Org. Agr. (2019) 9:107–116
https://doi.org/10.1007/s13165-018-0212-3

Exploration of essential oils as alternatives to conventional fungicides in lupin cultivation

Kevin Dewitte · Sofie Landschoot · Jasper Carrette · Kris Audenaert · Geert Haesaert

Received: 2 March 2018 / Accepted: 3 April 2018 / Published online: 17 April 2018

Abstract Lupin (*Lupinus* L.) has the potential to become a true alternative for soybean as protein source, especially in the more temperate regions in the world. However, diseases such as anthracnose (*Colletotrichum lupini*), gray mold (*Botrytis cinerea*), and root rot or brown spot (*Pleiochaeta setosa*) are important threats for lupin production, leading to yield and quality losses. Although conventional fungicides offer a solution to these problems, there is a growing interest in the use of alternative (biological) treatments. In this research, the applicability of four pure plant essential oils (clove oil, juniper oil, tea tree oil, and thyme essential oil) and timbor® (a *Thymus vulgaris*-derived plant extract) as alternatives for synthetic fungicides towards the lupin pathogens—*C. lupini*, *B. cinerea*, and *P. setosa*—was investigated. The anti-fungal effect of juniper oil was limited, whereas the other oils and timbor® clearly suppressed the growth and spore germination of all fungi. The in vitro experiments revealed that thyme essential oil and timbor® were most effective to inhibit conidial germination and mycelium growth. Furthermore, the results of the pot experiments demonstrated that these *Thymus*-derived compounds were able to suppress *P. setosa* brown spot and root rot symptoms. Additional trials are necessary to evaluate the effect of these compounds under field conditions. However, based on these in vitro and pot experiments, it can be concluded that pure essential oils and *Thymus*-derived plant extracts are promising anti-fungal agents, having the potential to become true alternatives for conventional fungicides in lupin cultivation. To the best of our knowledge, this is the first study demonstrating the potential of plant-derived compounds to treat the main diseases affecting lupin production.

Keywords Essential oils · Lupin · Fungi

K. Dewitte (✉) · J. Carrette · K. Audenaert · G. Haesaert
Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, BE-9000 Ghent, Belgium
e-mail: kevin.dewitte@ugent.be

S. Landschoot
Department of Data Analysis and Mathematical Modelling, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, BE-9000 Ghent, Belgium

Introduction

The genus *Lupinus* L. (*Fabaceae*) is a large and diverse genus comprising more than 200 species. The broad-leaved lupin (*Lupinus albus* L.), yellow lupin (*L. luteus* L.), and narrow-leaved lupin (*L. angustifolius* L.) are native European species that have been domesticated to serve as an alternative protein source (Glencross and Hawking, 2004). Due to the increasing demand of plant proteins for food and feed purposes, the lupin production in Europe has increased from 62,507 ha in 2000 to 198,871 ha in 2014 (FAOSTAT 2016). The interest in sweet lupin is growing as they have a number of attractive nutritional properties, high protein (up to 44%) and dietary fiber contents, and negligible starch or anti-nutritional factors (Lucas et al. 2015). Furthermore,

lupin has a great potential in cooler regions, where the cultivation of soybean is restricted due to climatic conditions (Wojakowska et al. 2015). Additionally, thanks to their deep root systems, lupin is an important crop in mobilizing nutrients from deeper soil levels unreachable for other crops. Furthermore, lupin can, in symbiosis with rhizobia bacteria, fix nitrogen and can thus serve as a nitrogen supplier to subsequent cereals and horticultural crops (Talhinhas et al. 2016).

Currently, efficient lupin production is hampered as lupin is prone to several diseases, which can lead to significant yield and quality losses. One of the most important diseases affecting lupin production is anthracnose caused by *Colletotrichum lupini* (var. *lupini* and var. *setosum*). This disease is mainly spread by infected seeds and rain-splash of spores from infected plants. It has the potential to cause complete crop losses in susceptible varieties (Thomas and Sweetingham 2004). The typical symptoms of anthracnose are bending and twisting of stems, petioles, and pods. The fungus produces orange masses of conidia on the surface of the affected areas. Seeds in infected pods become brown and wrinkled and, if able to germinate, the plantlet exhibits necrosis in the cotyledons or in the hypocotyl (Talhinhas et al. 2016).

Pleiochaeta setosa (Kirchn.) Hughes is the causal agent of brown leaf spot and root rot. The first symptoms usually appear on the leaves. The infections begin as small brown spots, which enlarge to form black networks on leaves and extensive brown areas on pods and stems. When leaves are severely infected, they die and drop off (Sweetingham 1999).

Another important threat of lupin is gray mold (*Botrytis cinerea*). The typical symptoms of this pathogen are water-soaked, irregular lesions initially on the stem bases or branches, which can extend upwards from the affected stems to the leaves. Furthermore, gray to grayish brown, velvety molds can appear on the lesions under moist conditions (Tomioka et al. 2008).

The most common method to tackle plant diseases is the application of synthetic fungicides. The dicarboximides iprodione and procymidone can be used to treat lupin seeds in order to control brown spot (*P. setosa*) (Sweetingham 1999). Furthermore, Thomas et al. (2008) showed that, when applied 1 day prior to infection, azoxystrobin, chlorothalonil, mancozeb, and copper oxychloride fungicides were highly effective against *C. lupini*, whereas tebuconazole, benomyl, and carbendazim were less effective. However, with the growing concern of potential environmental and health risks associated with the continuous use of synthetic chemicals and the fact that lupin is often introduced in organic farming systems, there is an increasing interest in alternative treatments. Additionally, integrated pest management emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural mechanisms for pest management (Lamichhane et al. 2016).

