농약과학회지 (Korean J. Pestic. Sci.)
Vol. 20, No. 1, pp. 30-34 (2016)
Open Access http://dx.doi.org/10.7585/kjps.2016.20.1.30
ORIGINAL ARTICLES / CONTROL

Online ISSN 2287-2051
Print ISSN 1226-6183

Chemical Compositions and Nematicidal Activities of Essential Oils on *Meloidogyne hapla* (Nematoda: Tylenchida) Under Laboratory Conditions

Ju-Hyun Jeon[1,2], Hyoung-Rai Ko[1], Se-Jong Kim[1] and Jae-Kook Lee[1,*]

Crop Protection Division, Department of Agro-food Safety and Crop Protection,
National Institute of Agricultural Sciences, RDA
[2]KM-Convergence Research Division, Korea Institute of Oriental Medicine

(Received on November 20, 2015. Revised on February 22, 2016. Accepted on February 28, 2016)

Abstract To evaluate the efficacy of natural nematicides for the control of root-knot nematode in strawberry greenhouses, commercial essential oils were examined by 24-well culture plate bioassay for their nematicidal activities against second-stage juveniles and eggs of *Meloidogyne hapla*. Based on the mortality of *M. hapla* juveniles at a concentration of 125 μg/mL, the most active essential oil was *Alpinia galanga* (100%), followed by *Carum carbi* (22.3%), *Eugenia caryophyllata* (9.4%), *Cinnamonum zeylanicum* (7.2%), *Mentha pulegium* (2.4%), and *Foeniculum vulgare* (2.1%). Moreover, *A. galanga* significantly reduced hatching at 7, 14, and 21 days after treatment. The volatile constituents identified in the *A. galangal* oil were methyl cinnamate (87.4%), 1,8-cineole (4.4%), β-pinene (2.5%), α-pinene (2.2%), and *p*-cymene (1.1%), as major constituents. Results of this study show that *A. galangal* essential oil and its major constituents may serve as an environmental friendly agent of a promising natural nematicide to control *Meloidogyne* spp.

Key words *Alpina galanga*, *Meloidogyne hapla*, Natural nematicide, Plant essential oil

Introduction

Today, increasing public awareness of the environmental effects of chemical pesticides, health hazards, and adverse effects on non-target organisms becomes more critical on continued use of synthetic nematicides (Pandey *et al*., 2000; Hong *et al*., 2010). These undesirable effects highlight the need for application of selective nematode control alternatives, because chemical nematicides such as 1,2-dibromo-3-chloropropane (DBCP) and 1,2-dibromoethane (ethylene dibromide) have been reserved from the agricultural chemical market due to their harmful effects on human health (Oka *et al*., 2000; Hong *et al*., 2010). It is necessary to develop new alternative control strategies for plant-parasitic nematodes, especially those with fumigant action in soil, because powders formulation are usually less effective and do not adequately penetrate deep layers (Hong *et al*., 2010). One possible alternative is utilization of natural pesticides based on plant-derived extracts such as essential oils or other nematicidal sources from plants (Chitwood, 2002; Nguyen *et al*., 2013; Kim *et al*., 2014). Consequently a large number of plants (flowers, leaves, and rhizomes) have been experimented for their nematicidal effects (Pandey *et al*., 2000). However, nematicidal effects of essential oils and its volatile constituents against *Meloidogyne hapla* have not been reported. In our present study, plant essential oils from nine species were evaluated for their nematicidal and hatching inhibition activities against *M. hapla* second-stage juveniles (J2) and eggs under laboratory conditions. We also analyze the volatile compounds of essential oil extracted from *Alpinia galanga* rhizomes.

*Corresponding author
E-mail: jk2lee@korea.kr

Materials and Methods

Essential oils

Nine plant essential oils (*Alpinia galanga*, *Carum carbi*, *Cinnamonum zeylanicum*, *Eugenia caryophyllata*, *Foeniculum*

vulgare, *Lavandula intermedia cv.* Grosso, *Melaleuca viridiflora*, *Mentha pulegium*, and *Myristica fragrans*) were purchased from a local herb market, Skinmate (Bucheon, Korea) and stored in a glass bottle at 4°C. Triton X-100 was supplied by Fluka (Buchs, Switzerland).

