J. Korean Wood Sci. Technol. 43(2): 274~285, 2015 pISSN: 1017-0715 eISSN: 2233-7180
http://dx.doi.org/DOI : 10.5658/WOOD.2015.43.2.274

# Insecticidal Activity of *Coptis chinensis* Extract Against *Myzus persicae* (Sulzer)[1]

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**ABSTRACT**

In view of the environmental and health hazards posed by synthetic insecticides, the use of plant products as botanical insecticides has gained increasing in recent years. In this study, we reported the insecticidal activity of extracts isolated from *Coptis chinensis*. On crude extraction, among the various solvent types tested (water, 1% (w/v) of sodium hydroxide, 70% ethanol), the 70% ethanol extract showed the best insecticidal activity (36.5%). Three different fractions (n-hexane, chloroform and ethyl acetate) were obtained from crude extract (70% ethanol) of the chloroform fraction and found to have noteworthy insecticidal activity (62.9%) by filter paper contact bioassay. Their chemical structures were identified as 2-methoxy-4-vinylphenol and aniline by head space-GC-MS analysis. Both compounds displayed a dose-dependent insecticidal activity of *Myzus persicae* (Sulzer). Insecticidal activity at the lowest concentration tested (500 ppm) approached 85.4% in the aniline compared with 79.9% in the 2-methoxy-4-vinylphenol. The insecticidal activity was greater for the aniline than 2-methoxy-4-vinylphenol. It is believed that the insecticidal activity is due mainly to the presence of aniline.

***Keywords :*** *Coptis chinensis*, *Myzus persicae* (Sulzer), extract, botanical insecticides

## 1. INTRODUCTION

The peach-potato aphid, *Myzus persicae* (Sulzer), is a common pest of many agronomic and vegetable crops and has a worldwide distribution (Ramsey *et al*. 2007). This highly poly-phagous insect can cause direct injury to the plants by feeding on the leaves and extracting sap or indirectly injure plants by transmitting viruses (Flanders *et al*. 1991; Tagu *et al*. 2008). It also secretes honeydew which attracts fungus causing the smutting of leaves and fruit (Gray and Gildow 2003). The growth of sooty molds also hampers photosynthesis. *M. persicae* has a very high reproductive potential and can cause substantial injury to young plants

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thus causing eventual death (Petitt and Smilowitz 1982).

*M. persicae* populations have developed resistance against synthetic insecticides (Blackman and Devonshire 1978; Bauernfeind and Chapman 1985). These synthetic insecticides are the organophosphates, carbamates, pyrethroids and neonicotinoids, but only three biochemically different molecular target sites are attacked: acetylcholinesterase (organophosphates and carbamates), voltage-gated sodium channels (pyrethroids) and nicotinic acetylcholine receptors (neonicotinoids). All chemical synthetic insecticides interfere agonistically with cholinergic nerve transmission (Eto 1974; Casida and Quistad 1998; Ishaaya 2001; Nauen *et al*. 2001). Synthetic insecticides are frequently used to suppress *M. persicae* populations but are less effective in cooler temperatures (McLeod 1991). Anstead *et al*. reported development of resistance in *M. persicae* to more insecticides than any other insect (Anstead *et al*. 2005). As a result, it has been subjected to heavy insecticide treatment. (Martinez *et al*. 1999). The indiscriminate use of chemical insecticides has given rise to many well-known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment (Ahmed *et al*. 1981). These problems coupled with the high cost of chemical pesticides have stimulated the search for biologically based alternatives.

Botanical insecticides, i.e., pest management products based on plant material, plant extracts, or natural products derived from plants, have long been touted as potential alternatives to conventional synthetic insecticides. (Malik *et al*. 2007).

Mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Balandrin 1985; Sukamar *et al*. 1991; Desneux *et al*. 2005; Jeon *et al*. 2011).

