Crop Protection 29 (2010) 295–299

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Activities of essential oils from *Asarum heterotropoides* var. *mandshuricum* against five phytopathogens

Yang Dan, Hai-Yan Liu, Wei-Wei Gao*, Shi-Lin Chen

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, No. 151 Malianwa North Road, Haidian District, Beijing 100193, China

ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form
26 November 2009
Accepted 6 December 2009

Keywords:
Asarum heterotropoides var. *mandshuricum*
Methyleugenol
Antiphytopathogenic activities
TEM
GC/MS

ABSTRACT

Some secondary metabolites of plants function as antimicrobial products against phytopathogens and constitute an increasingly important class of pesticides. In the present study, the essential oil of *Asarum heterotropoides* var. *mandshuricum* was analyzed by GC/MS and its antimicrobial activity was evaluated against five phytopathogenic fungi. Major components of the oil were methyleugenol (59.42%), eucarvone (24.10%), 5-allyl-1,2,3-trimethoxybenzene (5.72%), and 3,7,7-trimethylbicyclo(4.1.0)hept-3-ene (4.93%). The essential oil and the most abundant component, methyleugenol, were separately assayed for inhibition of 5 pathogens: *Alternaria humicola*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Phytophthora cactorum* and *Fusarium solani*. Both the oil and methyleugenol strongly inhibited the growth of the test pathogens (IC_{50} values <0.42 μg ml^{-1}) except *F. solani*, with the best activity against *P. cactorum* (IC_{50} values $= 0.073$ and 0.052 μg ml^{-1}, respectively). It is concluded that the essential oil of *A. heterotropoides* var. *mandshuricum* has a broad antiphytopathogenic spectrum, and that methyleugenol is largely responsible for the bioactivity of the oil. The mode of action of methyleugenol against *P. cactorum* is discussed based on changes in the mycelial ultrastructure.

1. Introduction

In recent decades, plant products have come to be exploited as botanical pesticides because they are more biodegradable than synthetic pesticides (Duke, 1990). In addition, many pathogens have become a serious threat to crops due to their resistance to known chemical control agents including benzimidazoles, demethylation inhibitors, Qo respiration inhibitors and dicarboximides (Ma and Michailides, 2005; Ishii, 2006). This has occasioned a growing effort in the search for new bioactive products (Duke, 1990; Gigante et al., 2002). A great number of plant essential oils exhibit antimycotic properties and are potential antifungal products (Deans et al., 1989; Lee et al., 2008; Chang et al., 2008). One such essential oil is that from *Asarum heterotropoides* F. Schmidt var. *mandshuricum* (Maxim.) Kitag (Maximowicz) Kitagawa (Aristolochiaceae) (Liu et al., 2007; Wang et al., 2008).

A. heterotropoides var. *mandshuricum*, a perennial herb endemic to China, is a traditional Chinese medicine by the name of Xixin (Huang et al., 2003). It can be found in forests, mountain slopes, valleys and moist shady areas. It is occasionally cultivated in the Heilongjiang, Jilin, Liaoning and South China regions (Huang et al., 2003). Its essential oil is used for analgesic, antitussive, and antiallergic purposes in China (Hashimoto et al., 1994). The components in the essential oil had been investigated (Zeng et al., 2004; Zhang et al., 2004) and a total of 82 components were identified, of which methyleugenol was found to be the most abundant (Kosuge et al., 1978; Zeng et al., 2004).

In previous investigations, this essential oil was found to possess the promising antifungal activity against a variety of plant pathogens (Liu et al., 2007; Wang and Ji, 2007; Wang et al., 2008). However, the antifungal constituents in the oil were not determined. Nevertheless, we could assume that methyleugenol might be the main constituent responsible for the bioactivity of the *A. heterotropoides* var. *mandshuricum* essential oil from the following facts: the compound is not only the most abundant component of this oil, but it is also widely distributed in the other aromatic plants which possess a wide spectrum of activities against microorganisms ranging from bacteria to fungi (Kivanç, 1988); its antifungal activity against *Botrytis cinerea* Pers and *Colletotrichum fragariae* Brooks had been reported in *Artemisia dracunculus* L. var. *dracunculus* by Meepagala et al. (2002). In order to verify our assumption and elucidate the main constituents responsible for the activity of the oil, we analyzed the essential oil of *A. heterotropoides* var. *mandshuricum* by GC/MS, evaluated the antifungal activity of

* Corresponding author. Tel.: +86 10 6289 9737; fax: +86 10 6289 6313.
E-mail address: wwgao411@sina.com (W.W. Gao).