Plant-derived compounds, such as essential oils, are a powerful alternative to conventional fungicides. The fungicidal effect of these compounds against several plant pathogens is widely reported (Daferera et al. 2003; Behtoei et al. 2012; Dikbas et al. 2008; Amini et al. 2016; Varo et al., 2017). The mode of action of these compounds is not yet completely unraveled. The anti-fungal effect includes the suppression of spore germination and reduction of hyphal growth. This can be attributed to the fact that the application of essential oils can lead to changes in cell wall composition, plasma membrane disruption, and mitochondrial structure disorganization. Furthermore, essential oils can interfere with the enzymatic reactions of the mitochondrial membrane, such as respiratory electron transport, proton transport, and coupled phosphorylation steps (Rasooli et al. 2006; Kishore et al. 2007).

The goal of this study is to evaluate the efficacy of four pure plant essentials oils: clove oil (*Syzygium aromaticum*), juniper oil (*Juniperus communis*), tea tree oil (*Melaleuca alternifoliai*), thyme essential oil (*Thymus vulgaris*), and timbor®, a *Thymus vulgaris*-derived plant extract, to control the lupin diseases: anthracnose (*C. lupini*), gray mold (*B. cinerea*), root rot, and brown spot (*P. setosa*). Therefore, in vitro tests were set up to screen the efficacy of these oils towards spore germination and mycelium growth of different isolates. Furthermore, the potential of these compounds to suppress the disease symptoms of *P. setosa* was tested by means of pot experiments.

Materials and methods

Fungal isolate collection

For this study, five *C. lupini* isolates, four *B. cinerea* isolates, and nine *P. setosa* isolates were used. An overview of the host crop, year, and location of isolates is represented in Table 1. All isolates were

Table 1 Overview of the *Colletotrichum lupini*, *Botrytis cinerea*, and *Pleiochaeta setosa* isolates, their host of origin, location, and year of isolation

Number	Host crop	Location	Year
Colletotrichum lupini			
CL1	*Lupinus albus*	Bottelare	2010
CL2	*Lupinus albus*	Bottelare	2010
CL3	*Lupinus mutabilis*	Bottelare	2010
CL4	*Lupinus mutabilis*	Bottelare	2010
CL5	*Lupinus mutabilis*	Bottelare	2010
Botrytis cinerea			
BC1	*Lupinus albus*	Bottelare	2010
BC2	*Lupinus albus*	Bottelare	2010
BC4	*Lupinus angustifolius*	Bottelare	2010
BC5	*Lupinus angustifolius*	Bottelare	2010
Pleiochaeta setosa			
PS1	*Lupinus luteus*	Bottelare	2010
PS2	*Cytisus scoparicus*	Koekelare	2010
PS3	*Lupinus angustifolius*	Pittem	2009
PS4	*Lupinus albus*	Koekelare	2009
PS5	*Lupinus albus (*var. *Dieta)*	Bottelare	2012
PS6	*Lupinus albus (*var. *Dieta)*	Vladslo	2012
PS7	*Lupinus albus*	Vladslo	2008
CBS 118.25	*Laburnum*	*Unknown*	
PS9	*Lupinus angustifolius var. Sonate*	Bottelare	2012

isolated from naturally infected *Fabaceae* species, except for *P. setosa*; one isolate from the CBS collection was included.

Essential oils

In this study, the anti-fungal effect of four 100% pure therapeutic grade essential oils (Omega Pharma): clove oil (*Syzygium aromaticum*), juniper oil (*Juniperus communis*), tea tree oil (*Melaleuca alternifoliai*), thyme essential oil (*Thymus vulgaris*) and one thyme extract timbor® (*Thymus vulgaris*) at different doses is evaluated. Timbor® (grupo agrotecnologia) is a natural plant extract derived from *Thymus vulgaris*; besides oil, this product also contains other natural plant components, e.g., polyphenolic compounds and flavonoids. In contrast to the pure oils, this product is intended for agricultural purposes. Since the main component of this extract is oil, this product is applied similarly as the pure oils in the experiments. The appropriate concentrations for the experiments were made in water with 0.1% Tween80.

Efficacy of plant essential oils on conidial germination

A 96-well plate assay was used to determine the effect of the five essential oils (four pure oils and timbor®) on the conidial germination. Spore suspensions of the different isolates were prepared with distilled water and diluted to 4×10^4 spores mL^{-1}. Twenty microliters of the oil dilution, 180 μL of potato dextrose broth (39 g L^{-1}), and 20 μL spore solution were mixed in each well of the 96-well plate. The final oil concentrations in the wells were as follows: 6.667, 3.333, 1.667, 0.833, and 0.417 μL mL^{-1}. These concentrations correspond to 2, 1, 0.5, 0.25, and 0.0625 L ha^{-1}, in case a spray volume of 300 L ha^{-1} is assumed. Each concentration was tested in six replicates and six replicates were used as negative controls (oil and medium with 20 μL water instead of spore solution). The absorbance at 620 nm was measured 1 and 2 weeks after incubation with a microplate Photometer (Asys UVM 330 Plate Reader). Based on the net optical density (density of the wells with spores subtracted by the density of the negative controls without spores), the growth percentage at each concentration

was calculated and the ED_{50} values were estimated using probit analysis.