*Coptis chinensis* ('Huanglian') is found mainly in the province of Szechwan in mainland China. It is frequently used in treating diabetes mellitus, and is particularly helpful in the treatment of diarrhea, acute enteritis and dysentery, delirium due to high fever, leukemia and otitis media, also known as Huang Lian (Anon 2010), and frequently found in traditional Chinese herbal formulae have been reported to exert a number of pharmacological actions including antihypertensive (Tsai *et al*. 2008), antibacterial (Kong *et al*. 2009), and antioxidative (Jung *et al*. 2009) and anti-inflammatory effects (Kim *et al*. 2010), among others. Literature reports indicate that *C. chinensis* itself exerts its anti-inflammatory effects by down regulation of inflammatory cytokines expression (Kim *et al*. 2010) and inhibition of the activator protein and the nuclear factor-kappa B pathways (Remppis *et al*. 2010). However, the insecticidal activities of extract from the *C. chinensis* have not been investigated. This study investigates the potential of extract from *C. chinensis* as an environmentally safe measure to control the peach-potato aphid, *M. persicae*.

## 2. MATERIALS and METHODS

### 2.1. Raw material and chemicals

*Coptis chinensis* was purchased from Traditional Korean Medicine Pharmacy Market in Sancheong, Korea. The *C. chinensis* was dried in an oven at 40℃ for 2 days and finely powdered using a laboratory Wiley mill. The particles that passed through a 20-mesh sieve but were retained by a 40-mesh sieve were stored in a sealed plastic bag at −72℃.

2-Methoxy-4-vinylphenol (98% purity, MW = 150.17) and aniline (99% purity, MW = 93.13) were purchased from Sigma-Aldrich (St. Louis, MO).

### 2.2. Extraction

Three kinds of solvents (water, 1% (w/v) of sodium hydroxide solution, 70% ethanol) were used to determine the optimum chemical solution for extraction.

Extracts from *C. chinensis* were prepared using water, 30% ethanol, 50% ethanol and 70% ethanol as solvents. Fifty grams of marc with 100 mℓ of solvent were mixed and shake (IS-97IR from Jeio-Tech Co., Korea) at the room temperature and 100 rpm for 2 days. The 70% ethanol was used for crude extraction. The crude extract was then concentrated on a rotary vacuum evaporator (Buchi, Switzerland) under reduced pressure at 35～40℃, and freeze-dried. The yield of each extract was calculated as follows :

Extraction yield (%)
= (weight of dry extract/ weight of raw material) × 100

### 2.3. Fractionation of crude extract

Fractionation of the crude extract (70% ethanol) was performed and respective fractions were obtained (Fig. 1) (Karawita *et al*. 2005). The fractionation was sequentially divided into *n*-hexane, chloroform and ethyl acetate fractions for the chemical composition and bioassay. The different fractions were concentrated using rotary vacuum evaporator at 45℃.

### 2.4. GC–MS analysis

The different fractions of crude extract were analyzed by static headspace gas chromatography-mass spectrometry (headspace-GC-MS). The headspace-GC-MS analysis was performed with Clarus 600 gas chromatograph (Perkinelmer, CA, USA) equipped with a static headspace sampler (Perkinelmer, CA, USA). The different fractions of crude extract were injected into a column DB-5MS (30 m × 0.25 mm × 0.25 μm film thickness; HP Agilent, USA). The injector temperature was set at 260℃, and helium (1 mℓ/min) was the carrier gas. The initial column temperature of 35℃ was held for 5 min, and then programmed at a rate of 5℃/min until reaching 280℃ and maintained at this temperature for further 10 min, next programmed again at a rate of 10℃/min until reaching 300℃ and maintained at this temperature for further 14