0261-2194/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2009.12.007

the oil and methyleugenol against five representatives of plant pathogens causing great damages in crops, and explored the mode of action of the main active component.

2. Materials and methods

2.1. Plant materials

Dried whole plant of *A. heterotropoides* var. *mandshuricum* was collected from Xinbin Manchus (N 125.6°, E 41.42°), Liaoning, China. A voucher specimen (No. 262) was deposited in the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China.

2.2. Preparation of essential oils and chemical reference substance

Dried plant material (600 g) was chopped into small pieces and subjected to hydrodistillation for 8 h, using a Clevenger-type apparatus to yield 6.87 g (1.15%) of a light brown oil. The essential oil was dissolved in methylene chloride, dried over anhydrous sodium sulfate, and stored in sealed glass vials at 4 °C prior to analysis. Methyleugenol (purity, 95%) was purchased from Sigma–Aldrich (Milwaukee, WI, USA).

2.3. Gas chromatography/mass spectrometry analysis

GC/MS analysis was performed on a gas chromatograph (ThermoQuest Trace 2000) coupled to a mass spectrometer (Thermo Finnigan Voyager GC/MS) equipped with a DB-5 MS capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness). Samples (0.4 μl) were injected in the split mode (1:50). The oven temperature was held at 50 °C for 1 min, raised to 290 °C at a rate of 8 °C min^{-1}, and then held at 290 °C for 20 min. Helium, used as the carrier gas, was passed through the column at a rate of 0.8 ml min^{-1}. The MS was operated in the El mode at 70 eV, in the *m*/*z* range 28–540. The source temperature was 230 °C and the transfer line was set at 250 °C. Compounds were identified by comparison of their retention indices and mass spectra with those found in the literature and the NIST Mass Spectral Library (2005 Version). Retention indices (RI) were calculated using an *n*-alkane series (C_9–C_{16}) under the same GC/MS conditions as used for sample. Components were quantified by integrating the peak areas on the chromatogram. The standard chemical, methyleugenol, was analyzed under the same GC/MS conditions.

2.4. Microorganism strains and culture conditions

Five plant pathogens were used: *Alternaria humicola* Oudem. (IMCAS 3.2917), *Fusarium solani* (Mart.) Sacc., *Rhizoctonia solani* J.G. Kühn, *Phytophthora cactorum* (Lebert & Cohn) J. Schröt., and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. *A. humicola* was offered by the Institute of Microbiology, Chinese Academy of Sciences. *C. gloeosporioides* was provided by the Plant Protection Institute of Ningxia, China. *F. solani*, *R. solani* and *P. cactorum* were isolated from ginseng shoots in our laboratory, and were incubated on potato dextrose agar (PDA) at 23 °C in the dark.

2.5. Bioassay and statistical analysis

The antimicrobial activities of the essential oil and methyleugenol against the plant pathogens were evaluated by the growth inhibition bioassay (Lee et al., 2008). The tested reagents were dissolved in acetone, and then added to the sterile culture medium (PDA) at the specified concentrations. Following thorough mixing, the media were poured into Petri dishes (9 cm i.d.). The small amount of acetone added with each reagent had no effect on the growth of pathogens. Then 5 × 5 mm agar plugs infected with fungi were incubated on each plate, one on each half, at 23 °C in the dark. Colony growth diameters were measured after 48 h. All treatments were tested in quadruplicate, and plates without any additives were used as controls. The IC_{50} values and p-test were carried out by SPSS 13.0 software, so did mean values and standard deviations.

Growth inhibition was calculated as follows:

$$\%\text{Inhibition} = (C - T)/(C - 5) \times 100$$

C and T were the averages from four replicates of hyphal extension (mm) in the control and the treatment, respectively.