In vitro efficacy of essential oils on mycelium growth

To test the inhibitory effect of the five essential oils (four pure oils and timbor®) on mycelium growth, they were added to autoclaved PDA medium (potato dextrose agar 39 g L^{-1}) cooled to 50 °C. The final oil concentrations in the medium were 6.667, 1.667, and 0.833 μL mL^{-1}. The medium with oil and the control medium were poured into Petri dishes. After solidification of the medium, a 5-mm mycelial disk was cut from the margin of an actively growing fungal colony with a cork borer and placed in the center of each Petri dish. Three replicates of each treatment were maintained at 22 °C. Based on the average colony diameter, measured in two perpendicular directions, 2, 5, and 20 days after inoculation, the growth rate was calculated. Finally, the growth rate on the media amended with oil was compared to the growth rate of the same isolate on the control medium to come to the relative growth rate. Remark that for this experiment, only two *P. setosa* isolates were used (isolates PS1 and PS2).

Effectivity of essential oils to control disease symptoms of *Pleiochaeta setosa*

To evaluate the effect of essential oils and timbor® to control *Pleiochaeta* root rot and brown leaf spot, pot trials with the broad-leaved lupin cultivar Dieta were set up. To induce root rot, 5-L pots were filled with standard potting soil (dry matter: 25%, organic matter: 20%, pH H_2O: 5–6.5, electrical conductivity: 1.5 mS/cm, NPK (14 + 16 + 18)) and 200 g wheat grains infected with a mixture of two *P. setosa* isolates (isolates PS1 and PS2). Infected wheat grains were prepared by mixing autoclaved wheat kernels with *P. setosa* mycelium from isolates grown on PDA medium. This mixture was maintained at 22 °C for 2 weeks and turned over and kneaded each day to ensure proper mixing and contact between wheat kernels and mycelium. After 3 weeks, five lupin seeds, treated with essential oils (5.7 mL per kg seed, i.e., 1 L ha^{-1}) (or untreated for the control objects) were sown in each pot. Seeds were coated with the products by use of a Hege 11 seed treater. Non-treated seeds were used as controls. The percentage emerging plants was evaluated 2 weeks after sowing and the length of the primary root and lateral roots were measured 3 weeks after sowing.

To induce brown leaf spot, five lupin seeds were sown in a 5-L pot filled with standard potting soil. Three weeks after emergence, the lupin plants were spray-infected with spores of the two *P. setosa* isolates (3×10^5 spores mL^{-1}). Two hours after inoculation, the plants were sprayed with essential oils at a dose rate of 1 L ha^{-1} (i.e., 3.33 μL mL^{-1}) (nozzle XR 110 03 (albuz)). Seven and 14 days after treatment, the percentage necrosis and chlorosis was assessed.

Both trials were set up in six replicates, i.e., six pots with five seeds for each essential oil and the control treatments. The plants were grown at 19 °C, 16 h light and 8 h dark.

Phytotoxicity of the essential oils

To gain insight into the potential phytotoxicity of the evaluated plant-derived components against lupin, a germination experiment was set up with seeds of broad-leaved, narrow-leaved, and yellow lupin (cv. Fortuna, Probor, and Lord). In order to obtain a high exposure to the plant-derived components, ten seeds were soaked in distilled water with an oil concentration of 6.667 μL mL^{-1} (2 L ha^{-1}) or 3.333 μL mL^{-1} (1 L ha^{-1}) for 12 h and subsequently air-dried at 20 °C for 2 h. Seeds soaked only in distilled water served as the control. For each oil and concentration, 40 seeds, 10 seeds per pot, were sown in 5-L pots filled with standard potting soil. At regular time points, the percentage seed germination was recorded.

Statistical analysis

For statistical evaluation, the R software package (R core Team 2014) version 2.15.3 was used. Since normality assumptions of parametric tests were not met, a non-parametric Kruskal-Wallis test was used to test if there were significant differences between groups of data (i.e., significant differences between oils and isolates). In case there was a significant difference (p value < 0.05), a pairwise comparison of the groups using a Dunn test was performed. The data are represented as box plots, which provide a graphical view of the median, quartiles, maximum, and minimum of the data. Above the box plots, a letter code distinguishes groups for which significant differences were observed.

Results

Efficacy of plant essential oils on conidial germination

In Fig. 1, the ED_{50} values for the spore germination for the five essential oils (four pure oils and timbor®) towards *C. lupini*, *P. setosa*, and *B. cinerea* are presented. For each species, there were significant differences between the ED_{50} values of the tested oils (Kruskal-Wallis test, p value < 0.05). Juniper oil was least effective, the ED_{50} values for *C. lupini* ranged between 3.99 and 5.84 μL mL^{-1}, for *P. setosa* between 1.64 and 5.01 μL mL^{-1}, and for *B. cinerea* between 4.48 and 12.03 μL mL^{-1}. The inhibitory effect of timbor® was the highest, with ED_{50} values between 0.34 and 0.53 μL mL^{-1} for *C. lupini*, between 0.42 and 0.58 μL mL^{-1} for *P. setosa*, and between 0.25 and 0.51 μL mL^{-1} for *B. cinerea*. However, the difference with thyme essential oil and clove oil was never significant. The effect of tea tree oil on the prevention of spore germination was intermediate. The ED_{50} values for this oil varied between 1.14 and 1.63 μL mL^{-1} for *C. lupini*, between 0.48 and 2.03 μL mL^{-1} for *P. setosa*, and between 0.60 and 1.48 μL mL^{-1} for *B. cinerea.*