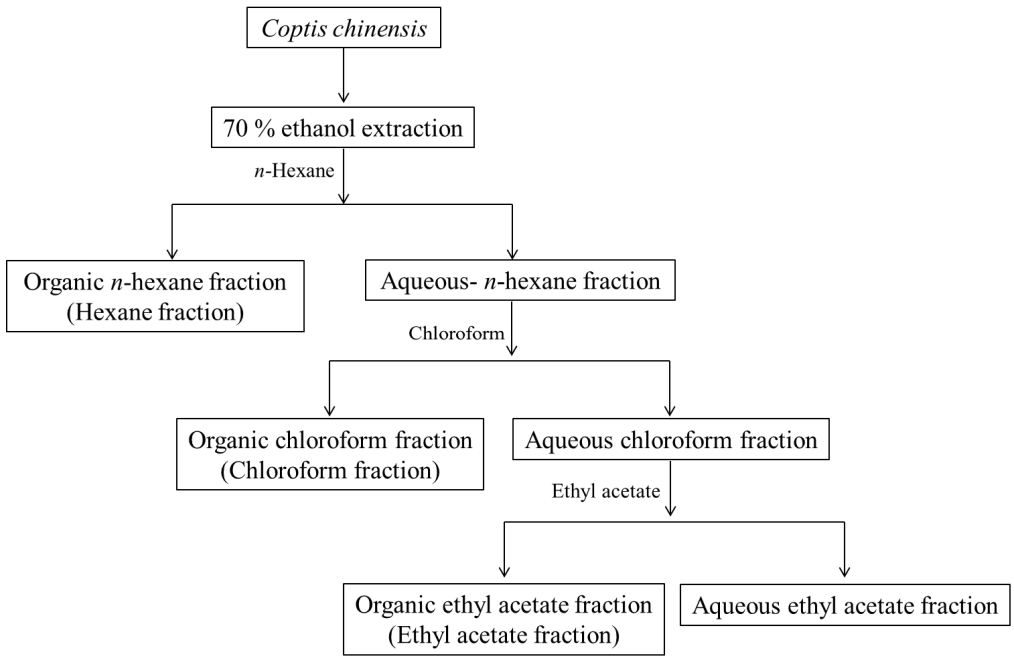


**Fig. 1.** Flow diagram of fractionation of crude extracts from *C. chinensis*.

min. The mass spectrometer operated in the electron impact ionization mode (70 eV), with a scan range of 50 to 300 amu. The ion source temperature was set at 230℃.

Identification of the constituents was performed on the basis of NIST library search. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

## 2.5. Bioassay

Adult alate of *Myzus persicae* (Sulzer), derived from a stock culture maintained on *Nicotiana tabacum* at 28 ± 1℃ in a greenhouse and a photo period of 14 : 10 (light : dark), were used for all experiments. Each treatment contains three replications and twenty insects were used for each replication.

The different extract isolated from *C. chinensis* and the derivatives of 2-methoxy-4-vinylphenol and aniline were tested for their insecticidal activities against *M. persicae* (Sulzer) using a filter paper contact bioassay. A dose of 500 ppm, 1,000 ppm and 2,000 ppm of each extracts was applied to filter papers (Whatman No. 1). After drying under a fume hood for 2 min, each filter paper was placed in the bottom of a petri-dish (10 cm diameter × 4 cm), and then 20 adults of each test materials were placed in each petri-dish which was covered with a lid. Treated insects were held at 28 ± 1℃, 50 - 60% relative humidity, and a 14 : 10 (light : dark) photoperiod. The insecticidal ac-

tivity was determined at 48 h after treatment. Test insects were considered dead if appendages did not move when prodded with a fine brush. Negative control was prepared using distilled water. 2-Methoxy-4-vinylphenol and aniline (commercial chemical) was used as a positive control with the tested insect. All treatments were replicated three times.

### 2.6. Statistical analysis

The insecticidal activity was transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P > 0.05$ (SAS 1990). Means (± SE) of untransformed data are reported.

## 3. RESULTS and DISCUSSION

### 3.1. Effect of solvent on extraction yields and insecticidal activity

Extraction is an important step for obtaining extracts with acceptable yields and strong insecticidal activity. The extraction yields and insecticidal activity of different solvent extracts were shown in Table 1. It was found that solvent types have a marked influence on the extract yield. The extract yielded 32.2, 62.7 and 60.8% in water, alkali and 70% ethanol extracts, respectively and yield of extract was maximized at alkali extract. When water extract, alkali extract and ethanol extract obtained from *C. chinensis* were laboratory assayed using filter paper contact bioassay, significant differences were observed in the toxicity to the *M. persicae* used. At a dose of 1,000 ppm, the insecticidal activity of the water extract, alkali extract and ethanol extract were 16.7%, 12.4% and 36.5%, respectively. No insecticidal activity was obtained in the untreated controls (data not shown).

