated under organic farming. In this regard, experiments were performed to screen organic (acetone and ethyl acetate) and water extracts of *Allium sativum* L. (garlic), *Carica papaya* L. (pawpaw), *Datura stramonium* L. (thorn apple), *Lantana camara* L. (Spanish flag), *Tagetes minuta* L. (Mexican marigold) and *Zingiber officinale* Roscoe (ginger) for their antifungal activities against growth of *A. alternata*. Thereafter, *in vivo* experiments were conducted to assess the effect of non-chemical seed treatments on seedling emergence and management of *Alternaria* leaf spot disease.

Materials and method

Source of materials

Pathogenic *Alternaria alternata* (PPRI 18133) isolated from a naturally infected coriander cv. American long seed lot (Mangwende et al. 2018) was used in this study. Plants used for extraction in this study were selected based on local availability and previous reports of efficacy against *Alternaria* spp. on other crops (Seetha Ramulu et al. 2010; Nashwa and Abo-Elyousr, 2012). *Datura*, *Lantana* and *Tagetes* leaves were collected at the Hillcrest campus Experimental farm of the University of Pretoria (Pretoria, South Africa). *Carica* leaves were sourced from a farm in Polokwane (Limpopo, South Africa). *Allium* cloves and *Zingiber* rhizomes were purchased from a fresh vegetable supermarket (Food Lover's Market, Brooklyn, Pretoria, South Africa). Syngenta South Africa (Pvt.) Ltd. supplied the systemic fungicide Celest[®]XL (25 ai/L fludioxonil and 10 g ai/L mefenoxam), which was used as a standard seed treatment chemical in this study. Two commercial biocontrol agents, viz. *Trichoderma harzianum* (EcoT[™]) and *Bacillus subtilis* strain MBI 600 (Integral[®]) were supplied by Plant Health Products (Pvt.) Ltd (Kwazulu-Natal, South Africa) and Becker Underwood (Pvt) Ltd (Kwazulu-Natal, South Africa), respectively. Commercial seed companies, Sakata and Starke Ayres (South Africa), provided untreated coriander, cv. American long, seed lots.

Preparation of materials

Pathogen inoculum was prepared from a 14 day old *A. alternata* culture by flooding the surface of plates with sterile distilled water amended with Tween 20 and rubbing the culture with a sterile glass rod to dislodge the mycelia. The mycelial suspension was

filtered through three layers of cheesecloth before adjusting the concentration to 1×10^5 spore/mL using a haemocytometer.

Plant parts were separately dried in the shade and were ground to a fine powder using a Macsalab mill (Model 200 LAB, Eriez[®], USA). Powdered plant material (1 kg for each sample) was submitted for extraction by dissolving in 2 L of organic solvent (acetone or ethyl acetate) and sterile distilled water (48 h in each). Thereafter, each mixture was thoroughly shaken at 1500 rpm with a hand held homogeniser (PRO 250, Monroe, CT USA) for 5 min. Solvent powder mixtures were left on a rotary shaker at 110 rpm for 48 h after which filtration was done using a 0.45 μ L filter funnel connected to a vacuum pump (Masangwa et al. 2013). For organic filtrates, organic solvents were vaporised in a Buchi-Rotavapor (Model R-200, Switzerland). Water filtrates were concentrated to powder using a freeze drier (Edwards High vacuum International, Sussex, England) at -80°C . Final plant extracts were stored in glass vials at -10°C until required for further tests.

In vitro* antifungal activities of plant extracts against *A. alternata

Powdered plant extracts were dissolved in the same solvent used to prepare them, viz. water, ethyl acetate or acetone, to yield stock solutions of 5, 10 and 15 mg/mL. Thereafter, stock solutions were filter sterilised and their antifungal activity examined using a modified disk diffusion method used by Murray et al. (1995). In this assay, 100 μ L of *A. alternata* inoculum concentrated to 10^5 spore/mL was spread on 90 mm Petri dishes containing PDA. Sterile filter paper disks (6 mm in diameter) were impregnated with 10 μ L of sterile stock solution of plant extracts and aligned on the surface of an inoculated Petri dish. Sterile disks impregnated with the same solvents used to dissolve

the plant extracts served as negative controls. The experimental units were all compared against the positive treatments, which consisted of discs impregnated with 5, 10 and 15 mg/mL of Celest[®] XL. Treatments were replicated three times and randomly arranged inside a 25°C incubator. The entire experiment was repeated twice.

***In vivo* antifungal assay**

The experiments were conducted during the summer season (sowing dates: 04 September and 18 October 2014) in the Plant Pathology greenhouse (Hillcrest Experimental farm, latitude: 25° 45' 6.94" S, longitude: 28°15' 34.69'' E, and at an elevation of 1 380 m above sea level). Coriander seed lot 34184, which was naturally infected with *A. alternata* (73%) was treated with the following seed treatments, viz. a liquid formulation of *Bacillus* sp. applied at the recommended dosage of 3.23 mL/kg seed, dried *Trichoderma* sp. granules applied at a rate of 0.1 g/g of seed, hot water seed treatments at temperature-time combinations of 48°C for 60 min and 54°C for 15 min as determined by a preliminary study following the protocol of Masum et al. (2009).