2.6. Ultrastructure observation

The mycelia of *P. cactorum* from the control and the treatments were examined by transmission electron microscopy (TEM). Small segments of agar ($D = 7$ mm) with mycelia were fixed in 3% glutaraldehyde and 2% formaldehyde in 0.1 mol l^{-1} phosphate buffer and prepared for electron microscopy as previously described (Brewster et al., 1997).

3. Results

3.1. Major chemical components of the essential oil

In total, 32 peaks were detected by GC/MS and only the major constituents are shown in Table 1. Seven compounds were identified by GC/MS and by the retention indexes, including terpenes and phenylpropanes. The oil was dominated by methyleugenol (59.42%), eucarvone (24.10%), 5-allyl-1,2,3-trimethoxybenzene (5.72%) and 3,7,7-trimethylbicyclo(4.1.0)hept-3-ene (4.93%), and also included 2,6,6-trimethylbicyclo(3.1.1)hept-2-ene (0.42%), (1S)-(1)-beta-pinene (0.75%), and 1,3-dimethoxy-5-methylbenzene (0.63%).

3.2. Antimicrobial activity

The antimicrobial effects of the essential oil of *A. heterotropoides* var. *mandshuricum* and the major constituent methyleugenol

Table 1
Constituents of the essential oil of *Asarum heterotropoides* var. *mandshuricum*, identified by GC/MS and Kovats retention indexes (RI) and listed in order of elution from a DB-5 MS column.

Compounds	RI_a	RI_b	Proportion (%)
2,6,6-Trimethylbicyclo(3.1.1)hept-2-ene	952	953 (Jirovetz et al., 2003)	0.42
(1S)-(1)-beta-Pinene	994	990 (Jirovetz et al., 2003)	0.75
3,7,7-Trimethylbicyclo(4.1.0)hept-3-ene	1007	1005 (Sefidkon and Omidbaigi, 2004)	4.93
Eucarvone	1225	1223 (Leffingwell and Alford, 2005)	24.1
1,3-Dimethoxy-5-methylbenzene	1266	1264 (Hamm et al., 2004)	0.63
Methyleugenol	1412	1410 (Pino et al., 2005)	59.42
5-Allyl-1,2,3-trimethoxybenzene	1556	1556 (da Silva et al., 1999)	5.72

Kovats indices (RI_a) were calculated against C_9–C_{16} *n*-alkanes on DB-5 MS column. RI_b values were from literature.

against the five pathogens are shown in Figs. 1 and 2, and their IC_{50} values are present in Table 2. The bioactivities varied depending on the phytopathogens tested. Both the essential oil and methyleugenol exhibited the most potent activity against *P. cactorum* and had weak activity against *F. solani*. The IC_{50} value of methyleugenol against *P. cactorum* was 0.052 μg ml^{-1}, lower than that of the essential oil (0.073 μg ml^{-1}). Methyleugenol also exhibited stronger activities against all of other fungi than the essential oil.

3.3. Ultrastructure of mycelium of *P. cactorum* exposed to methyleugenol

In order to understand the mode of methyleugenol against phytopathogens, changes in the ultrastructure of hyphae of *P. cactorum* after treatment with the compound were examined under TEM. As can be seen in Fig. 3, healthy hyphae of *P. cactorum* contained a finely granulated cytoplasm, small vacuoles, and the usual complement of micro-organelles (Fig. 3a and b). Further, few mitochondria were present, the endoplasmic reticulum was not distinguished, and the plasmalemma was thin (Fig. 3a) in the healthy hyphae. By contrast, in the hyphae of *P. cactorum* exposed to methyleugenol, the plasmalemma was slightly invaginated and the cell wall was thickened (Fig. 3c and d), membranous micro-organelles such as vacuoles and mitochondria were losing their structures, membranes were becoming partly obscured, numerous mitochondria appeared, the endoplasmic reticulum became distinguished, and irregular cavae were presented. However, the lomasome and electron-dense structures were absent (Fig. 3c and d), and lipid globules showed little change.

