Asian Journal of Plant Pathology, 2012
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2012.

Activity of Some Essential Oils against Pathogenic Seed Borne Fungi on Legumes

Enzo Marinelli, Laura Orzali, Elvira Lotti and Luca Riccioni
Consiglio per la ricerca e la sperimentazione in agricoltura, Centro di ricerca per la patologia vegetale (Plant Pathology Research Centre), Via C.G. Bertero, 22, I-00156 Rome, Italy

Corresponding Author: Enzo Marinelli, Consiglio per la ricerca e la sperimentazione in agricoltura, Centro di ricerca per la patologia vegetale (Plant Pathology Research Centre), Via C.G. Bertero, 22, I-00156 Rome, Italy Fax: +390682070249

ABSTRACT

Melaleuca alternifolia (tea tree), *Thymus vulgaris* (thyme), *Laurel nobilis* (laurel), *Mentha x piperita* (peppermint), *Origanum vulgare* (oregano), *Syzygium aromaticum* (clove) and *Rosmarinus officianalis* (rosemary) essential oils were tested for their antifungal activity to investigate the possibility of their use for legumes seed treatment. The ability of the seven oils to inhibit mycelial growth was studied by *in-vitro* assay on agar medium containing different concentrations of the essential oils. Six seed-borne pathogen fungi of large interest *Peyronellaea pinodella*, *Peyronellaea pinodes*, *Diaporthe phaseolorum* var. *caulivora*, *Phomopsis longicolla*, *Ascochyta lentis* and *Colletotrichum gloeosporioides* were transferred on the modified medium in order to test the oils antifungal activity, by calculating the percentage of Mycelial Growth Inhibition (MGI) and the Minimum Inhibitory Concentration (MIC). Results showed a clear reducing effect of the oils on fungal growth, that was dose-dependent and it differed depending on the fungal species. The most effective were thyme, clove, peppermint and oregano oils. Therefore, the essential oils tested can be considered very interesting for developing alternative natural fungicides to the synthetic chemicals and can be potentially used in organic agriculture to prevent and control seed-borne diseases for safe and low environmental impact seed treatments.

Key words: Oil efficacy, mycelial growth, minimum inhibitory concentration, organic farming, seed treatment, antifungal activity

INTRODUCTION

In order to reduce the problems associated with the use of synthetic fungicides such as pollution, phytotoxicity and selection of resistant pathogen populations, scientific research is undertaken to evaluate the effectiveness and reliability of different techniques to contain pathogens of crops and seeds like agronomics, physical methods, biological agents and chemicals with low environmental impact such as essential oils (Tinivella *et al*., 2009). Essential oils are products extracted from various parts of aromatic plants, in particular from the flowering tops, leaves, fruits, roots and rhizomes. They contain aromatic essences produced by plants to carry out allelopathic, antimicrobial and attraction of pollinator functions. These substances have long been studied for their characteristic bactericidal and fungicidal properties due to the presence of active compounds classifiable among alkaloids, phenols, flavonods, monoterpenes, sesquiterpenes and isoprenoids) (Alilou *et al*., 2008; Barrera-Necha *et al*., 2008; Kalemba and Kunicka, 2003; Scarito *et al*., 2002;

Zaccardelli *et al.*, 2006; Zambonelli *et al.*, 1996; Elisabeth *et al.*, 2008). In particular, tea tree oil (*Melaleuca alternifolia* Cheel), thyme oil (*Thymus vulgaris* Linnaeus), laurel oil (*Laurel nobilis* Linnaeus), peppermint oil (*Mentha x piperita),* oregano oil (*Origanum vulgare* Linnaeus), clove oil (*Syzygium aromaticum* Linnaeus*)* and rosemary oil (*Rosmarinus officianalis* Linnaeus) have been reported to possess antifungal activity (Carson and Riley, 1995; Dzamic *et al.*, 2008; El-Mougy, 2009; Korukluoglu *et al.*, 2009; Ozcan and Chalchat, 2008; Pina-Vaz *et al.*, 2004; Pinto *et al.*, 2006; Pintore *et al.*, 2002; Salamone *et al.*, 2002; Scarito *et al.*, 2003; Simic *et al.*, 2004; Sipailienee *et al.*, 2006; Soto-Mendivil *et al.*, 2006; Terzi *et al.*, 2007; Viuda-Martos *et al.*, 2007) and are considered to be potentially important for the agriculture, thanks to their effectiveness, non-toxicity to humans, biodegradability and easy availability (Nguefack *et al.*, 2008).