In addition, some coriander seeds were treated with plant extracts that showed high activity from the aforementioned antifungal tests, viz. ethyl acetate extract of *Allium*, acetone extract of *Datura* and *Zingiber* or extract of *Lantana* prepared in 1% dimethyl sulfoxide (DMSO). Treated coriander seeds were sown in 6-cavity plastic seedling trays filled with pasteurized loam soil. In this study, each seedling tray represented a replicate with one seed sown per cavity. The experimental units were replicated four times and arranged on greenhouse benches in a complete randomized block design. Seedling trays with seeds treated with Celest[®] XL applied at the

recommended rate of 1 mL/kg seed served as standard positive control seed treatment; whereas, seedling trays with untreated seeds served as the untreated controls.

The number of emerged seedlings was recorded and assessments for the presence and severity of *Alternaria* leaf spot disease were done at 21 and 42 days after sowing, respectively. The severity of *Alternaria* leaf spot disease was evaluated using a disease rating scale described by Mangwende et al. (2018). Plants were harvested (three plants/replicate) and fresh mass of aerial vegetative parts (stem and leaves) determined. Measurements of shoot length were taken and the surface area of coriander leaves determined using the standard graph paper method (five leaves/plant) (Tagliipour and Salehi, 2008). The plants were then oven dried at 85°C for 48 h until constant weight and the dry mass was recorded (Marichali et al. 2014).

Statistical analysis

Statistical analysis of variance of experimental data was done using SAS 9.3 (SAS institute, 2010), in which differences between means were compared using the Fisher's LSD test ($p < 0.05$). Since repeats of antifungal assays were similar, experimental data was pooled for statistical analysis. Antifungal activities of plant extracts were determined by measuring inhibition zones around *A. alternata* plugs after three and six days of incubation, where; percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition of growth of fungi} = \frac{D_t}{D_c} \times 100$$

Where, D_c = zone of inhibition around disc impregnated with Celest® XL.

D_t = zone of inhibition around disc impregnated with plant extract or solvent

Results

Antifungal activity of plant extracts

Mean percentage inhibition of plant extracts are displayed in Table 1. Discs impregnated with acetone extracts of *Allium*, *Carica*, *Datura* and *Zingiber* at 15 mg/mL had similar antifungal activities compared to discs impregnated with Celest[®] XL. Discs impregnated with acetone extracts of *Lantana* and *Tagetes* at 15 mg/mL had a significantly lower antifungal activity against *A. alternata* compared to discs impregnated with Celest[®] XL. At a concentration of 10 mg/mL, only discs impregnated with *Allium*, *Zingiber* and *Datura* extracts were comparable with Celest[®] XL. Compared to other plant extracts extracted with acetone, discs impregnated with *Tagetes* extracts at 5, 10 and 15 mg/mL had the lowest inhibition zones against growth of *A. alternata*.

Discs impregnated with ethyl acetate extracts showed an increase in antifungal activity with an increase in concentration, except for a slight decrease of 1.6 and 3.0% recorded when the concentration was elevated from 10 to 15 mg/mL on discs impregnated with *Tagetes* and *Zingiber* extracts, respectively. At the highest concentration (15 mg/mL), performance of discs impregnated with ethyl acetate extracts was statistically similar to that of Celest[®] XL, except for discs impregnated with *Carica* and *Tagetes* in which antifungal effects were significantly lower (Table 1).

Table 1 Antifungal activity of plant extracts against growth of *Alternaria alternata*, after six days of incubation, using the disk diffusion method.