The alkali could dissolve the carbohydrate with high molecular weights. Alkaline extraction has attracted growing interest because they can release hemicelluloses from different fiber sources in an environmentally friendly manner. However, it was found that insecticidal substances could be dissolved in water-ethanol mixtures (Hoogenboom *et al*. 2007).

According to the results of extraction yields and insecticidal activity of different solvent extracts (Table 1), 70% ethanol extract (crude extract) was chosen for the subsequent fractionation.

### 3.2. Insecticidal activity of *M. persicae* treated with different solvent fractions by filter paper contact bioassay

In the current study, the toxicities of hexane fraction, chloroform fraction and ethyl acetate fraction isolated from *C. chinensis* were tested on *M. persicae* at 500, 1,000 and 2,000 ppm (Fig. 2).

The hexane fraction, chloroform fraction and ethyl acetate fraction displayed a dose-dependent response against *M. persicae*. The in-

**Table 1.** Extract yield and insecticidal activity of different solvent extracts from *C. chinensis* [a]

| Sample | Yield (%) | Insecticidal activity (%) [b] |
|---|---|---|
| Water extract | 32.2 ± 0.1 b [c] | 16.7 ± 1.5 b |
| Alkali extract [d] | 62.7 ± 0.8 a | 12.4 ± 1.5 c |
| Ethanol extract [e] | 60.8 ± 0.1 a | 36.5 ± 1.4 a |

[a] Values are presented as means ± SD of three measurements
[b] Insecticidal activity was observed for mortality 48 h after treatment
[c] Values followed by different letters in the same column differ significantly at $P < 0.05$.
[d] 1% sodium hydroxide solution
[e] 70% ethanol

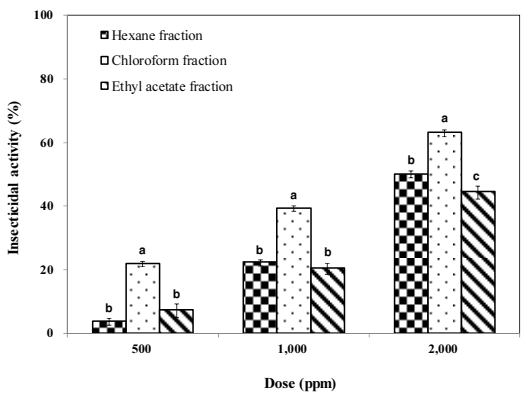


**Fig. 2.** Insecticidal activity of *M. persicae* (Sulzer) treated with different fractions of *C. chinensis* by filter paper contact bioassay. Values followed by different letters in the same column differ significantly at $P < 0.05$.

secticidal activity of *M. persica* was observed to be increased with increase in concentration 500, 1,000 and 2,000 ppm of hexane fraction, chloroform fraction and ethyl acetate fraction isolated from *C. chinensis*.

When the hexane fraction, chloroform fraction and ethyl acetate fraction of *C. chinensis* were examined for toxicity against *M. persica*, significant differences were observed. Among the different fractions, the chloroform fraction showed the highest insecticidal action. However, weak insecticidal activities were observed from the hexane and ethyl acetate fractions. Both hexane and ethyl acetate fractions showed a similar insecticidal activity against *M. persicae* at concentrations of 500 ppm (3.7 - 7.1%) and 1,000 ppm (20.3 - 22.2%).

The insecticidal activity of chloroform fraction was 21.7%, 39.2% and 62.9% in 500, 1,000 and 2,000 ppm respectively. It was highly effective in insecticidal activity of tested *M. persicae* at a 2000 ppm dose of the chloroform fraction.