In vitro efficacy of essential oils on mycelium growth

The spread of the relative growth rate, i.e., the growth rate on medium amended with an essential oil compared to growth rate on control medium, is depicted in Fig. 2. It can be seen that the growth rate of each species on each dose of clove oil and thyme essential oil was always significantly lower compared to growth rate on control medium. Even at the lowest concentration of 0.833 μL mL^{-1}, thyme essential oil completely inhibited the radial mycelium growth of the *C. lupini* isolates and *B. cinerea* isolates. Thyme essential oil was also most effective against the *P. setosa* isolates; however, to completely inhibit mycelium growth of this species, 6.667 μL mL^{-1} was necessary. As mentioned above, clove oil was very effective in suppressing mycelium growth rate; however, remark that for *C. lupini* and *B. cinerea*, a dose rate of 6.667 μL mL^{-1} clove oil was less effective than a dose rate of 0.833 μL mL^{-1}; however, the difference between the doses was not significant. Tea tree oil at the highest dose (6.667 μl mL^{-1}) and timbor® at a dose rate of 1.667 and 6.667 μL mL^{-1} resulted in a significantly reduced growth rate compared to the growth rate on control medium. At the highest concentration, tea tree oil completely inhibited the growth rate of *C. lupini* and *B. cinerea* isolates, whereas at lower doses, the mycelium growth rate was equal to the growth rate on control medium. The inhibitory effect of juniper oil was minimal, except at a concentration of 6.667 μL mL^{-1}, the growth rate of the *P. setosa* isolate isolated from *L. anagyroides* was suppressed. Remark that at a dose rate of 0.833 μL mL^{-1}, tea tree oil and juniper oil promoted the mycelium growth of *P. setosa* isolates, i.e., relative growth rates higher than 100%.

Concerning the differences between pathogens, it can be seen that the applied essential oils were most effective against *C. lupini* and *B. cinerea*. Furthermore, there was a considerable variation in effectiveness of the different oils against the two *P. setosa* isolates. All tested oils were most effective in suppressing the mycelium growth of the isolate from *L. albus*, except juniper oil, which was most effective against the isolate from *L. anagyroides*.

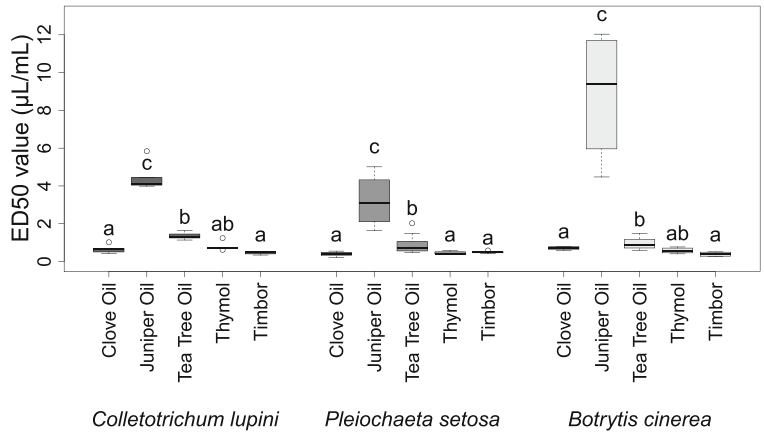

Fig. 1 ED_{50} values (μL mL^{-1}) indicating the sensitivity of *Colletotrichum lupini*, *Pleiochaeta setosa*, and *Botritys cinerea* for the five essential oils (clove oil, juniper oil, tea tree oil, thyme essential oil (thymol), and timbor®). Different letters per oil per species refer to significant differences between essential oils for the different species according to a Dunn test

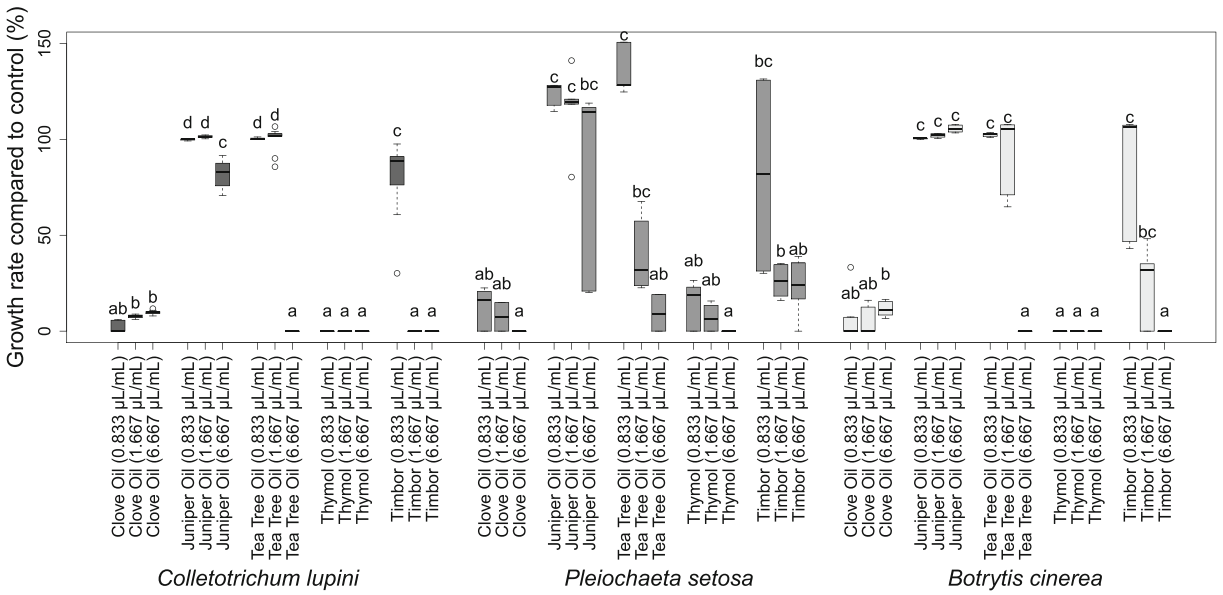

Fig. 2 Relative mycelium growth rate (%) of *Colletotrichum lupini*, *Pleiochaeta setosa*, and *Botritys cinerea* isolates on different essential oils and doses compared to the growth rate of these isolates on control medium. Different letters per species refer to significant differences in growth rate between the different oils and doses (Dunn test)