Plant	Concentration (mg/mL)	Inhibition of <i>A. alternata</i> [(%) ^a , mm*]		
		Acetone solvent	Ethyl acetate solvent	Water solvent
<i>Allium sativum</i>	5	(97.5) 17.8 c	(98.1) 18.1 ab	(18.4) 3.2 j
	10	(99.6) 18.4 b	(100.2) 18.7 ab	(32.5) 5.7 i
	15	(100.4) 18.6 ab	(99.8) 18.7 ab	(37.9) 6.7 h
<i>Carica papaya</i>	5	(79.7) 14.5 e	(64.6) 11.9 h	(5.6) 1.0 o
	10	(97.07) 17.9 c	(78.8) 14.7 g	(12.9) 2.3 kl
	15	(101.4) 18.8 ab	(80.7) 15.1 fg	(17.9) 3.2 j
<i>Datura stramonium</i>	5	(77.5) 14.2 fg	(98.5) 18.2 ab	(53.4) 9.3 f
	10	(100.02) 18.4 ab	(100.01) 18.7 ab	(65.6) 11.6 e
	15	(101.04) 18.8 ab	(99.1) 18.6 ab	(71.1) 12.6 d
<i>Lantana camara</i>	5	(60.6) 11.1 i	(83.03) 15.3 ef	(44.0) 7.7 g
	10	(71.9) 13.3 h	(94.97) 17.8 c	(81.6) 14.4 c
	15	(79.4) 14.8 ef	(98.8) 18.6 ab	(93.2) 16.5 b
<i>Tagetes minuta</i>	5	(10.3) 1.9 l	(19.8) 3.7 i	(1.4) 0.3 p
	10	(24.7) 4.6 k	(18.95) 3.5 i	(9.0) 1.6 m
	15	(33.2) 6.2 j	(17.3) 3.3 i	(18.7) 3.3 j
<i>Zingiber officinale</i>	5	(83.5) 15.3 d	(88.7) 16.4 d	(7.5) 1.3 mn
	10	(100.9) 18.6 ab	(100.4) 18.8 a	(13.8) 2.4 k
	15	(102.1) 19.0 a	(98.4) 18.4 ab	(12.6) 2.2 l
Celest® XL	5	(100.0) 18.3 ab	(100.0) 18.4 ab	(100.0) 17.5 a
Celest® XL	10	(100.0) 18.4 ab	(100.0) 18.7 ab	(100.0) 17.6 a
Celest® XL	15	(100.0) 18.6 ab	(100.0) 18.7 ab	(100.0) 17.7 a
Control		(1.3) 0.2 m	(1.1) 0.2 j	(0.0) 0.0 p
LSD		0.027	0.020	0.013
CV%		3.14	3.78	5.021

In each column, values followed by the same letter do not differ significantly according to Fisher's LSD test (at $p < 0.05$)

*Values not in brackets are mean zones of inhibition measured around discs impregnated with different treatments

^aValues in brackets are percentage inhibition of treatments

Discs impregnated with water extracts recorded the lowest zones of inhibition against *A. alternata* compared to those measured around discs impregnated with acetone and ethyl acetate plant extracts. Similarly to other plant extracts obtained using the two

solvents, there was an increase of antifungal activity on discs impregnated with water extracts, with an increase in concentration, except for a significant decrease of 1.3% observed when the concentration of *Zingiber* was elevated from 10 to 15 mg/mL.

Compared to other water extracts, discs impregnated with extracts of *Lantana* at 15 mg/mL recorded the highest zones of inhibition against *A. alternata* (16.5 mm); whereas, discs impregnated with *Tagetes* at a concentration of 5 mg/mL yielded the lowest antifungal activity (0.3 mm) compared with other plant extracts against *A. alternata*.

Effect of seed treatments on seedling growth

All seed treatments significantly improved seedling emergence, except for seeds treated with *Lantana* extracts and hot water at 48°C for 60 min, compared to untreated seeds (Table 2). Sowing seeds treated with *Bacillus* resulted in a higher seedling emergence compared to seeds treated with Celest[®] XL; although, the difference was not significant. Seedling emergence of seeds treated with *Allium* and *Lantana* extracts and hot water at 48°C for 60 min were significantly lower compared to seedling emergence recorded for seeds treated with Celest[®] XL.

In general, there was a significant difference between length, fresh and dried mass of seedlings sown in October compared with seedlings sown in September ($p < 0.05$). In this regard, *in vivo* evaluations of efficacy of seed treatments were based on results taken from the October crop as it had a significantly better growth (Table 2).

Table 2 Effect of seed treatments on emergence, shoot length, leaf area, fresh and dry mass of coriander seedlings from seed infected with *A. alternata*.

Treatment	Seedling emergence (%) [*]	Shoot length (mm) [*]		Leaf area (mm ²) [*]		Fresh mass (g) [*]		Dry mass (g) [*]	
		Sept	Oct	Sept	Oct	Sept	Oct	Sept	Oct
<i>Allium sativum</i> ethyl acetate	66.5 cd	85.4 b	93.6 e	3.36 de	3.83 f	1.25 c	1.34 e	0.080 de	0.083 ef
<i>Datura stramonium</i> acetone	66.7 bcd	76.9 cd	94.9 e	3.12 fg	2.63 i	0.90 e	1.04 f	0.067 fgh	0.076 g
<i>Lantana camara</i> water	54.2 cde	94.4 a	117.8 ab	3.62 bc	4.90 a	1.22 c	1.41 de	0.081 de	0.093 d
<i>Zingiber officinale</i> acetone	70.8 b	76.2 cd	73.3 f	3.54 cd	3.09 h	1.52 b	1.60 ab	0.101 ab	0.106 b
<i>Bacillus subtilis</i>	83.3 a	98.2 a	114.7 b	4.16 a	4.82 ab	1.50 b	1.64 a	0.098 bc	0.113 a
<i>Trichoderma harzianum</i>	72.8 ab	79.3 c	119.8 a	3.74 b	4.62 bc	1.44 b	1.45 cd	0.089 cd	0.100 cd
Hot water 48 °C-60 min	50.0 de	68.7 e	64.1 g	2.88 hi	4.47 cd	0.75 f	0.57 g	0.060 h	0.050 j
Hot water 54 °C-15 min	75.0 ab	87.1 b	92.0 e	3.28 def	4.53 bc	1.05 d	1.00 f	0.074 efg	0.079 fg
Water	62.5 cd	74.0 d	109.6 c	2.97 gh	4.21 de	0.77 f	0.99 f	0.065 gh	0.069 h
Celest® XL	79.2 ab	96.2 a	102.2 d	3.98 a	4.16 e	1.67 a	1.52 bc	0.119 a	0.102 bc
Untreated control	45.8 e	57.0 f	52.2 h	2.74 i	3.41 g	0.66 g	0.65 g	0.057 h	0.063 i
LSD	2.83	4.51	4.58	0.22	0.31	0.08	0.1	0.01	0.01
CV%	17.847	6.973	6.146	8.202	9.281	9.053	10.315	15.485	8.665