In many crops, including legumes, the problem of seed-borne diseases is one of the most important threats for the success of the production, as it can cause low germinability, seedlings blight and reducing yields (Tivoli and Banniza, 2007). Therefore, the control of pathogen organisms present on/in seeds is of vital significance especially for the organic production, where less efficient plant protection agents are available for managing plant diseases. In this work, some seed-borne pathogen fungi of large interest on legume (soybean, lentil, pea, lupin) crops were studied: *Ascochyta lentis* (Vassiljevsky), *Colletotrichum gloeosporioides* (Penzig) Saccardo, *Peyronellaea pinodella* (L.K. Jones) Aveskamp, Gruyter and Verkley (syn. *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema), *Peyronellaea pinodes* (Berk. and A. Bloxam) Aveskamp, Gruyter and Verkley (syn. *Mycosphaerella pinodes* (Berk and A. Bloxam) Vestergr.), *Phomopsis longicolla* (Hobbs), *Diaporthe phaseolorum* var. *caulivora* (Athow and Caldwell).

The aim of this study was to test the efficacy of the antifungal activity of seven different essential oils against these pathogenic seed-borne fungi by *in-vitro* assay, in order to evaluate the potential use of the oils for safe and eco-friendly seed treatments.

MATERIALS AND METHODS

The experiments were carry out during 2011, after having collected oils and fungal strains necessary to run the test at the laboratory of the Plant pathology research center in Rome, Italy (CRA-PAV).

Oils: Stocks of the following medicinal oils were purchased; Thyme Oil (TO), Laurel Oil (LO), Peppermint Oil (PO), Oregano Oil (OO), Rosemary Oil (RO) (Esperis s.p.a., Milan, Italy), Tea Tree oil (TTO) (Sovimpex, Marseille, France) and Clove Oil (CO) (Cedax Srl, Forlì-Cesena, Italy). The main active components of the oils used for *in vitro* trials are: terpinen-4-ol (40.7%) and γ-terpinene (20.3%) for TTO, thymol (41%) for TO, eugenol (16,3%) for CO, menthol (63,4%) for PO, 1,8 cineole (41,8%) for RO, thymol and carvacrol (67%) for OO, 1,8 cineole (40%) for LO. The concentrations were determined by the respective producer Companies, except for LO that was analysed by an accredited laboratory (Agri-bio-eco s.r.l., Pomezia, Italy).

Fungi: A total of 6 fungi (*P. pinodella*, *P. pinodes*, *D. phaseolorum* var. *caulivora*, *P. longicolla*, *A. lentis* and *C. gloeosporioides*), maintained on solid PDA medium (potato dextrose agar), were used for antifungal activity evaluation of TTO, TO, CO, LO, RO, PO and OO. The strain 1 of *P. pinodella* was provided from A.T.C.C. 16235-USA, while the others belong to CRA-PAV collection and were isolated from lentil seed (*A. lentis*), chickling vetch seed (*P. pinodes* and *P. pinodella*) soybean seeds (*P. longicolla*, *D. phaseolorum* var. *caulivora*) and from Banksia serrata leaves (*C. gloeosporioides*).

***In-vitro* activity:** Mycelial growth was determined on solid PDA medium amended with different oils at the following concentrations (v/v): 0% (control), 0.01, 0.025, 0.05 and 0.1% for TO, CO and OO, 0% (control), 0.25, 0.5, 1 and 1.5% for LO and RO, 0% (control), 0.1, 0.25, 0.5 and 1% for tea tree oil and finally 0% (control), 0.025, 0.05, 0.1 and 0.25% for PO, in presence of Tween 20 (0.5% v/v) as emulsifying agent. The experiments were conducted twice in duplicate in 90 mm Petri dishes containing the medium modified and inoculated with 6 mm plugs of PDA from actively growing cultures; after inoculations dishes were kept in the dark at 24°C. Radial growth of colonies was measured the seventh day after inoculation at two perpendicular points along the diameter of the plate and the means was used to calculate the mean daily radius of the fungal colony. Percentage inhibition of mycelial growth (MGI) by the oils was calculated using the equation:

$$\text{Inhibition}(\%) = \frac{(C - T)}{C} \times 100$$

where, C and T are the average of the colonies radius values in control and treatments, respectively, measured for each fungus at the seventh day after inoculation, obtained in both experiments. The Minimum Inhibitory Concentration (MIC), that produced a 90% reduction of growth compared with the growth of the oil-free control, was computed for each combination of fungus/compound at the seventh day after inoculation by second-degree polynomial regression.

Statistical analysis: Analysis of variance (ANOVA) was performed on the data obtained using CoStat-Statistics Software version 6.4. The significance of the differences among treated samples was evaluated using the Student-Newman-Keuls test (SNK) for multiple comparisons (significance level 0.05).

RESULTS AND DISCUSSION

The antifungal activity of the oils was evaluated considering the percentage of MGI (Table 1) and the MIC (Table 2-3), computed for each combination fungus/compound. All the essential oils were significantly ($p \leq 0.05$) active against all the fungal isolates tested at variable degree. About TO and CO, the concentration of 0.1% was able to completely inhibit the growth of all the fungi

Table 1: *In vitro* activity of oil as percentage of mycelial growth inhibition of fungus at different oil concentrations

	Thyme oil							
Fungus sp.	0.01%	SD	0.025%	SD	0.05%	SD	0.1%	SD
P. pinodella strain 1	-2^{e}	6.6	41^{b}	9.9	96^{a}	4.5	100^{a}	0.0
P. pinodella strain 2	20^{d}	5.9	49^{c}	7.3	90^{b}	3.7	100^{a}	0.0
P. pinodes	18^{b}	18.2	20^{b}	20.5	85^{a}	11.3	100^{a}	0.0
D. phaseolorum var. *caulivora*	73^{c}	1.0	91^{c}	2.4	97^{b}	3.9	100^{a}	0.0
P. longicolla strain 1	39^{d}	1.9	68^{c}	1.6	92^{b}	6.4	100^{a}	0.0
P. longicolla strain 2	56^{c}	5.6	76^{b}	1.2	99^{a}	1.4	100^{a}	0.0
A. lentis	36^{c}	6.2	62^{c}	4.3	94^{b}	3.9	100^{a}	0.0
C. gloeosporioides	9^{e}	24.0	30^{c}	10.7	73^{b}	4.2	100^{a}	0.0