4. Discussion

The main results in the present study was consistent with those previous investigations in which, the essential oil of *A. heterotropoides* var. *mandshuricum* exhibited antifungal activities against pathogens such as *Gloeosporium* sp., *Pestalotiopsis* sp., *Actinonema rosae* (Lib.) Fr., *Alternaria* spp., *Fusarium* spp., *Bipolaris* spp., *Curvularia lunata* (Wakker) Boedijn *Ustilago maydis* (DC.) Corda with IC_{50} values <400 μg ml^{-1} (Liu et al., 2007; Wang and Ji, 2007). However, the activity of the essential oil against *F. solani* was found to be weak in the present study, in disagreement with the previous study. This was probably due to differences in plant sample sources, extraction methods or strains of *Fusarium*. Therefore, we analyzed the essential oil and determined the bioactive components and their relative abundances (Tables 1 and 2). In the present study, methyleugenol was dominant in the oil, which is consistent with Kosuge et al. (1978) and Zeng et al. (2004). Meanwhile it is similar with the oil in terms of the tendency towards the test phytopathogens and

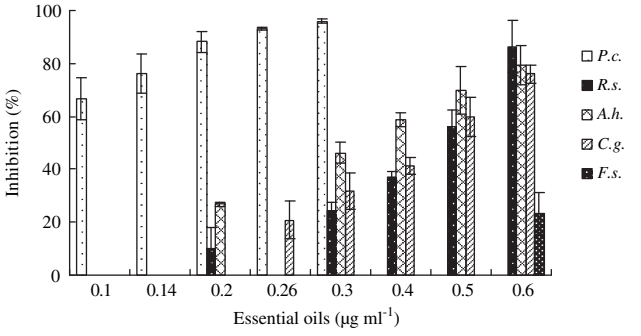

Fig. 1. Antiphytopathogenic activities of the essential oil of *Asarum heterotropoides* var. *mandshuricum*. *P.c.*: *Phytophthora cactorum*; *R.s.*: *Rhizoctonia solani*; *A.h.*: *Alternaria humicola*; *C.g.*:*Colletotrichum gloeosporioides*; *F.s.*: *Fusarium solani*.

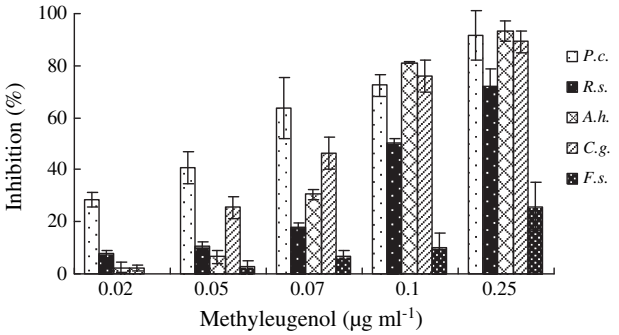

Fig. 2. Antiphytopathogenic activities of methyleugenol. *P.c.*: *Phytophthora cactorum*; *R.s.*: *Rhizoctonia solani*; *A.h.*: *Alternaria humicola*; *C.g.*:*Colletotrichum gloeosporioides*; *F.s.*: *Fusarium solani*.

the antifungal potencies, indicating that it was the major constituent responsible for the antifungal activity of the oil. However, it was not excluded that other main constituents, such as eucarvone (24.1%) and 5-allyl-1,2,3-trimethoxybenzene (5.72%), might also cooperated with the activity because the determined IC_{50} value (0.052 μg ml^{-1}) of methyleugenol was slightly higher than the theoretic value (0.043 μg ml^{-1}) calculated in consideration of the IC_{50} value of the oil and the abundance of methyleugenol.

Methyleugenol is such a cyclic hydrocarbon commonly found in the essential oils of a number of plants such as asarum, nutmeg, pimento, lemongrass, tarragon, basil, star anise and fennel. Although methyleugenol exhibited activities against food-born microorganisms (Pauli and Knobloch, 1987; Kivanç, 1988), few studies have considered the inhibitory properties of methyleugenol against phytopathogens (Meepagala et al., 2002). In addition, knowledge of the inhibitory action of purified methyleugenol is limited. We demonstrated that methyleugenol inhibited the growth of mycelia in phytopathogens *in vitro* and observed the mycelial ultrastructure in *P. cactorum*, the pathogen most sensitive to methyleugenol among the five studied.