Effectivity of essential oils to control disease symptoms of *Pleiochaeta setosa*

The emergence of the lupin seeds significantly differed between treatments. Almost all seeds (97%) sown in pots without *P. setosa* infected wheat kernels emerged (uninfected control), whereas only 33% of the untreated seeds sown in pots with *P. setosa*-infected wheat kernels (infected control) emerged. The percentage growth of the oil-treated seeds sown in inoculated pots depended on the applied oil. Ninety percent of the timbor® and thyme essential oil-treated seeds developed into plants. The emergence percentages of the seeds treated with clove oil, juniper oil, and tea tree oil were 60, 47, and 43%, respectively. Figure 3 depicts the length of the primary root and length of the lateral roots of the lupin seedlings. The length of the primary root of plants that developed from the uninfected seeds or seeds treated with thyme essential oil or timbor® was significantly shorter compared to the plants from seeds treated with other oils. The primary root length of untreated plants grown in infected soil was the longest; however, the difference with juniper oil was not significant. The results concerning the lateral root length are opposite compared to the primary root length. Plants grown in uninfected soil or from seeds treated with thyme essential oil or timbor® had the longest lateral roots; however, the difference with the lateral root length of the plants developing from seeds treated with the other oils was not significant.

The results concerning the potential of the oils to suppress leaf chlorosis and necrosis were in line with those from the previous experiments (Fig. 4). Timbor® and thyme essential oil were most effective; these oils almost completely inhibited the development of disease symptoms. The effect of clove oil was intermediate. The symptom development of plants sprayed with juniper oil was similar to the untreated plants, illustrating that the anti-fungal effect of this compound is limited.

Phytotoxicity of the essential oils

The germination percentage of seeds soaked in essential oil was in most cases lower compared to the untreated seeds. Furthermore, there was a dose effect, soaking in an oil concentration of 2 L ha^{-1} resulted, in most cases, in a lower germination percentage compared to 1 L ha^{-1}. Clove oil seemed to be the most phytotoxic, whereas the effect of juniper oil and tea tree oil on the germination percentage was in most cases not significant. Concerning the *Thymus vulgaris*-based products, timbor® and thymol, it was seen that although both mostly resulted in a significantly reduced germination, timbor® was the least phytotoxic of both (Fig. 5).

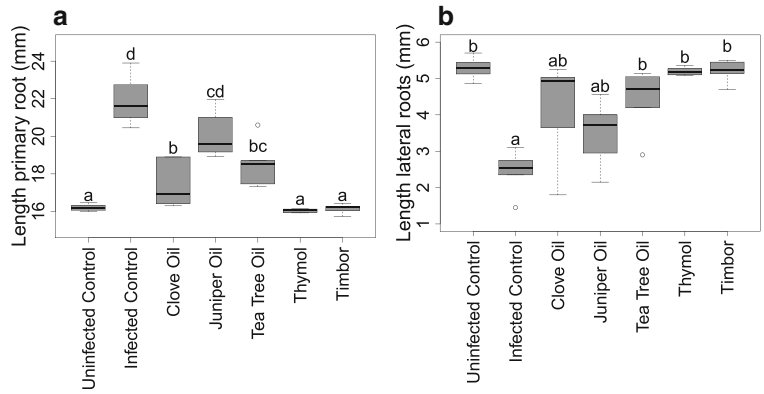

Fig. 3 Distribution of the length of the primary root (**a**) and lateral roots (**b**) of the uninfected control plants (without infected grain kernels and without essential oils), infected control (with infected grain kernels and without essential oils), and the plants grown with infected grain kernels and treated with the five essential oils. Different letters per oil refer to significant differences in length (Dunn test)

Discussion

Last years, the performance of synthetic fungicides is decreasing due to the frequent development of resistance by the pathogens towards certain chemicals. Furthermore, in the frame of integrated pest management, it is important to gain insight into the potential of alternative disease management strategies. Plant-derived components provide a promising and more environmentally-friendly alternative (or supplement) to conventional treatments. Therefore, in this study, the efficacy of five essential oils, four pure oils and the commercial *T. vulgaris* extract timbor®, for the treatment of the most frequently occurring diseases of lupin, caused by *C. lupini*, *P. setosa*, and *B. cinerea*, was evaluated.