Table 1: Continue

	Tea tree oil							
Fungus sp.	0.1%	SD	0.25%	SD	0.5%	SD	1%	SD
P. pinodella strain 1	57^{b}	8.3	97^{a}	4.0	100^{a}	0.0	100^{a}	0.0
P. pinodella strain 2	66^{b}	5.1	100^{a}	0.0	100^{a}	0.0	100^{a}	0.0
P. pinodes	47^{b}	21.1	97^{a}	4.5	100^{a}	0.0	100^{a}	0.0
D. phaseolorum var. *caulivora*	16^{c}	7.0	45^{b}	24.2	100^{a}	0.0	100^{a}	0.0
P. longicolla strain 1	20^{c}	16.5	64^{b}	19.4	100^{a}	0.0	100^{a}	0.0
P. longicolla strain 2	48^{b}	15.8	86^{a}	16.5	100^{a}	0.0	100^{a}	0.0
A. lentis	60^{b}	22.6	99^{a}	1.2	100^{a}	0.0	100^{a}	0.0
C. gloeosporioides	19^{c}	10.2	49^{b}	8.4	100^{a}	0.0	100^{a}	0.0
	Clove oil							
Fungus sp.	0.01%	SD	0.025%	SD	0.05%	SD	0.1%	SD
P. pinodella strain 1	27^{c}	10.7	67^{b}	15.0	98^{a}	1.1	100^{a}	0.0
P. pinodella strain 2	25^{c}	11.1	60^{b}	13.6	95^{a}	0.9	100^{a}	0.0
P. pinodes	9^{c}	18.5	55^{b}	9.7	87^{a}	1.2	100^{a}	0.0
D. phaseolorum var. *caulivora*	15^{c}	31.7	57^{b}	11.3	99^{a}	1.4	100^{a}	0.0
P. longicolla strain 1	12^{d}	15.9	45^{c}	25.0	71^{b}	9.2	100^{a}	0.0
P. longicolla strain 2	50^{b}	38.3	61^{b}	7.5	97^{a}	3.3	100^{a}	0.0
A. lentis	-14^{b}	24.3	12^{b}	25.3	70^{a}	23.1	100^{a}	0.0
C. gloeosporioides	18^{d}	2.7	29^{c}	8.6	82^{b}	2.6	100^{a}	0.0
	Laurel oil							
Fungus sp.	0.25%	SD	0.5%	SD	1%	SD	1.5%	SD
P. pinodella strain 1	73^{b}	18.1	86^{ab}	9.2	98^{a}	1.7	100^{a}	0.0
P. pinodella strain 2	79^{b}	7.2	91^{a}	10.3	98^{a}	4.8	100^{a}	0.0
P. pinodes	44^{b}	12.1	95^{a}	8.2	100^{a}	0.0	100^{a}	0.0
D. phaseolorum var. *caulivora*	23^{c}	3.0	53^{b}	10.7	95^{a}	8.8	100^{a}	0.0
P. longicolla strain 1	24^{d}	1.7	42^{c}	8.0	73^{b}	4.2	100^{a}	0.0
P. longicolla strain 2	79^{a}	16.6	80^{a}	24.7	100^{a}	0.0	100^{a}	0.0
A. lentis	75^{b}	10.0	93^{a}	8.1	100^{a}	0.0	100^{a}	0.0
C. gloeosporioides	53^{b}	7.8	95^{a}	4.0	100^{a}	0.0	100^{a}	0.0
	Rosemary oil							
Fungus sp.	0.25%	SD	0.5%	SD	1%	SD	1.5%	SD
P. pinodella strain 1	50^{c}	12.9	81^{b}	7.1	99^{a}	1.3	100^{a}	0.0
P. pinodella strain 2	77^{a}	3.6	95^{a}	5.6	100^{a}	0.0	100^{a}	0.0
P. pinodes	59^{b}	19.3	88^{a}	4.6	100^{a}	0.0	100^{a}	0.0
D. phaseolorum var. *caulivora*	17^{c}	14.0	75^{b}	10.3	99^{a}	1.0	100^{a}	0.0
P. longicolla strain 1	31^{d}	9.8	63^{c}	1.9	81^{b}	6.1	100^{a}	0.0
P. longicolla strain 2	42^{c}	5.9	75^{b}	13.3	100^{a}	0.0	100^{a}	0.0
A. lentis	78^{b}	10.4	97^{a}	2.9	100^{a}	0.0	100^{a}	0.0
C. gloeosporioides	47^{c}	17.2	76^{b}	2.2	100^{a}	0.0	100^{a}	0.0
	Peppermint oil							
Fungus sp.	0.025%	SD	0.05%	SD	0.1%	SD	0.25%	SD
P. pinodella strain 1	1^{c}	18.0	50^{b}	13.7	97^{a}	3.1	100^{a}	0.0
P. pinodella strain 2	17^{c}	7.9	62^{b}	8.5	99^{a}	1.5	100^{a}	0.0
P. pinodes	2^{c}	7.6	44^{b}	11.4	98^{a}	4.6	100^{a}	0.0