TEM showed responses of *P. cactorum* to methyleugenol in membrane integrity, mitochondria, lomasome and electron density. Loss of micro-organellar membrane integrity has been observed in several instances (Brewster et al., 1997; Xu et al., 2007), which shows that cyclic hydrocarbons interact with hydrophobic parts of the cell due to their lipophilic character. This interaction plays an important role in the mechanism of toxicity (Knobloch et al., 1989; Sikkema et al., 1995). As we know, mitochondria in plant cells should be activated to inhibit hydrolyzing enzyme activity when exposed to pathogens. Thus, numerous mitochondria occurring in hyphae may be involved in the cell's defenses in the early stages of methyleugenol treatment. The lomasome was not observed in hyphae exposed to methyleugenol. Similar effects were seen in cells of *Phytophthora capsici* Leonian exposed to oligochitosan (Xu et al., 2007) and metalaxyl (Li, 1994). The lomasome exists between the thickened cell wall and the plasmalemma, is abundant during the period of active cell growth, and is closely involved in cell wall synthesis (Rajasingham and Cawson, 1984). We are not, however, able to address the linkage between the absence of the lomasome and the thickening of the cell wall. The electron-dense body disappeared in treated cells, which was consistent with results from *P. capsici* (Xu et al., 2007). Some cellular components exhibit high electron density in intact cells when observed with an electron microscope. We support that chemicals negatively impact their roles in iron storage and homeostasis (Nagasaka et al., 2003), but further studies should be carried out.

Phytophthora is the genus of plant–pathogenic oomycetes that have devastating effects on crops. Due to the lack of appropriate

Table 2
Toxicities of the essential oil of *A. heterotropoides* var. *mandshuricum* and methyleugenol against phytopathogens.

Species	Source of oil	Toxicity regression equation	R^2	IC_{50} (μg ml^{-1})
Phytophthora cactorum	Full oil	$Y = 1.194X + 2.625$	0.988	0.073
	Methyleugenol	$Y = 0.872X + 1.558$	0.979	0.052
Rhizoctonia solani	Full oil	$Y = 1.976X - 2.363$	0.913	0.415
	Methyleugenol	$Y = 0.634X + 1.425$	0.930	0.279
Alternaria humicola	Full oil	$Y = 1.284X + 0.523$	0.997	0.327
	Methyleugenol	$Y = 1.138X - 0.660$	0.980	0.144
Colletotrichum gloeosporioides	Full oil	$Y = 1.698X - 1.344$	0.971	0.419
	Methyleugenol	$Y = 0.573X + 2.423$	0.968	0.090

X denotes the concentration (natural logarithm). Y denotes the probability of death. R denotes the correlation coefficient. ($P < 0.05$).