The essential oils derived from *Thymus vulgaris*, timbor® and thyme essential oil, were the most effective in suppressing the conidial germination and mycelium growth of the studied pathogens. Even at the lowest dose rate of 0.25 L ha^{-1} (0.833 μL mL^{-1}), thyme essential oil completely inhibited the mycelium growth of *B. cinerea* and *C. lupini*. To completely inhibit the mycelium growth of *P. setosa*, a dose rate of 2 L ha^{-1} (6.667 μL mL^{-1}) was necessary. Timbor® had to be applied at a higher dosage (2 L ha^{-1}) to completely suppress mycelium growth the growth rate of *B. cinerea* and *C. lupini.* The differences between the ED_{50} values of both thyme-derived oils were never significant. The mean ED_{50} values for thyme essential oil for *C. lupini*, *P. setosa*, and *B. cinerea* were, respectively, 0.81 μL mL^{-1} (0.24 L ha^{-1}), 0.46 μL mL^{-1} (0.14 L ha^{-1}), and 0.59 μL mL^{-1} (0.18 L ha^{-1}) and for timbor® 0.46 μL mL^{-1} (0.14 L ha^{-1}), 0.48 μL mL^{-1} (0.15 L ha^{-1}), and 0.39 (0.12 L ha^{-1}). These values are clearly lower compared to the recommended dose of

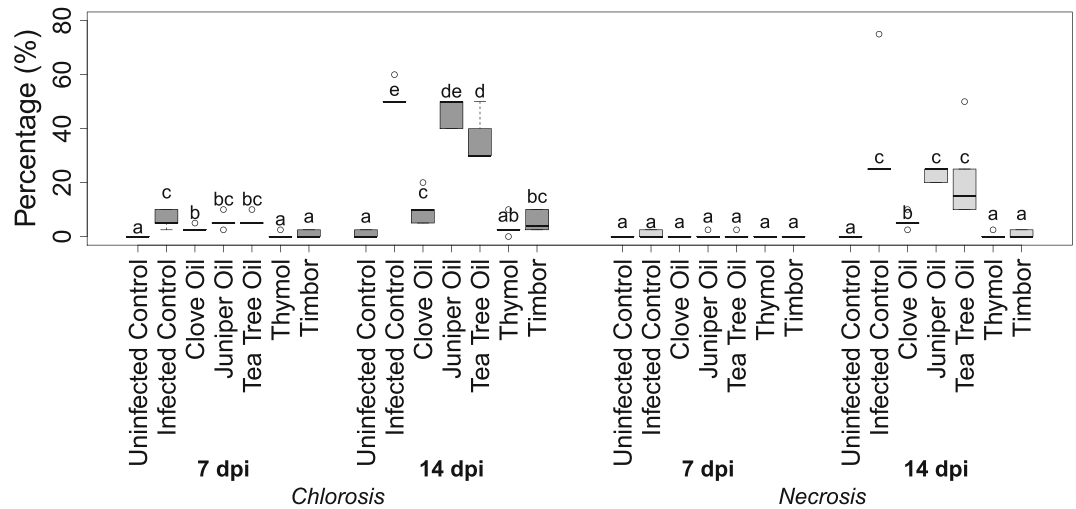

Fig. 4 Percentage chlorosis or necrosis caused by *Pleiochaeta setosa* 7 or 14 days post-inoculation. Different letters per oil and time point refer to significant differences between oils (Dunn test)

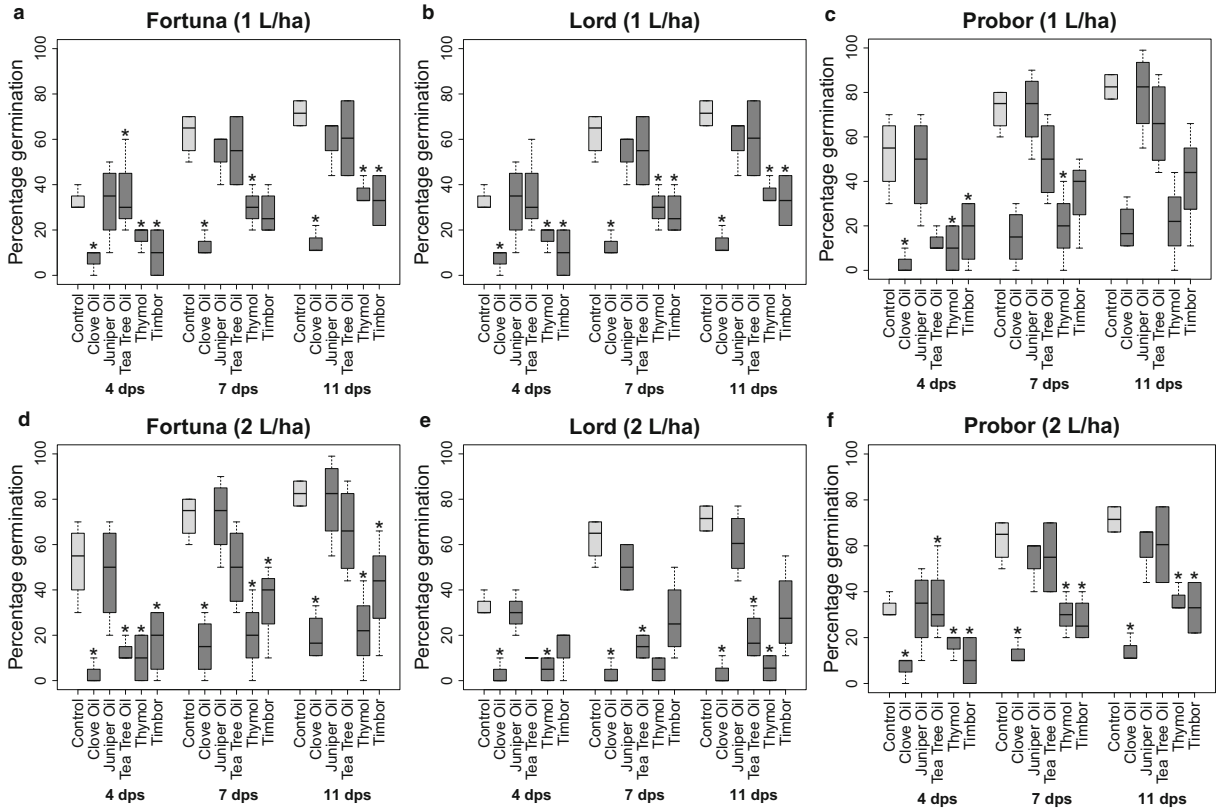

Fig. 5 Percentage seed germination (4, 7, and 11 days post-sowing) of lupin (cv. Fortuna (a,d), Lord (b,e), Probor (c,f)) seeds previously soaked in distilled water amended with essential oil at a concentration of 1 L ha^{-1} (**a**–**c**) or 2 L ha^{-1} (**d**–**f**). Treatments resulting in a significantly lower germination percentage are indicated with "*"