Table 1: Continue

	Peppermint oil							
Fungus sp.	0.025%	SD	0.05%	SD	0.1%	SD	0.25%	SD
D. phaseolorum var. *caulivora*	35^{b}	17.4	46^{b}	7.2	100^{a}	0.0	100^{a}	0.0
P. longicolla strain 1	20^{c}	19.4	49^{b}	17.4	96^{a}	5.6	100^{a}	0.0
P. longicolla strain 2	4^{c}	17.7	53^{b}	15.3	99^{a}	1.0	100^{a}	0.0
A. lentis	30^{c}	18.0	69^{b}	14.0	97^{a}	3.0	100^{a}	0.0
C. gloeosporioides	45^{b}	17.1	57^{b}	23.1	90^{a}	6.5	100^{a}	0.0
	Oregano oil							
Fungus sp.	0.01%	SD	0.025%	SD	0.05%	SD	0.1%	SD
P. pinodella strain 1	2^{c}	20.3	35^{b}	9.4	99^{a}	1.2	100^{a}	0.0
P. pinodella strain 2	8^{c}	19.3	40^{b}	13.0	99^{a}	1.4	100^{a}	0.0
P. pinodes	-49^{c}	42.0	5^{b}	10.7	99^{a}	1.2	100^{a}	0.0
D. phaseolorum var. *caulivora*	37^{c}	2.5	69^{b}	6.2	99^{a}	0.9	100^{a}	0.0
P. longicolla strain 1	26^{c}	7.8	45^{b}	16.5	99^{a}	0.8	100^{a}	0.0
P. longicolla strain 2	32^{b}	26.4	43^{b}	14.8	100^{a}	0.0	100^{a}	0.0
A. lentis	36^{c}	9.1	47^{b}	9.7	98^{a}	2.5	100^{a}	0.0
C. gloeosporioides	48^{b}	23.1	64^{b}	18.7	96^{a}	1.6	100^{a}	0.0

Hundred value refers to complete inhibition with no mycelial growth, SD: Standard deviation, a-d: Significance differences by Student-Newman-Keuls test ($p \leq 0.05$), Value followed by the same letter within the same row do not differ significantly

Table 2: Minimum inhibitory concentration of oils at the 7th day after inoculation

Fungus sp.	TO	CO	OO	PO
P. pinodella strain 1	0.05	0.04	0.07	0.09
P. pinodella strain 2	0.05	0.04	0.07	0.08
P. pinodes	0.06	0.05	0.07	0.09
D. phaseolorum var. *caulivora*	0.03	0.04	0.06	0.09
P. longicolla strain 1	0.05	0.07	0.06	0.09
P. longicolla strain 2	0.04	0.05	0.06	0.09
A. lentis	0.05	0.07	0.07	0.08
C. gloeosporioides	0.07	0.06	0.06	0.11

TO: Thyme, CO: Clove, PO: Peppermint and OO: Oregano

Table 3: Minimum inhibitory concentration of oils at the 7th day after inoculation

Fungus sp.	TTO	RO	LO
P. pinodella strain 1	0.30	0.77	0.68
P. pinodella strain 2	0.28	0.65	0.67
P. pinodes	0.31	0.72	0.71
D. phaseolorum var. *caulivora*	0.48	0.86	0.98
P. longicolla strain 1	0.40	1.02	1.25
P. longicolla strain 2	0.34	0.78	0.70
A. lentis	0.28	0.61	0.64
C. gloeosporioides	0.46	0.80	0.70

TTO: Tree oil, RO: Rosemary oil and LO: Laurel oil

tested. For some fungal strains, even the lower concentration of 0.05% was sufficient to ensure a complete inhibition statistically comparable ($p<0.05$) to the higher concentration, in particular: *P. pinodella* strain 1, *P. pinodes, P. longicolla* strain 2 for each oils, *D. phaseolorum* var. *Caulivora*,

P. pinodella strain 2 and *A. lentis* for CO. PO and OO gave steady results as they induced the total inhibition of growth for all fungi tested, at the concentration of 0.1 and 0.05%, respectively. TTO provided a total inhibition of fungal growth at concentrations much higher, achieving maximum results against all isolates at the concentration of 0.5%. For some fungi like *P. pinodella*, *P. pinodes*, *P. longicolla* strain 2 and *A. lentis* even the lower concentration of 0.25% was effective (Table 1).

The results regarding LO showed a different effectiveness among two different strains of *P. longicolla*, as they showed a different behavior in presence of the same oil, demonstrating a possible variability intra-species: for *P. longicolla* strain 1, the percentage of maximum inhibition (100%) was 1.5%, while for *P. longicolla* strain 2 was 1%, but no statistical differences were found up to 0.25% of LO; for all other isolates the optimum concentration was 0.5% (Table 1).