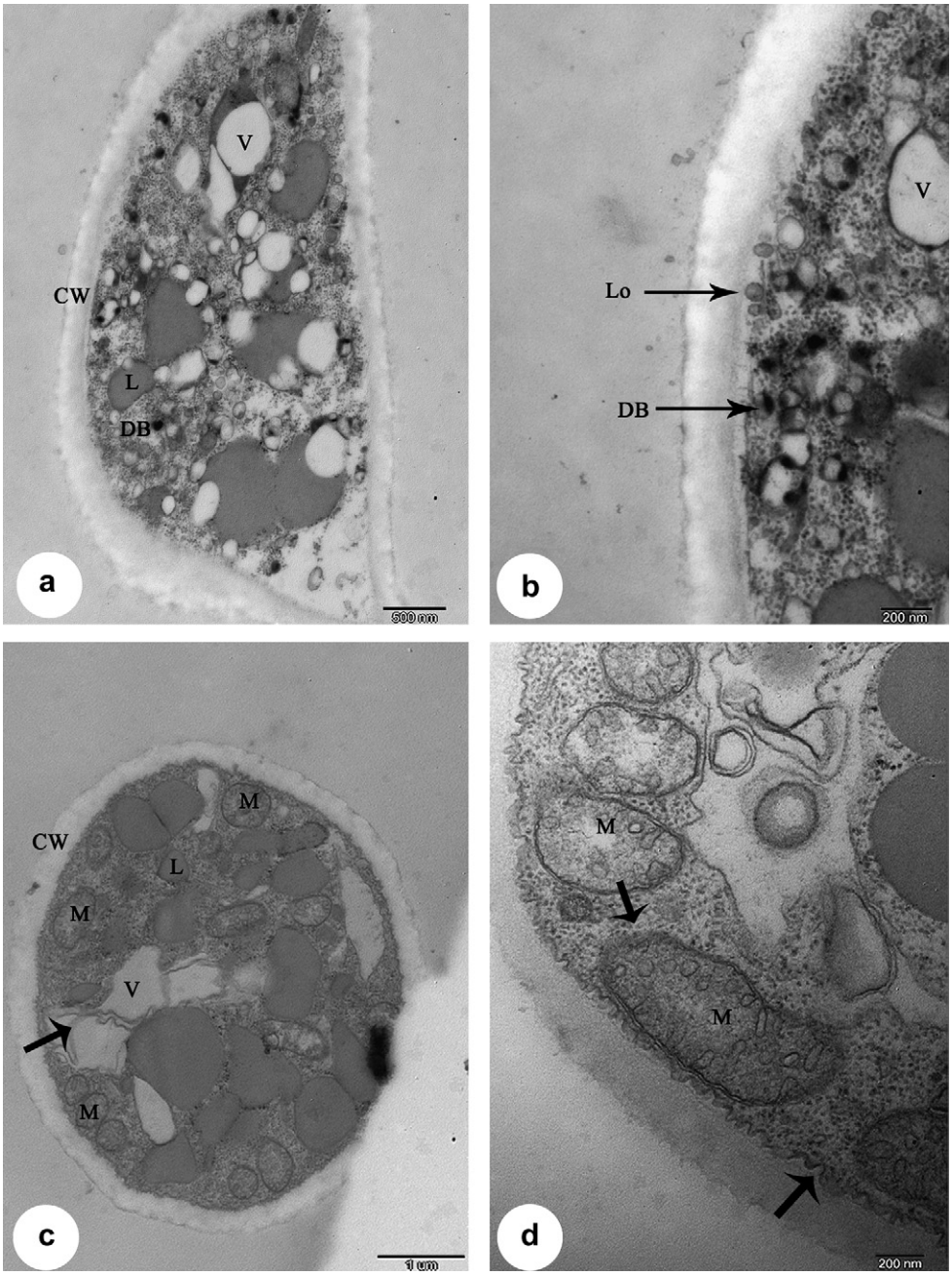

Fig. 3. Transmission electron micrographs of *P. cactorum*. Sections of *P. cactorum* hyphae grown on the PDA medium in the absence of methyleugenol (a, ×30,000; bar = 500 nm), showing normal cell wall (CW), vacuoles (V), lipid bodies (L), dense body (DB), and lomasomes (Lo). Details of a normal cell (b, ×60,000; bar = 200 nm). Hyphae of *P. cactorum* grown on PDA medium exposed to 0.052 μg ml^{-1} methyleugenol (c, ×20,000; bar = 1 μm). Details of an abnormal cell (d, ×60,000; bar = 200 nm). Note distorted vacuoles (c, d), thickening of the cell wall (c,d), invaginated plasmalemma (arrowheads in d), and abundant mitochondria (c,d). Lomasomes are absent, and the mitochondrial membrane is disrupted (d).

fungicides, no efficient treatments against diseases caused by these pathogens are presently available (Latijnhouwers et al., 2003; Attard et al., 2008). Recently, pesticides used against oomycetes have relied on the phenylamide metalaxyl, which specifically inhibits RNA polymerase-1 (Sukul and Spiteller, 2000). However, the emergence of resistant isolates is widespread (Parra and Ristanio, 2001). Methyleugenol is a potential pesticide with the IC_{50} value of 0.052 $\mu g\,ml^{-1}$, while the growth inhibition of metalaxyl against *Phytophthora* ranges from 0.013 to 4.61 $\mu g\,ml^{-1}$ (Coffey and Bower, 1984; Bashan et al., 1989). In addition, methyleugenol is an alk-2-enyl-benzene compound, largely different from metalaxyl in chemical structure, which suggests that methyleugenol has no cross-resistance with metalaxyl. We will investigate its efficiency, chemical stability, etc. in field, which is a premier for a promising antimicrobial natural product in plant protection.