Nematode

Meoloidogyne hapla, originally extracted from a strawberry field in Gongju (Chungnam province, South Korea), was cultured on tomato (*Lycopersicon esculentum* cv. Rutgers) in a glasshouse. Egg masses were isolated from plant roots, collected in water, and sterilized by agitation for 3 min in 1% sodium hypochlorite (NaOCl) solution (Kim *et al*., 2014). The eggs were collected on a 500 mesh sieve, refrigerated overnight at 4°C in sterile distilled water and used for bioassay. Juveniles were obtained by Baermann funnel method (Barker, 1985), and used in this study were less than 5 days old.

Bioassay

The nematicidal activity of essential oils against *M. hapla* second-stage juveniles (J2) was evaluated by 24-well culture plate bioassay as described previously (Kim and Whang, 2012). The test solution containing 120 to 150 J2 per 0.98 mL of distilled water was prepared by diluting the test solution. Nematodes were placed in each well prior to adding the essential oils. Four concentrations (1,000, 500, 250, and 125 μg/mL) of each materials (0.02 mL) composed of ethanol and Triton X-100 (0.9:0.1, v/v) were added to the wells and incubated at 24 ± 1°C. The percentage mortality of J2 was recorded by counting them after 24 h under a binocular microscope (×40). Essential oils at a concentration of 1,000 μg/mL that showed nematicidal activities more than 90% of the *M. hapla* J2 population were selected for the hatching inhibition experiments.

The hatching inhibition assays of six active essential oils against *M. hapla* eggs were evaluated under laboratory conditions. Approximately 130 to 170 nematode eggs in were introduced into six active essential oil solutions at a concentration of 500 μg/mL and incubated at 24 ± 1°C. Inhibitory effect of hatching was recorded after 7, 14, and 21 days, respectively. Controls consisted of the ethanol/Triton X-100 carrier solution in distilled water. All treatments had three replicated and were performed at least twice.

Gas chromatography mass spectroscopy (GC/MS)

Gas chromatography-mass spectroscopy (GC/MS) analyses of the active *Alpinia galanga* oil were performed using Agilent GC model 6890 fitted with MS detector (Agilent 5973) and a DB-5 silica capillary column (inner diameter 0.32 mm; length 30 m; film thickness 0.5 μm). Helium was used as carrier gas at the flow rate of 1.5 mL/min. The analytic conditions were as follows: ion source temperature, 280°C; injector temperature, 250°C; column temperature, 90°C. Mass spectra were recorded over 50 to 600 amu range with 70 eV. The split ratio use was 20:1. The identification of volatile constituents was based on the retention indices on the DB-5 silica capillary column, retention times, and mass spectra with the Wiley Registry of Mass spectral Data 7th edition.

Data analysis

The percentage mortality values of J2 were calculated from the formula: % = (number of dead J2 / total number of J2) × 100. Data were analyzed statistically by analysis of variance and means compared with Duncan's Multiple Range test ($P<0.05$).