^{*}Means within each column followed by the same letters are not significantly different according to Fisher's LSD test ($p < 0.05$)

Seeds treated with *Trichoderma* sown in October yielded the longest seedlings and were significantly higher compared to seedlings raised from seeds treated with Celest® XL. In addition, seedlings raised from seeds treated with *Lantana* and *Bacillus* yielded seedlings that were significantly longer than seedlings sown from seeds treated with Celest® XL.

There was a significant enhancement of mean leaf area of seedlings from treated seeds compared to untreated seeds, except for significantly lower mean leaf areas of seedlings raised from seeds treated with extracts *Datura* and *Zingiber*. The highest leaf

area was recorded for seedlings raised from seeds treated with an extract of *Lantana* (4.90 mm²). Seedlings treated with *Bacillus*, *Trichoderma*, and hot water bath (both, 48°C for 60 min and 54°C for 15 min) had significantly broader leaves (4.82, 4.62, 4.53 and 4.47 mm², respectively) compared to mean leaf area of seedlings raised from seeds treated with Celest[®] XL (4.16 mm²).

Seedling fresh mass of coriander was significantly improved compared to the untreated control by most non-chemical seed treatments except for hot water treated seeds (at 48°C for 60 min) (Table 2). Seedlings from seeds treated with *Bacillus* had the highest fresh mass, which was significantly higher than seedlings from Celest[®] XL treated seeds. Fresh mass of seedlings grown from seeds treated with *Trichoderma* and *Zingiber* were comparable to fresh mass of seedlings grown from seeds treated with Celest[®] XL.

In contrast to the untreated seeds, all seed treatments significantly increased the seedling dry mass, except for seedlings from seeds soaked in a hot water bath at 48°C for 60 min. The highest seedling dry mass was obtained from seeds treated with *Bacillus*. Dry mass of seedlings from seeds treated with *Trichoderma* and *Zingiber* extract were comparable to those from seeds treated with Celest[®] XL.

Effect of seed treatments on incidence and severity of *Alternaria* leaf spot

All seed treatments significantly reduced the incidence of *Alternaria* leaf spot when compared with the untreated seeds. Celest[®] XL, *Zingiber*, *Bacillus* and *Allium* extract seed treatments were most effective at reducing disease incidence, with the latter two having zero disease incidence (Figure 1).