## 3.3. Chemical composition of fractions isolated from *C. chinensis*

The investigation of chemical component of hexane fraction, chloroform fraction and ethyl acetate fraction isolated from *C. chinensis* was carried out by means of headspace-GC-MS to identify the odorous target component responsible for characteristic odor of food flavoring products.

The chemical compositions of the hexane fraction, chloroform fraction and ethyl acetate fraction obtained from the crude extract of *C. chinensis* presented in Table 2.

The hexane fraction on analysis showed the

**Table 2.** Chemical composition of different fractions from *C. chinensis*

| Constituent | R.T [a] (min) | Hexane fraction (%) | Chloroform fraction (%) | Ethyl acetate fraction (%) |
|---|---|---|---|---|
| Cyclopentanone | 4.9 | 2.6 | - [b] | - |
| 2-Ethylhexyl tetradecyl ester | 9.0 | 1.8 | - | - |
| Sydnone | 9.7 | - | 4.8 | 1.7 |
| Hydroquinone | 10.5 | - | 2.6 | - |
| Thiophene | 11.0 | - | 1.1 | - |
| 2-Methoxy-4-vinylphenol | 12.2 | 0.7 | 13.0 | 6.1 |
| 1,2,3-Benzenetriol | 13.0 | - | 1.9 | - |
| Acetaldehyde | 13.3 | 2.6 | - | - |
| Vanillin | 13.4 | - | - | 4.3 |
| Heptadecane | 14.5 | 0.6 | - | - |
| D-allose | 14.9 | - | 2.1 | - |
| Acetamide | 15.0 | - | - | 1.0 |
| Phosphoric acid | 15.4 | 0.5 | 2.5 | 0.7 |
| Aniline | 15.5 | - | 14.4 | - |
| Cyclooctasiloxane | 16.1 | - | 4.6 | 0.9 |
| Pentanoic acid | 16.7 | - | - | 1.8 |
| Disulfide | 17.0 | 0.6 | - | - |
| 3-Methyl-2-Butenoic acid | 17.4 | - | 4.5 | - |
| 1-Methylene-2B-hydroxymethyl-3 | 17.5 | - | - | 4.6 |
| Butanone | 17.9 | - | - | 1.6 |
| Phenylpropanoic acid | 18.1 | 0.9 | - | - |
| 2-Propenoic acid | 18.5 | - | - | 1.3 |
| 2,4-Dimethoxybenzyl alcohol | 19.0 | 0.4 | - | - |
| Levodopa | 19.3 | - | 2.4 | - |
| Thujone | 19.5 | - | - | 1.1 |
| Ascorbic acid | 19.6 | - | 2.0 | - |
| 7-Methanoazulene | 19.7 | 1.1 | - | - |
| Cyclopropane | 20.0 | - | - | 5.1 |
| 3-Decanynoic acid | 20.5 | - | - | 8.3 |
| Tetrazole | 20.6 | - | 4.0 | - |
| 9,12-Octadecadien-1-ol | 21.4 | - | 0.8 | 4.0 |
| 8-Heptadecene | 22.0 | 0.9 | - | - |
| Pyrrolo(1,2-A)Pyrazine-1,4-dione | 23.5 | - | - | 0.6 |
| 1-Hentetracontanol | 23.7 | 0.4 | - | - |
| Didodecyl phthalate | 24.6 | - | - | 2.2 |
| Bis(2-ethylhexyl)phthalate | 24.7 | 2.3 | 2.7 | - |
| Retinal | 25.6 | 6.3 | 6.3 | - |