Results and discussion

Nematicidal activities of nine essential oils against *M. hapla* J2 were evaluated by 24-well culture plate bioassay (Table 1). Based on the mortality of juveniles at a concentration of 125 μg/mL, the most active essential oil was *A. galanga* (100%), followed by *C. carbi* (22.3%), *E. caryophyllata* (9.4%), *C. zeylanicum* (7.2%), *M. pulegium* (2.4%), and *F. vulgare* (2.1%). However, *L. intermedia cv. Grosso*, *M. viridiflora*, and *M. fragrans* had no activity at the same concentration against *M. hapla* juveniles. Because of potent activity of six essential oils (*A. galanga*, *C. carbi*, *E. caryophyllata*, *C. zeylanicum*, *M. pulegium*, and *F. vulgare*) against *M. hapla* juveniles, the hatching inhibition of selected oils to *M. hapla* eggs was investigated using 24-well culture plate bioassay at a concentration of 500 μg/mL (Fig. 1). *A. galanga* was also effective at hatching inhibition, followed by *C. zeylanicum*, *E. caryophyllata*, *F. vulgare*, *M. pulegium*, and *C. carbi*. These results indicate that toxicity differences were related to that of susceptibility varies with the developmental stage. According to Wharton (2002), egg stage is the most resistant stage in the nematode developmental stages, due to its three-layer shell (vitelline layer, chitinous layer, and lipid layer).

Volatile constituents accounting 98.1% of *A. galanga*

Table 1. Nematicidal activity of essential oils against *Meloidogyne hapla* second-stage juveniles[a]

Samples (μg/mL)	Mortality (%) ± SE[b]			
	1,000	500	250	125
Alpinia galanga	100a	100a	100a	100a
Carum carbi	100a	100a	59.0±2.3b	22.3±5.8b
Cinnamonum zeylanicum	100a	95.9±0.5a	27.1±2.6c	7.2±1.4c
Eugenia caryophyllata	100a	98.2±0.4a	30.1±4.5c	9.4±2.6c
Foeniculum vulgare	100a	67.4±7.8b	7.8±2.4d	2.1±0.8d
Lavandula intermedia cv. Grosso	41.5±5.6b	23.1±7.2c	0.0±0.0e	0.0±0.0d
Melaleuca viridiflora	29.0±4.9c	14.1±2.2d	0.0±0.0e	0.0±0.0d
Mentha pulegium	100a	98.7±1.2a	10.3±3.4d	2.4±0.7d
Myristica fragrans	31.3±4.6c	15.6±6.1d	0.0±0.0e	0.0±0.0d
Negative control	2.6±0.5d	-	-	-

[a]Exposed for 24 h.
[b]Different letters next to the values in the column indicate significant differences at $p<0.05$.

Table 2. Volatile constituents from rhizomes of *Alpinia galanga* oil identified by GC/MS

Peak number	RI[a]	Retention time (min)	Components	Relative composition (%)
1	943	6.54	α-Pinene	2.23
2	965	7.41	β-Pinene	2.54
3	1010	8.25	*p*-Cymene	1.05
4	1031	8.42	1,8-Cineole	4.37
5	1121	9.45	Fenchone	0.53
6	1267	16.65	Methyl cinnamate	87.39
			Total identified	98.11
			Major grouped compounds	
			Monoterpene esters	87.39
			Monoterpene hydrocarbons	5.82
			Monoterpene ketones	0.53
			Monoterpene oxides	4.37

[a]Retention indices (RI) were determined on DB-5 capillary column.

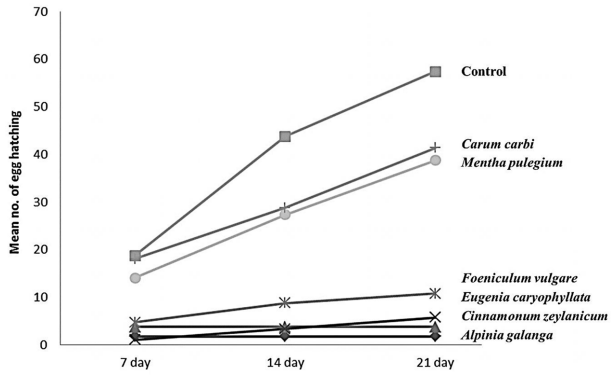

Fig. 1. Inhibitory effect of 500 μg/mL essential oils on hatching of *Meloidogyne hapla* eggs.