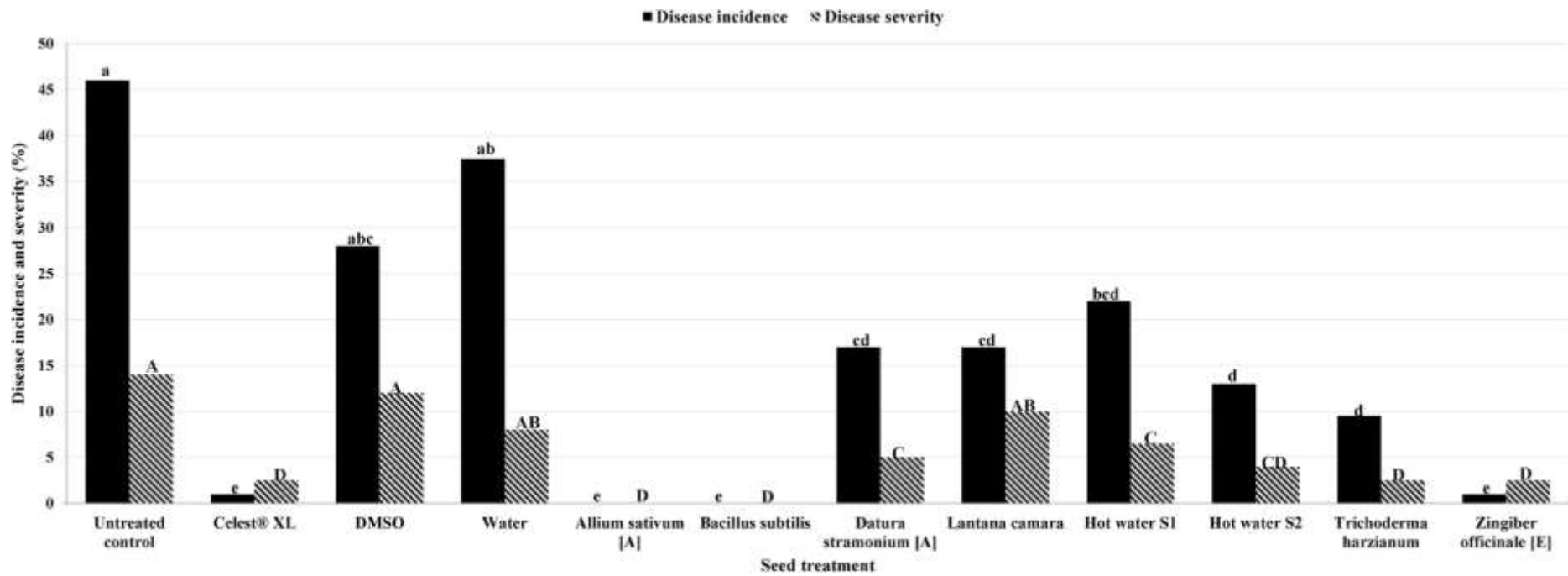


Figure 1 Effect of different seed treatments on the incidence and severity of *Alternaria* leaf spot on coriander seedlings grown from seeds infected with *Alternaria alternata*.

Coriander seedlings from untreated seeds had the highest severity of leaf spot disease, which did not differ significantly from those of seeds soaked in 1% DMSO, *Lantana* extract and sterile distilled water. Seeds treated with Celest[®] XL, *Trichoderma*, *Zingiber* and hot water soak at 54°C for 15 min recorded the lowest severity of *Alternaria* leaf spot, but did not differ from seeds treated with *Bacillus* and *Allium* extract, which yielded disease-free seedlings.

Discussion

Alternaria alternata was recently reported on coriander in South Africa (Mangwende et al. 2018). Since the pathogen is both seed-borne and seed-transmitted, application of effective seed treatments is crucial to minimise extensive defoliation for cost-effective crop production. In the present study, the use of hot water, plant extracts and biological agents as seed treatments were evaluated for the management of *Alternaria* leaf spot disease of coriander produced under organic farming.

In vitro tests showed that regardless of concentration, organic extracts of *Allium* and *Datura* demonstrated high antifungal activities that were comparable with the commercial fungicide Celest[®] XL. All plant extracts exhibited the most inhibition of mycelial growth of *A. alternata* at six days after incubation. Ethyl acetate extracts applied at 15 mg/mL showed antifungal activities in descending order of *Allium*> *Datura*> *Lantana*> *Zingiber*> *Carica* and lastly *Tagetes*; whilst for acetone extracts the order was *Zingiber*> *Carica*> *Allium*> *Datura*> *Lantana*> *Tagetes*. On the contrary, none of the water extracts could compare to the Celest control even at the highest concentration tested.

The difference in degree of the antifungal activity exhibited by the plant extracts may be due to the different composition of phytochemicals synthesised by the respective plant species. For instance, allicin (diallyl thio sulphinate) and alliin (S-allyl-L-cysteine sulphoxide) are the two bioactive compounds that give *Allium* its fungistatic and fungicidal properties (Lanzotti et al. 2013); whereas, 5,6-dihydro-6-pentyl-2H-pentyl-2-H-pyran-2-one is the principle antifungal component of *Datura* extracts (Zhen-guo et al. 2012). Since these metabolites are non-polar, organic solvents favour exhaustive extraction of the bioactive compounds that translates to higher antifungal activities out-performing aqueous plant extracts (Curtis et al. 2004). Moderate antifungal activities exhibited by *Carica* and *Lantana* might be due to weaker fungistatic or fungicidal activities of their bioactive compounds against *A. alternata*. Generally, antifungal activities of these extracts were concentration dependent. It is possible that bioactive compounds were present in insufficient amounts at low concentrations, and as concentration was increased it significantly enhanced antifungal activities of the extracts.

Seeds infected with seed-borne *Alternaria* sp. often rot affecting seed germination and reduce seedling emergence (Naik et al. 2004). Fungi might be transmitted into few of the seedlings that emerged and cause leaf blight disease at later growth stages of the plants (Mangwende et al. 2018). Observations of improved stand establishment and plant health with reduced incidence of seed-borne *A. alternata* on seeds treated with plant extracts of *Allium* and *Zingiber* are in agreement with findings by other researchers, for example, Lima et al. (2016) and Rahmatzai et al. (2017). However, Mahapatra and Das (2013) recorded high incidences of *Alternaria* blight disease on mustard (*Brassica juncea* L.) despite seed treatment and foliar applications with an extract of *Zingiber*.