The results about RO were more contrasting among the different isolates: *P. pinodella* strain 2 underwent to a complete inhibition of fungal growth at 0.25% concentration; the optimum concentration rose to 0.5% for *A. lentis* and *P. pinodes* and to 1% for *D. phaseolorum* var. *caulivora*, *P. pinodella* strain 1, *P. longicolla* strain 2 and *C. gloeosporioides*; finally the growth of *P. longicolla* strain 1 was totally inhibited at the highest concentration tested of 1.5% (Table 1).

In general, data obtained at the lower concentrations possessed higher variability other than an unsatisfying antifungal activity; as the concentrations (and consequently the effectiveness) raise, the standard deviations became lower, obtaining an higher robustness of the data. Comparing MIC values, the oils tested can be split into two distinct groups on the basis of their activity. TO, CO, PO and OO presented a comparable effectiveness, with very low MIC values (Table 2), while TTO, LO and RO showed MIC values around 10 times higher than the previous (Table 3). However, the oils activity was proved to be dependent on the fungal specie and, in the case of LO and RO, even on the strain, as reported above. The highest MIC value was 1.25% of LO for *P. longicolla* strain 1 while the lowest one was 0.03% of TO for *D. phaseolorum* var. *caulivora*.

Broadly, our results demonstrated that all the oils possess a remarkable antifungal activity, with TO, CO, PO and OO being more effective at the same concentration than TTO, LO and RO in mycelial growth inhibition and this occurred with all the fungi tested. Previous results (Dzamic *et al*., 2008) indicate that the antimicrobial activity of essential oils is affected by their composition and the higher antifungal effect could be attributed to the higher amount of compounds with the phenol structure such as thymol, carvacrol, eugenol and menthol, that are indeed the main components of TO, CO, PO and OO.

The oils tested posses different commercial values and different volumes are needed to achieve the highest MIC concentration effective against fungi. In order to provide an effective and affordable solution that combines the best effectiveness and the lowest price, the cost of 1 liter treatment solution was calculated for each oil considering the concentration of the active component, the efficacy (MIC values) and the value (Table 4): CO and PO seem to give the best perspective considering the volume needed for one litre solution treatment, followed by TO and OO; LO is the oil with less perspective because of the high cost.

Table 4: Cost of 1 litre solution treatment, calculated on the basis of its cost and the volume needed to achieve the highest MIC concentration

	Thyme oil	Clove oil	Oregano oil	Peppermint oil	Tea tree oil	Rosemary oil	Laurel oil
Highest MIC	0.07	0.07	0.07	0.11	0.48	1.02	1.25
Cost*	€ 0.12	€ 0.03	€ 0.13	€ 0.06	€ 0.38	€ 0.50	€ 2.38

*€: Euro currency

Many other studies were focused on the antifungal activities of essential oils against plant pathogenic fungi of agricultural interest. In a recent paper (Riccioni and Orzali, 2011) the antifungal activity of thyme and tea tree oil was showed against seven seed borne pathogens of other crops: *F. graminearum*, *F. culmorum*, *A. radicina*, *A. dauci*, *A. rabiei*, *C. lindemuthianum* and *D. avenae*. Velluti *et al.* (2004) tested cinnamon, clove, oregano, palmarosa and lemongrass oils against *F. graminearum*, one of the main causal agents of the root and foot rot and fusarium ear blight in wheat; Faria *et al.* (2006) investigated the activity of *Ocimum gratissimum* essential oil against some phytopatogenic fungi like *Botryosphaeria rhodina*, *Rhizoctonia* sp. and *Alternaria* sp. Terzi *et al.* (2007) reported the in vitro antifungal activity of the tea tree essential oil and its major components against some cereals pathogen fungi as *Blumeria graminis* f. sp. *hordei*, *F. culmorum*, *F. graminearum*, *Pyrenophora graminea*. Simic *et al.* (2004) tested the chemical composition of some lauraceae essential oils and their antifungal activities against 17 micromycetes.