rhizomes oil were identified by GC/MS (Table 2). The identified compounds in *A. galanga* oil were methyl cinnamate (87.39%), 1,8-cineole (4.37%), β-pinene (2.54%), α-pinene (2.23%), *p*-cymene (1.05%), and fenchone (0.53%). The volatile constituents from *A. galanga* rhizomes oil were grouped as monoterpene ester (methyl cinnamate), monoterpene hydrocarbon (α-pinene, β-pinene, and *p*-cymene), monoterpene oxide (1,8-cineole), and monoterpene ketone (fenchone). Compared with the previous studies (Raina *et al*., 2014), the major components of *A. galanga* oil were 1.8-cineole (63.4%), (*E*)-β-farnesene (8.4%), eugenol acetate (3.3%), α-terpineol (2.8%), terpinen-4-ol (2.8%), and (*E*)-methyl cinnamate (0.2%). However, the occurrence of methyl cinnamate as major component in the *A. galanga* oil in the present study is not matched those of previous reports. The quantitative and compositional differences of chemical composition of plants

are attributed to geographical origin, extraction methods, parts of herbs, chemotypes, and harvest conditions (Raina *et al*., 2002; Yang and Lee, 2013). Essential oil from various plants is used commercially sources of pesticides for insect control (Oka *et al*., 2000; Jeon *et al*., 2015). Several essential oils and their constituents have been reported to their nematicidal effect, such as *Mentha* spp., *Origanum spp*., *Eucalypus* spp., and *Tagetes* spp., and their components (alcohols, alkaloids, phenolics, and terpenoids) (Uhlenbroek *et al*., 1958; Belcher and Hussey, 1977; Oka *et al*., 2000; Pandey *et al*., 2000; Lee *et al*., 2011). Our study also demonstrated that essential oil from *A. galanga* rhizome had nematicidal effective against *M. hapla* juveniles and eggs. Therefore, natural nematicides based on plant extracts, essential oils, and phytochemicals could be beneficial as alternatives to pesticides for control nematodes. Further experiments are needed to evaluate the nematicidal efficacy under greenhouse and field conditions for the practical use of *A. galanga* rhizome-derived oil and its volatile constituents. Additionally, nematicidal constituents derived from *A. galanga* oil and mode of action need to be conducted.

Acknowledgments

This research was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project title: Distribution and occurrence of root-knot nematodes under greenhouse cultivation, Project No: PJ009309), Rural Development Administration, Republic of Korea.

Literature Cited

Barker, K. R. (1985) Nematode extraction and bioassays. In: Barker KR, Carter CC & Sasser JN (eds). An advanced treatise on *Meloidogyne*, volume 2, pp. 19-35. North Carolina State University, Raleigh, NC, USA.

Belcher, J. V. and R. S. Hussey (1977) Influence of *Tagetes patula* and *Arachis hypogaea* on *Meloidogyne incognita*. Plant Dis. Reptr. 61:525-528.

Chitwood, D. J. (2002) Phytochemical-based strategies for nematode control. Ann. Rev. Phytopathol. 40:221-249.

Hong, T. K., J. K. Lee, J. W. Heo, S. I. Kim, D. R. Choi and Y. J. Ahn (2010) Toxicity of *Kaempferia galanga* rhizome-derived extract and steam distillate to *Meloidogyne incognita* juveniles and eggs, and their effects on *Lycopersicon esculentum* germination and growth. Nematol. 12:775-785.

Jeon, J. H., S. G. Lee and H. S. Lee (2015) Isolation of insecticidal constituent from *Ruta graveolens* and structure-activity relationship studies against stored-food pests (Coleoptera). J. Food Prot. 78:1536-1540.

Kim, S. I., J. K. Lee, Y. E. Na, S. T. Yoon and Y. J. Oh (2014) Nematicidal and ovicidal activities of *Dryobalanops aromatic* and *Mentha haplocalyx* var. piperascens-derived materials and their formulations against *Meloidogyne incognita* second-stage juveniles and eggs. Nematol. 16: 193-200.

Kim, S. J. and K. S. Whang (2012) Nematicidal effect of root-knot nematode (*Meloidogyne incognita*) by amino acids biochemical agent extracted from chicken feather. J. Appl. Biol. Chem. 55:247-252.