Acknowledgements

This study was supported by the IMPLAD foundation (1391) and the National Key Technology R&D Program (2006BAI09B03). We thank Ms. Chen H. J. in Chinese Forest University for GC/MS analysis, and Mr. Jia W.J. and Ms. Liu M.F. for TEM. Authors are thankful to Prof. Wei X.Y. and Prof. Liu X.Z. for reading the manuscript.

References

Attard, A., Gourgues, M., Galiana, E., Panabières, F., Ponchet, M., Keller, H., 2008. Strategies of attack and defense in plant–oomycete interactions, accentuated for *Phytophthora parasitica* Dastur (syn. *P. nicotianea* Breda de Haan). J. Plant Physiol. 165, 83–94.

Bashan, B., Badish, D., Levy, Y., Cohen, Y., 1989. Infectivity to potato, sporangial germination, and resperation of isolates of *Phytophthora infestans* from metalaxyl-sensitive and metalaxyl-resistant population. Phytopathology 79, 832–836.

Brewster, D.T., Spiers, A.G., Hopcroft, D.H., 1997. Biocontrol of *Phytophthora cactorum in vitro* with *Enterobacter aerogenes*. N.Z. J. Crop Hortic. Sci. 25, 9–18.

Chang, H.T., Cheng, Y.H., Wu, Ch.L., Chang, Sh.Tz., Chang, T.T., Su, Y.C., 2008. Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* florin leaf against plant pathogenic fungi. Bioresour. Technol. 99, 6266–6270.

Coffey, M.D., Bower, L., 1984. *In vitro* variability among isolates of eight *Phytophthora* species in response to metalaxyl. Phytopathology 74, 738–742.

da Silva, M.H.L., Zoghbi, M.G.B., Andrade, E.H.A., Maia, J.G.S., 1999. The essential oils of *Peperomia pellucida* Kunth and *P. circinnata* Link var. *circinnata*. Flavour. Frag. J. 14, 312–314.

Deans, S.G., Svoboda, K.P., Kennedy, A.I., 1989. Biological activity of plant volatile oils and their constituents. Planta Med. 55, 588.

Duke, S.O., 1990. Natural pesticides from plants. In: Janick, J., Simon, J.E. (Eds.), Advances in New Crops. Timber Press, Portland, OR, pp. 511–517.

Gigante, B., Silva, A.M., Marcelo Curto, M.J., Savluchinske Feio, S., Roseiro, J.C., Reis, V.R., 2002. Structural effects on the bioactivity of dehydroabietic acid derivatives. Planta Med. 68, 680–684.

Hamm, S., Bleton, J., Tchapla, A., 2004. Headspace solid phase microextraction for screening for the presence of resins in Egyptian archaeological samples. J. Sep. Sci. 27, 235–243.

Hashimoto, K., Yanagisawa, T., Okui, Y., Maruno, M., Fujita, T., 1994. Studies on anti-allergic components in the roots of *Asiasarum sieboldi*. Planta Med. 60, 124–127.

Huang, S.M., Kelly, L.M., Gilbert, M.G., 2003. Aristolochiaceae. In: Flora China, vol. 5. Science Press, Beijing and Missouri Botanical Garden, St Louis. 246–269.

Ishii, H., 2006. Impact of fungicide resistance in plant pathogens on crop disease control and agricultural environment. Jpn. Agric. Res. Q. 40, 205–211.

Jirovetz, L., Buchbauer, G., Stoyanova, A.S., Georgiev, E.V., Damianova, S.T., 2003. Composition, quality control, and antimicrobial activity of the essential oil of long-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. J. Agric. Food Chem. 51, 3854–3857.

Kivanç, M., 1988. Antimicrobial activity of "Çörtük" (*Echinophora sibthorpiana* Guss.) spice, its essential oil and methyl-eugenol. Food/Nahrung 32, 369–373.