Sowing coriander seeds treated with *Datura* plant extracts resulted in significantly lower percentage emergence compared to the other plant extracts. Similar observations were reported when *Alternaria* spp. infected seeds of sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) were treated with *Datura* extracts (Lovett and Potts, 1987). This may have been caused by toxic allelopathic compounds contained in *Datura* extracts that suppressed seed germination. Lovett et al. (1981) showed the presence of tropane alkaloids, scopolamine and hyoscyamine as allelopathic chemicals in seeds and leaves of *Datura*.

Soaking coriander seeds in a hot water bath at 54°C for 15 min resulted in a significant reduction of incidence of *A. alternata* and yielded disease-free seedlings, which compared well with seeds treated with *Bacillus* and *Allium*. Although there was significant improvement on seedling emergence for coriander seeds soaked in a 54°C hot water bath for 15 min, fresh and dry mass of seedlings were significantly lower compared to seedlings raised from seeds treated with Celest[®] XL. However, sowing coriander seed lots treated with biocontrol agents and plant extracts resulted in a significant improvement of seedling growth parameters that were comparable with Celest[®] XL treatments. In fact, coriander seeds treated with *Trichoderma* recorded the longest seedlings and a considerably broader leaf surface area. Similarly Kleifeld and Chet (1992) showed that *Trichoderma* spp. induced an increase in emergence of seeds, plant height, leaf area and dry weight of bean (*Phaseolus vulgaris* L. Brittle Wax), radish (*Raphanus sativus* L.) and tomato [*Lycopersicon lycopersicum* (L.) H. Karst.]. Many studies have also reported plant growth characteristics of *Bacillus* sp. on several plants (Mena-Violante and Olalde-Portugal, 2007; Haiyambo et al. 2015; Wu et al. 2016; Breedts et al. 2017; Huang et al. 2017). As a plant growth promoting rhizobacterium, *Bacillus* might have induced systemic resistance and improved overall

plant growth through increased uptake, solubilisation and mobilisation of phosphate (Richardson et al. 2009; Wang et al. 2009). The same might have happened with seeds treated with plant extracts, as some secondary metabolites have been reported to induce systemic resistance (Kagale et al. 2004; Guleria and Kumar, 2006; Latha et al. 2009). For instance, sulphur containing secondary compounds of *Allium* are known to induce systematic resistance (Nashwa and Abo-Elyousr, 2012; Mancini and Romannazzi, 2014), which might have contributed to the significant increase in number of healthy plants regardless of level of contamination and/or infection of coriander seeds.

Since there are limited options in the management of herb diseases in South Africa, it appears safe to conclude that non-chemical seed treatments evaluated in this study are effective alternatives against *A. alternata* associated with commercial coriander seed lots. Because biocontrol agents, plant extracts of *Allium* and *Zingiber* and a hot water seed treatment at 54°C for 15 min were at least equally effective and as reliable as that of the commercial chemical Celest[®] XL, they may be recommended for the management of *Alternaria* leaf spot on coriander produced under organic farming. However, the authors advise further studies to investigate toxicity effects of plant extracts and biopesticides as they may contain active substances that could enter the human food chain.

Conflict of Interest: The authors declare that they have no conflict of interest.

Compliance with Ethical Standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the

institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding: This work was funded by the European Union's Seventh Framework Programme (KBBE.2012.1.2-05: 311875) TESTA (Seed health: Development of seed treatment methods, evidence for seed transmission and assessment of seed health).

Acknowledgments: We wish to acknowledge commercial seed companies for supplying untreated herb seed. Syngenta, Plant Health Products (Pvt.) Ltd and Becker Underwood (Pvt.) Ltd for supplying Celest[®] XL, *Trichoderma harzianum* (EcoT[™]) and *Bacillus subtilis* (Integral[®]), respectively. Special thanks also to Mrs N. Joubert and Dr Z. Pieterse for help with editing and statistical analysis.

References

- Anitescu, G., Doneanu, C. and Radulescu, V. (1997). Isolation of coriander oil: comparison between steam distillation and supercritical CO₂ extraction. *Flavour and Fragrance Journal*, 12, 173–176.
- Boedo, C., Benichou, S., Berruyer, R., Bersihand, S., Dongo, A., Simoneau, P. and Poupard, P. (2012). Evaluating aggressiveness and host range of *Alternaria dauci* in a controlled environment. *Plant Pathology* 61, 63–75.
- Breedt, G., Labuschagne, N. and Coutinho, T.A. (2017). Seed treatment with selected plant growth-promoting rhizobacteria increases maize yield in the field. *Annals of Applied Biology* 171, 229-236.
- CBI. (2017). Trade statistics of spices and herbs. Available at: <https://www.cbi.eu/market-information/spices-herbs/trends/>. Accessed: 11/05/2018.