timbor® (0.9–1.5 L ha^{-1}). However, it has to be added that these in vitro values are an under estimation of the concentrations needed to effectively control the fungi in the field. In the in vitro trials, there is an optimal contact between the species and the oils, and there are no side effects (e.g., weather conditions, unequal distribution of product, etc.) affecting the performance of the products. To establish appropriate doses for field applications, field trials have to be performed.

The anti-fungal activity of essential oils is approved by previous research. In vitro and in vivo experiments conducted by Vitoratos et al. (2013) indicated that *Penicillium italicum* did not show any mycelium growth in the presence of thyme essential oils at a concentration of 0.13 μL mL^{-1}. Thyme essential oil is also able to inhibit the growth of *Alternaria alternata*, to reduce fungal viability, and to prevent the penetration in fruit (Perina et al. 2015). Furthermore, thyme essential oil showed almost 100% growth inhibition of the wheat pathogens, *Oculimacula yallundae*, *Microdochium nivale*, *Zymoseptoria tritici*, *Pyrenophora teres*, and *Fusarium culmorum*, at a concentration of 1 μL mL^{-1} (Matusinsky et al. 2015). Oils derived from *Thymus* spp. are also very effective to control *Verticillium* wilt in olive plants (Varo et al., 2017). The strong anti-fungal effect of *Thymus*-derived compounds can be attributed to the main component thyme essential oil. This monoterpene phenol induces oxidative stress resulting in fungal membrane destruction (Khan et al. 2015).

Additionally, it was seen that at the lowest dos rate (0.833 μL mL^{-1}), tea tree oil and juniper oil promoted the mycelium growth of *P. setosa* isolates. There are several reports showing that suboptimal doses of fungicides or other chemicals used in plant disease management can stimulate growth, pathogenicity, or toxin production. Zhou et al. (2014) demonstrated that sub-lethal doses of dimethachlon could stimulate mycelial growth of some *Sclerotinia sclerotiorum* isolates and increased virulence. In *Fusarium* species, sub-optimal fungicide concentrations can lead to increased toxin production (Audenaert et al. 2011). In McMahon et al. (2007), the efficacy of tea tree oil against several

pathogenic bacterial strains was evaluated; the results of this study suggest that the use of tea tree oil at sub-lethal concentrations can lead to the development of resistance towards this compound.

The pot trials approved the strong anti-fungal effect of thyme essential oil and timbor®. In the presence of these oils, at a concentration of 1 L ha^{-1}, the primary and lateral root length of the infected lupin plants was similar to the root length of the uninfected plants. Furthermore, the *P. setosa* leaf symptom development was almost completely suppressed when these oils were applied. Similar to the in vitro experiments, the effect of juniper oil was limited. Furthermore, from this trial, it can be concluded that, under *P. setosa* disease pressure, the germination percentage of lupin seeds treated with oils was higher compared to the germination percentage the untreated lupin seeds. An additional germination experiment, where seeds were soaked in essential oil, showed that, especially at higher concentrations, phytotoxicity problems can occur. This can be explained by the fact that the seeds were soaked into water with oil for 12 h, which is a considerably higher exposure compared to a conventional seed treatment with essential oils. So, to avoid problems with a reduced germination, for seed treatment, a proper mode of application is essential. Phytotoxicity is indeed an important aspect in the evaluation of natural products, since in previous research, it has been shown that some essential oils can cause phytotoxicity at the concentrations appropriate for the control of plant diseases. In vivo tests conducted by Sturchio et al. (2016) demonstrated phytotoxic effects of a clove plus rosemary oil mixture on *Vicia faba* roots when seeds were directly exposed to the essential oils.

Although there has been a lot of research in the field of alternative management strategies for plant diseases, this work, for the first time, illustrates the potential of plant extracts and essential oils to combat the main diseases of lupin. The fact that the oils are effective against both spore germination and mycelium growth indicates that these oils can be used preventive (by acting against spore germination) and curative (by acting against mycelium growth). To ensure yield and quality of upcoming, promising protein crops, such as lupin, research on sustainable disease control strategies is extremely important. Especially, in the frame of integrated pest management and the fact that lupin is often introduced in organic farming, research on the potential of various plant-derived anti-fungal compounds towards this crop is crucial.

Our findings, based on in vitro and in vivo experiments, suggest that essential oils, especially, thyme essential oil, and timbor®, are promising agents to control fungal diseases affecting lupin cultivation. However, before essential oils can become true alternatives to synthetic fungicides, field studies are necessary to evaluate the appropriate doses and suitable formulation, application interval, and possible effects on sensory parameters of the lupin crop.

Acknowledgements The authors gratefully acknowledge the technical staff of the experimental farm (Bottelare).