Knobloch, K., Pauli, A., Iberl, B., 1989. Antibacterial and antifungal properties of essential oil. J. Essent. Oil. Res. 1, 119–128.

Kosuge, T., Yokota, M., Nukaya, H., Gotoh, Y., Nagasawa, M., 1978. Studies on antitussive principles of asiasari radix. Pharm. Bull. Chem. 26, 2284.

Latijnhouwers, M., de Wit, P.J.G.M., Govers, F., 2003. Oomycetes and fungi: similar weaponry to attack plants. Trends Microb. 11, 462–469.

Lee, Y.S., Kim, J., Shin, S.C., Lee, S.G., Park, I.K., 2008. Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. Flavour. Frag. J. 23, 23–28.

Leffingwell, J.C., Alford, E.D., 2005. Volatile constituents of *Perique tobacco*. J. Agric. Food Chem. 4, 899–915.

Li, B.D., 1994. Study on Resistance of *Phytophthora capsici* to Metalexyl. Beijing, China: Chinese Agriculture University, PhD thesis, pp. 97.

Liu, H.Y., Gao, W.W., Fan, Y., Chen, S.L., 2007. Inhibitory effect of essential oil from *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag against plant pathogenic fungi. Acta Phytopathol. Sin. 37, 95–98.

Ma, Z.H., Michailides, T.J., 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 24, 853–863.

Meepagala, K.M., Sturtz, G., Wedge, D.E., 2002. Antifungal constituents of the essential oil fraction of *Artemisia dracunculus* L. var. *dracunculus*. J. Agric. Food Chem. 50, 6989–6992.

Nagasaka, S., Nishizawa, N.K., Watanabe, T., Mori, S., Yoshimura, E., 2003. Evidence that electron-dense bodies in *Cyanidium caldarium* have an iron-storage role. Biometals 16, 465–470.

Parra, G., Ristanio, J.B., 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of beel pepper. Plant Dis. 85, 1069–1075.

Pauli, A., Knobloch, K., 1987. Inhibitory effects of essential oil components on growth of food-contaminating fungi. Z. Lebensm. Unters. Forsch. 185, 10–13.

Pino, J.A., Mesa, J., Munoz, Y., Marti, M.P., Marbot, R., 2005. Volatile components from mango (*Mangifera indica* L.) cultivars. J. Agric. Food Chem. 53, 2213–2223.

Rajasingham, K.C., Cawson, R.A., 1984. Plasmalemmasomes and lomasomes in *Candida albicans*. Cytobios 40, 21–25.

Sefidkon, F., Omidbaigi, R., 2004. Essential oil composition of *Cephalophora aromatica* cultivated in Iran. Flavour. Fragr. J. 19, 153–155.

Sikkema, J., De Bont, J.A., Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59, 201–222.

Sukul, P., Spiteller, M., 2000. Metalaxyl: persistence, degradation, metabolism, and analytical methods. Rev. Environ. Contam. T. 164, 1–26.

Wang, G.Q., Ji, L.Z., 2007. The antibacterial effects of asarum essential oils on diseases in Ligneous flower *in vitro*. Northern Hortic. 2007 (9), 220–222.

Wang, G.Q., Zhang, J.H., Zhang, M., Ji, L.Z., 2008. The antibacterial effects of *Asarum heterotropoides* extracts to *Pestaloliopsis sp. in vitro*. Henan Agr. Sci. 3, 60–63.

Xu, J.G., Zhao, X.M., Han, X.W., Du, Y.G., 2007. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. Pestic. Biochem. Phys. 87, 220–228.

Zeng, H.Y., Jin, Y.Z., Bao, L.T., Wang, P., 2004. Analysis on fingerprint chromatogram of the volatile oils obtained from *Asarum heterotropoides* with different methods. J. Test Meas. Tech. 18, 232–236.

Zhang, F., Fu, S.P., Xu, Q., Xiao, H.B., Cai, S.Q., Liang, X.M., 2004. Study on GC fingerprint of the constituents of *Herba asari*. China J. Chin. Mater. Med. 29, 411–413.