Lee, J. S., H. Y. Choo and D. W. Lee (2011) Nematicidal efficacy of herbal extracts against *Meloidogyne hapla*. Kor. J. Appl. Entomol. 50:315-324.

Nguyen, D. M. C., D. J. Seo, V. N. Nguyen, K. Y. Kim, R. D. Park and W. J. Jung (2013) Nematicidal activity of gallic acid purified from *Terminalia nigrovenulosa* bark against the root-knot nematode *Meloidogyne incognita*. Nematol. 15:507-518.

Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv and Y. Spiegel (2000) Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathol. 90:710-705.

Pandey, R., A. Kalra, S. Tandon, N. Mehrotra, H. N. Singh and S. Kumar (2000) Essential oils as potent sources of nematicidal compounds. J. Phytopathol. 148:201-202.

Raina, A. P., S. K. Verma and Z. Abraham (2014) Volatile constituents of essential oils isolated from *Alpinia galanga* Willd. (L.) and *A. officinarum* Hance rhizomes from North East India. J. Ess. oil Res. 26:24-28.

Raina, V. K., S. K. Srivastava and K. V. Syamasunder (2002) The essential oil of 'greater galanga' [*Alpinia galalga* (L.) Willd.] from the lower Himalayan region of India. Flavour. Fragr. J. 17:358-360.

Uhlenbroek, J. H. and J. D. Bijloo (1958) Investigations on nematicides. I. Isolation and structure of a nematicidal principle occurring in *Tagetes* roots. Recl. Trav. Chim. Pays. Bas. 77:1004-1008.

Wharton, D. A. (2002) Nematode survival strategies. In: Lee DL (ed.), The biology of nematodes, pp. 389-411. Taylor & Francis, New York, USA.

Yang, J. Y. and H. S. Lee (2013) Verbenone structural analogues isolated from *Artemesia aucheri* as natural acaricides against *Dermatophagoides* spp. and *Tyrophagus putrescentiae*. J. Agric. Food Chem. 61:12292-12296.

식물정유의 당근뿌리혹선충(*Meloidogyne hapla*)에 대한 살선충활성 및 방향성성분 분석

전주현[1,2] · **고형래**[1] · **김세종**[1] · **이재국**[1,*]

[1]농촌진흥청 국립농업과학원 작물보호과, [2]한국한의학연구원 한의약융합연구부

요 약 본 연구는 뿌리혹선충의 친환경적 방제를 위하여 9종의 식물정유를 선발하여 당근뿌리혹선충 2령기 유충과 알에 대한 살선충 및 부화억제 효과를 검정하였다. 당근뿌리혹선충의 2령기 유충에 대해 125 μg/mL로 검정한 결과, *Alpinia galanga* 정유가 100% 살선충활성을 나타냈고, *Carum carbi* (22.3%), *Eugenia caryophyllata* (9.4%), *Cinnamonum zeylanicum* (7.2%), *Mentha pulegium* (2.4%) 및 *Foeniculum vulgare* (2.1%) 순으로 활성을 나타내었다. 이들 중 *A. galanga* 정유를 이용하여 당근뿌리혹선충 알의 부화억제 활성을 검정한 결과, 500 μg/mL 농도로 처리 후 7, 14, 21 일째까지 낮은 부화 활성을 나타내었다. 우수한 활성을 보인 *A. galanga* 정유의 활성 성분을 분석한 결과 methyl cinnamate 함유율이 가장 높았으며, 1,8-cineole, β-pinene, α-pinene, *p*-cymene이 주요 방향성 물질로 구성되어 있음을 확인할 수 있었다. 따라서 본 연구에서 선발한 *A. galanga* 정유가 당근뿌리혹선충의 친환경적 방제 수단으로 활용가능성이 있을 것으로 생각된다.

색인어 갈랑갈, 당근뿌리혹선충, 식물정유, 천연살선충제