- Croplife. (2017). A list of fungicides registered in South Africa. Available at: <http://www.croplife.co.za/images/croplife/initiatives/Pesticides.pdf>. Accessed: 11/05/2018.
- Curtis, H., Noll, U., Störmann, J. and Slusarenko, A.J. (2004). Broad spectrum activity of the volatile phytoanticipin allicin in extracts of garlic *Allium sativum* L. against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and Molecular Plant Pathology* 65, 79–89.
- DAFF, South Africa. (2016). Explorative review on opportunities for the trade of herbs, spices and berries. Department of Forest Fisheries (South Africa). Available at: [http://webapps.daff.gov.za/AmisAdmin/upload/Exploringagriculturalexportopportunities \(herbs, spices and berries\).pdf](http://webapps.daff.gov.za/AmisAdmin/upload/Exploringagriculturalexportopportunities (herbs, spices and berries).pdf). Accessed: 12/05/2018.
- Diederichsen, A. (1996). Coriander (*Coriandrum sativum* L.) promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben: International Plant Genetic Resources Institute, Rome, pp. 1–82.
- Gilardi, G., Demarchi, S., Garibaldi, A. and Gullino, M.L. (2013). Management of downy mildew of sweet basil (*Ocimum basilicum*) caused by *Peronospora belbahrii* by means of resistance inducers, fungicides, biocontrol agents and natural products. *Phytoparasitica* 41, 59–72.
- Guleria, S. and Kumar, A. (2006). *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot disease. *Journal of Cell and Molecular Biology* 5, 9-21.
- Haiyambo, D.H., Reinhold-Hurek, B. and Chimwamurombe, P.M. (2015). Effects of plant growth promoting bacterial isolates from Kavango on the vegetative

- growth of *Sorghum bicolor*. African Journal of Microbiology Research 9, 725-729.
- Hashmi, H.M. and Ghaffar, A. (1991). *Phoma multirostrata* on coriander in Pakistan. Pakistan Journal of Botany 23, 127–130.
- Huang, P., de-Bashan, L., Crocker, T., Kloepper, J.W. and Bashan, Y. (2017). Evidence that fresh weight measurement is imprecise for reporting the effect of plant growth-promoting rhizobacteria on growth promotion of crop plants. Biology and Fertility of Soils 53, 199-208.
- Kagale, S., Marimuthu, S., Nandakumar, R. and Samiyappan. (2004). Antimicrobial activity induction of systematic resistance in rice by leaf extracts of *Datura* sp. against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiology and Molecular Plant Pathology 65, 91-100.
- Kleifeld, O. and Chet, I. (1992). *Trichoderma harzianum*, interaction with plants and effect on growth response. Plant and Soil 144, 267–272.
- Lanzotti, V., Bonanomi, G. and Scala, F. (2013). What makes *Allium* species effective against pathogenic microbes? Phytochemistry Reviews 12, 751–772.
- Latha, P. Anand, T., Ragupathi, N. Prakasamand, V., Samiyappan, R. (2009). Antimicrobial activity of plant extracts and induction of systematic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biological Control 50, 85-93.
- Lima, C.B.D., Rentschler, L.L.A., Bueno, J.T. and Boaventura, A.C. (2016). Plant extracts and essential oils on the control of *Alternaria alternata*, *Alternaria dauci* and on the germination and emergence of carrot seeds (*Daucus carota* L.). Ciência Rural, Santa Maria 46, 764-770.

- Lovett, J.V. and Potts, C.W. (1987). Primary effects of allelochemicals of *Datura stramonium* L. Plant and Soil Journal 98, 137–144.
- Lovett, J.V., Levitt, J., Duffield, A.M. and Smith, N.G. (1981). Allelopathic potential of thorn-apple (*Datura stramonium* L.). Weed Research 21, 165–170.
- Mahapatra, S. and Das, S. (2013). Bio-efficacy of botanicals against *Alternaria* leaf blight of mustard under field condition. The Bioscan 8, 675-679.
- Mancini, V. and Romannazzi, G. (2014). Seed treatments to control seed-borne fungal pathogens of vegetable crops. Pest Management Science 70, 860-868.
- Mangwende, E., Kritzing, Q., Truter, M. and Aveling, T.A.S. (2018). *Alternaria alternata*: A new seed-transmitted disease of coriander in South Africa. European Journal of Plant Pathology 152, 409-416.
- Manoranjitham, K.S., Rabindran, R. and Doraiswamy, S. (2003). Management of seed-borne pathogens and wilt disease of coriander. Madras Agricultural Journal 90, 298–300.
- Marichali, A., Dallali, S., Ouerghemmi, S., Sebei, H. and Hosni, K. (2014). Germination, morpho-physiological and biochemical responses of coriander (*Coriandrum sativum* L.) to zinc excess. Industrial Crops and Products 55, 248–257.
- Masangwa, G.I.J., Aveling, T.A.S. and Kritzing, Q. (2013). Screening of plant extracts for antifungal activities against *Colletotrichum* species of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp). The Journal of Agricultural Science 151, 482–491.
- Masum, I.M.M., Islam, M.M.S. and Fakir, A.G.M. (2009). Effect of seed treatment practices in controlling of seed-borne fungi in sorghum. Scientific Research and Essays 4, 22–27.