References

Amini J, Farhang V, Javadi T, Nazemi J (2016) Antifungal effect of plant essential oils on controlling *Phytophthora* species. Plant Pathol J 32:16–24

Audenaert K, Landschoot S, Vanheule A, Waegeman W, De Baets B, Haesaert G (2011) Impact of fungicide timing on the composition of the fusarium head blight disease complex and the presence of deoxynivalenol (DON) in wheat. In: Thajuddin N (ed) Fungicides-beneficial and Harmfull aspects, INTECH, pp 79–98

Behtoei H, Amini J, Javadi T, Sadeghi A (2012) Composition and in vitro antifungal activity *of Bunium persicum*, *Carum copticum* and *Cinnamomum zeylanicum* essential oils. J Med Plant Res 6:5069–5076

Daferera DJ, Ziogas BN, Polission MG (2003) The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp and *Clavibacter michiganensis* subsp *michiganensis*. Crop Prot 22:39–44

Dikbas N, Kotan R, Dadasoglu F, Sahin F (2008) Control of *Aspergillus flavus* with essential oil and methanol extract of Sartureja hortensis. Int J Food Microbiol 124:179–182

FAOSTAT (2016) Database collections. Food and agriculture organization of the United Nations. http://faostat.fao.org. Accessed 6 Jan 2018

Glencross B, Hawking W (2004) A comparison of the digestibility of lupin *(Lupinus* sp) kernel meals as dietary protein resources when fed to either, rainbow trout, *Oncorhynchus mykiss* or red seabream, *Pagrus auratus*. Aquac Nutr 10: 65–72

Kishore GK, Pande S, Harish S (2007) Evaluation of essential oils and their components for broad-spectrum antifungal activity and control of late leaf spot and crown rot diseases in peanut. Plant Dis 91:375–379

Khan A, Ahmad A, Khan A, Padoa LCJ, van Vuuren S, Manzoor N (2015) Effect of two monoterpene phenols on antioxidant defense system in Candida albicans. Microb Pathog 80:50–56

Lamichhane JR, Dachbrodth-Saaydeh S, Kudsk P, Messéan A (2016) Towards a reduced reliance on conventional pesticides in European agriculture. Plant Dis 100(1): 10–24

Lucas ML, Stoddard FL, Annicchiarico P, Frías J, Martínez-Villaluenga C, Sussmann D, Duranti M, Seger A, Zander PM, Pueyo JJ (2015) The future of lupin as a protein crop in Europe. Front Plant Sci 6:705

Matusinsky P, Zouhar M, Pavela R, Novy P (2015) Antifungal effect of five essential oils against important pathogenic fungi of cereals. Ind Crop Prod 67:208–215

McMahon MAS, Blair IS, Moore JE, McDowell DA (2007) Habituation to sub-lethal concentrations of tea tree oil (*Melaleuca alternifolia*) is associated with reduced susceptibility to antibiotics in human pathogens. J Antimicrob Chemother 59:125–127

Perina FJ, Amaral DC, Fernandes RS, Labory CR, Teixeira GA, Alves E (2015) *Thymus vulgaris* essential oil and thyme essential oil against *Alternaria alternata* (Fr) Keissler: effects on growth, viability, early infection and cellular mode of action. Pest Manag Sci 71(10): 1371–1378

R Core Team (2014) R: a language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria URL: http://wwwR-projectorg/

Rasooli I, Rezaei MB, Allameh A (2006) Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. Food Control 17:359–364

Sturchio E, Boccia P, Zanellato M, Meconi C, Donnarumma L, Mercurio G, Mecozzi M (2016) Molecular and structural changes induced by essential oils treatments in *Vicia faba* roots detected by genotoxicity testing. J Toxic Environ Health A 79:143–152

Sweetingham MW (1999) Low rates of dicarboximide seed treatment reduce brown spot in narrow-leafed lupin Australian journal of experimental. Agriculture 39(2):195–201

Talhinhas P, Baroncelli R, Le Floch G (2016) Anthracnose of lupins caused by *Colletotrichum Lupini*: a recent disease and a successful worldwide pathogen. J Plant Pathol 98(1): 5–14

Thomas GJ, Sweetingham MW (2004) Cultivar and environment influence the development of lupin anthracnose caused by *Colletotrichum lupini*. Australas Plant Pathol 33:571–577

Thomas GJ, Sweetingham MW, Adcock KG (2008) Application of fungicides to reduce yield loss in anthracnose-infected. Crop Prot 27(7):1071–1077

Tomioka K, Sawada H, Aoki T, Sato T (2008) Gray mold of pearl lupin caused by *Botrytis cinerea*. J Gen Plant Pathol 74:405–407

Varo A, Mulero-Aparicio A, Adem M, Roca LF, Raya-Ortega MC, López-Escudero FJ, Trapero A (2017) Screening water extracts and essential oils from Mediterranean plants against *Verticillium dahliae* in olive. Crop Prot 92:168–175

Vitoratos A, Bilalis B, Karkanis A, Efthimiadou A (2013) Antifungal activity of plant essential oils against *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41(1): 86–92

Wojakowska A, Kułak K, Jasiński M, Kachlicki P, Stawiński S, Stobieck M (2015) Metabolic response of narrow leaf lupine (*Lupinus angustifolius*) plants to elicitation and infection with *Colletotrichum lupini* under field conditions. Acta Physiol Plant 37:152

Zhou F, Liang H-J, Di Y-L, You H, Zhu F-X (2014) Stimulatory effects of sublethal doses of dimethachlon on *Sclerotinia sclerotiorum*. Plant Dis 98:1364–1370