CONCLUSION

Results achieved in this study were consistent with the previous ones, confirming the essential oils antifungal broad-spectrum efficacy and showing promising prospects for the utilization of natural plant essential oils in crop production systems as alternative safe natural antifungal agents, on the basis of their efficacy, non-toxicity to humans, systemicity, biodegradability, easy availability and cost. Seed treatment against seed-borne fungi could be one of this potentially use.

However, because the in vitro effects did not always provide a good criterion for their *in-vivo* performances, further investigations are needed as well as field experiments to verify their possible phytotoxicity on plant/seed material and their effectiveness in field conditions as seed treatment.

ACKNOWLEDGMENT

The authors thank the Ministero delle Politiche Agricole Alimentari e Forestali for financial support the "Piano Sementiero Nazionale per l'Agricoltura Biologica" coordinated by INRAN-ENSE.

REFERENCES

Alilou, H., M. Akssira, L.I. Hassani, B. Chebli and A. El Hakmoui *et al.*, 2008. Chemical composition and antifungal activity of *Bubonium imbricatum* volatile oil. Phytopathol. Mediterr., 47: 3-10.

Barrera-Necha, L.L., S. Bautista-Banos, H.E. Flores-Moctezuma and A.R. Estudillo, 2008. Efficacy of essential oils on the conidial germination, growth of *Colletotrichum gloeosporioides* (Penz.) penz. and sacc and control of postharvest diseases in papaya (*Carica papaya* L.). Plant Pathol. J., 7: 174-178.

Carson, C.F. and T.V. Riley, 1995. Antimicrobial activity of the major components of the essential oil of *M. alternifolia*. J. Applied Bacteriol., 78: 264-269.

Dzamic, A., M. Sokovic, M.S. Ristic, S. Grujic-Jovanovic, J. Vukojevic and P.D. Marin, 2008. Chemical composition and antifungal activity of *Origanum heracleoticum* essential oil. Chem. Nat. Compounds, 44: 659-660.

El-Mougy, N.S., 2009. Effect of some essential oils for limiting early blight (*Alternaria solani*) development in potato field. J. Plant Prot. Res., 49: 57-62.

Elisabeth, Z.P., S. Paco, L. Vibeke, S. Philippe, S. Irenee and N. Adama, 2008. Importance of seed-borne fungi of sorghum and pearl millet in burkina faso and their control using plant extracts. Pak. J. Biol. Sci., 11: 321-331.

Faria, T.J., R.S. Ferreira, L. Yassumoto, J.R.P. de Souza, N.K. Ishikawa and A.M. Barbosa, 2006. Antifungal activity of essential oil isolated from *Ocimum gratissimum* L. (eugenol chemotype) against phytopathogenic fungi. Braz. Arch. Biol. Technol., 49: 867-871.

Kalemba, D. and A. Kunicka, 2003. Antibacterial and antifungal properties of essential oils. Curr. Med. Chem., 10: 813-829.

Korukluoglu, M., O. Gurbuz, Y. Sahan, A. Yigit, O. Kacar and R. Rouseff, 2009. Chemical characterization and antifungal activity of *Origanum onites* L. essential oils and extracts. J. Food Saf., 29: 144-161.

Nguefack J., V. Leth, J.B.L. Dongmo, J. Torp, P.H.A. Zollo and S. Nyasse, 2008. Use of three essential oils as seed treatments against seed-borne fungi of rice (*Oryza sativa* L.). Am. Eurasian J. Agric. Environ. Sci., 4: 554-560.

Ozcan, M.M. and J.C. Chalchat, 2008. Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. Int. J. Food Sci. Nutr., 59: 691-698.

Pina-Vaz, C., A.G. Rodfigues, E. Pinto, H.S. Costa-de-Oiiveira and C. Tavares *et al.*, 2004. Antifungal activity of thymus oils and their major compounds. J. Eur. Acad. Dermatol. Venereol., 18: 73-78.

Pinto, E., C. Pina-Vaz, L. Salgueiro, M.J. Goncalves and S. Costa-de-Oliveira *et al.*, 2006. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida, Aspergillus* and *dermatophyte* species. J. Med. Microbiol., 55: 1367-1373.