- McFrederick, Q.S., Kathilankal, C.J. and Fuentes, D.J. (2008). Air pollution modifies floral scent trails. *Atmospheric Environment* 42, 202–209.
- Meena, S.S. and Malhotra, K.S. (2006). Effect of sowing time, nitrogen and plant growth regulators on green leaf yield of coriander. *Journal of Spices and Aromatic Crops* 15, 115–117.
- Mena-Violante, H.G. and Olalde-Portugal, V. (2007). Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. *Scientia Horticulturae* 113, 103-106.
- Murray, P.R., Baron, E.J., Pfaller, A.M., Tenover, C.F. and Tenover, H.R. (1995). *Manual of Clinical Microbiology*, 6th edition, ASM: Washington, DC, p. 211.
- Naik, M.K., Patil, R.G., Suvarna, Ajithkumar, K. (2004). Yield loss model and blight prediction model in *Alternaria* blight of sesame. *Indian Phytopathology* 57, 106.
- Nashwa, S. and Abo-Elyousr, A.K. (2012). Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science* 48, 74–79.
- Pathak, L.N., Kasture, B.S., Bhatt, M.N. and Rathod, D.J. (2011). Phytopharmacological properties of *Coriander sativum* as a potential medicinal tree. *Journal of Applied Pharmaceutical Science* 1, 20–25.
- Ramadan, F.M. and Mörsel, J.T. (2002). Oil composition of coriander (*Coriandrum sativum*) fruit seeds. *European Food Research and Technology* 36, 273–276.
- Rahmatzai, N., Zaitoun, A.A., Madkour, M.H., Ahmady, A., Hazim, Z. and Mousa, M.A. (2017). *In vitro* and *in vivo* antifungal activity of botanical oils against *Alternaria solani* causing early blight of tomato. *International Journal of Biosciences* 10, 91-99.

- Richardson, A.E., Barea, J.M., McNeill, A.M. and Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth-promotion by microorganisms. *Plant and Soil Journal* 321, 305–339.
- SADC Trade (2014). Trade information brief of spices. <http://www.sadctrade.org/files/TIB-Spices-final.pdf>, Date accessed: 21/010/2014.
- SAS Institute. (2010). Analysis Software/Stat software version 9.3 for windows. SAS institution Inc., Cary NC North Carolina, USA.
- Seetha Ramulu, S., Raja, G.R.C. and Ramanjaneyulu, R. (2010). Evaluation of certain plant extracts and antagonists against *Fusarium solani* and *Alternaria tenuissima*, the Incitants of root rot and die-back diseases of mulberry. *International Journal of Industrial Entomology* 20, 1–5.
- Sher, H. (2013). Collection and marketing of high value medicinal and aromatic plants from district Swat, Pakistan. International Food Policy Research Institute (IFPRI). Available at: www.ifpri.org/sites/default/files/publications/psspwp//pdf. Accessed: 29/10/2014.
- Sriti, J., Talou, T., Wannes, A.W., Cerny, M. and Marzouk, B. (2009). Essential oil, fatty acid and sterol composition of Tunisian coriander fruit different parts. *Journal of the Science of Food and Agriculture* 89, 1659–1664.
- Tagliipour, F. and Salehi, M. (2008). The study of salt tolerance of Iranian barley (*Hordeum vulgare*) genotypes in seedling growth stages. *Biodiversity Conservation* 172, 53–58.
- Wang, S., Wu, H., Qiao, J., Ma, L., Liu, J., Xia, Y. and Gao, X. (2009). Molecular mechanism of plant growth-promotion and induced systemic resistance to

tobacco mosaic virus by *Bacillus* spp. *Journal of Microbiology and Biotechnology* 19, 1250–1258.

Wu, Y.N., Feng, Y.L., Paré, P.W., Chen, Y.L., Xu, R., Wu, S., Wang, S.M., Zhao, Q., Li, H.R., Wang, Y.Q. and Zhang, J.L. (2016). Beneficial soil microbe promotes seed germination, plant growth and photosynthesis in herbal crop *Codonopsis pilosula*. *Crop and Pasture Science* 67, 91-98.

Zhen-guo, J., Yu-liang, J., Huan-ying, Y. and Feng-ying, C. (2012). Determination on the main chemical components and antibacterial activities of volatile oil from *Datura stramonium*. *Medicinal Plant* 3, 45–47.