Pintore, G., M. Usai, P. Bradesi, C. Juliano and G. Boatto *et al.*, 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour Fragrance J., 17: 15-19.

Riccioni, L. and L. Orzali, 2011. Activity of tea tree (*Melaleuca alternifolia*, Cheel) and thyme (*Thymus vulgaris*, Linnaeus.) essential oils against some pathogenic seed borne fungi. J. Essent. Oils Res., 23: 43-47.

Salamone, A., G. Scarito and V. Somma, 2002. Attivita antifungina *in vitro* e *in planta* di olio essenziale di Origano (*Origanum heracleoticum* L.) [Antifungal activity *in vitro* and *in planta* of essential oil of oregano (*Origanum heracleoticum* L.)]. Atti Giornate Fitopatologiche, 2: 533-537.

Scarito, G., A. Salamone, G.V. Zizzo and S. Agnello, 2003. Indagini preliminari sullattivita inibente *in vitro* e in serra dellolio essenziale di chiodi di garofano nei confronti di *Sphaerotheca pannosa* (var. rosae) su rosa [Preliminary investigations on the activity inhibiting *in vitro* and in greenhouse of the essential oil of cloves against *Sphaerotheca pannosa* (var. rosae) of pink]. Notiziario sulla Protezione delle Piante, 17: 147-151.

Scarito, G., A. Salamone, V. Somma and G. Pirajno, 2002. Effetto inibente di alcuni oli essenziali sull'accrescimento *in vitro* di funghi fitopatogeni [Inhibitory effect of some essential oils on growth *in vitro* of phytopathogenic fungi]. Atti Giornate Fitopatologiche, 2: 529-532.

Simic, A., M.D. Sokovic, M. Ristic, S. Grujic-Jovanovic, J. Vukojevic and P.D. Marin, 2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytother. Res., 18: 713-717.

Sipailienee, A., P.R. Venskutonis, R. Baranauskiene and A. Sarkinas, 2006. Antimicrobial activity of commercial samples of thyme and marjoram oils. J. Essent. Oils Res., 18: 698-703.

Soto-Mendivil, E.A., J.F. Moreno-Rodriguez, M. Estarron-Espinosa, J.A. Garcia-Fajardo and E.N. Obledo-Vasquez, 2006. Chemical composition and fungicidal activity of the essential oil of *Thymus vulgaris* against *Alternaria citri*. e-Gnosis, Vol. 4.

Terzi, V., C. Morcia, P. Faccioli, G. Vale, G. Sacconi and M. Malnati, 2007. *In vitro* antifungal activity of the tea tree (*Melaleuca alternifolia*) essential oil and its major components against plant pathogens. Lett. Applied Microbiol., 44: 613-618.

Tinivella, F., L.M. Hirata, M.A. Celan, S.A.I. Wright and T. Amein *et al.*, 2009. Control of seed-borne pathogens on legumes by microbial and other alternative seed treatments. Eur. J. Plant Pathol., 123: 139-151.

Tivoli, B. and S. Banniza, 2007. Comparison of the epidemiology of *Ascochyta* blights on grain legumes. Eur. J. Plant Pathol., 119: 59-76.

Velluti, A., V. Sanchis, A.J. Ramos, C. Turon and S. Marin, 2004. Impact of essential oils on Growth rate, zearalenone and deoxynivalenol production by *Fusarium graminearium* under different temperature and water activity conditions in maize grain. Food Microbiol., 21: 313-318.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez-Alvarez, 2007. Antifungal activities of thyme, clove and oregano essential oils. J. Food Saf., 27: 91-101.

Zaccardelli, M., E. Mancini, F. De Nicola, F. Campanile, E. De Feo and E. De Falco, 2006. Potential use of esential oil, extracted from officinal plants cultivated in Mediterraneo area, to control phytopathogenic bacteria and fungi. J. Plant Pathol., 88: 63-63.

Zambonelli, A., A.Z. D'Aulerio, A. Bianchi and A. Albasini, 1996. Effects of essential oils on phytopathogenic fungi *in vitro*. J. Phytopathol., 144: 491-494.