**Table 2.** To be Continued

| Constituent | R.T [a] (min) | Hexane fraction (%) | Chloroform fraction (%) | Ethyl acetate fraction (%) |
|---|---|---|---|---|
| 2-Butenoic acid | 26.0 | 2.8 | 9.1 | 1.2 |
| Desacetylanguidine | 26.7 | 4.6 | 8.4 | 1.3 |
| 5-Methyl-z-5-docosene | 26.8 | 0.7 | - | - |
| Tetrapentacontane | 28.3 | 1.7 | - | - |
| Carinol | 28.4 | - | - | 2.0 |
| Corydine | 29.2 | 2.6 | - | 3.9 |
| Cyclotrisiloxane | 30.9 | 0.6 | - | 3.5 |
| Lopan-3-ol | 31.1 | 2.8 | - | - |
| Propenyl guaethol | 31.6 | - | - | 2.1 |
| D-mannitol | 32.2 | 0.9 | - | - |
| (+)-Lariciresinol | 32.3 | - | - | 2.7 |
| Squalene | 32.7 | 2.3 | - | 1.6 |
| $\beta$ -Sitosterol | 33.1 | 4.6 | - | - |
| Farnesyl bromide | 33.5 | 7.2 | - | - |
| $\beta$ -Amyrin | 33.8 | 9.6 | - | 6.4 |
| Silicic acid | 33.9 | - | - | 1.2 |
| Carvone | 34.4 | 15.5 | - | - |
| Distearyl thiodipropionate | 35.7 | - | - | - |
| Oxoberberine | 36.6 | 3.3 | - | 21.6 |
| Acetate | 38.1 | 9.2 | - | - |
| Methyltrissilane | 38.8 | 2.7 | - | - |
| 2-Pteridinamine | 39.8 | 4.4 | - | - |

[a] R.T : Retention time
[b] Not detected.

presence of 31 identified components (Table 1), which account for 97.2% of the total amount. Carvone (15.5%) was found as a major component along with $\beta$-amyrin (9.6%), acetate (9.2%), farnesyl bromide (7.2%), retinal (6.3%), and several other components in minor percentages. Moreover, chloroform fraction showed the presence of 18 identified components, which account for 87.2% of the total amount. The major component was aniline (14.4%), 2-methoxy-4-vinylphenol (13.0%), 2-butenoic acid (9.1%), desacetylanguidine (8.4%), retinal (6.3%). Ethyl acetate fraction showed the presence of 27 identified components, which account for 92.8% of the total amount. The major compounds found in the ethyl acetate fraction were oxoberberine (21.6%), 3-decanynoic acid (8.3%), 2-methoxy-4-vinylphenol (6.1%). The chloroform fraction had highest aniline and 2-methoxy-4-vinylphenol. These results, the insecticidal activity of *C. chinensis* is mainly due to its major compound

aniline and 2-methoxy-4-vinylphenol. Aniline-based derivatives were organic compounds that possess widespread chemical, agrochemical, and pharmaceutical applications (Pelucchi *et al*. 2006). Also, aromatic amine was characterized by numerous toxicological manifestations (Williams *et al*. 2000). Aniline showed strong contact toxicity.

2-Methoxy-4-vinylphenol was an aromatic substance used as a flavoring agent (Chu *et al*. 2011). However, no reports on insecticidal activity 2-methoxy-4-vinylphenol against insects were available so far.

### 3.4. Insecticidal activity of *M. persicae* treated with aniline and 2-methoxy-4-vinylphenol by filter paper contact bioassay

The insecticidal activities of aniline and 2-methoxy-4-vinylphenol were shown in Fig. 3.

Both aniline (85.4 - 98.1%) and 2-methoxy-4-vinylphenol (79.9 - 95.4%) showed a similar insecticidal activity against *M. persicae* at concentrations of 500, 1,000 and 2,000 ppm. The insecticidal activity at the lowest concentration tested (500 ppm) approached 85.4% in the aniline compared with 79.9% in the 2-methoxy-4-vinylphenol. The insecticidal activity was decreased in all treatments with the mixing of aniline and 2-methoxy-4-vinylphenol. The different mixing ratios of aniline and 2-methoxy-4-vinylphenol gave decreased insecticidal activity compared to control. This may be mainly due to the increase of 2-methoxy-4-vinylphenol. Therefore, the insecticidal activity was greater for the aniline than 2-methoxy-4-vinylphenol.

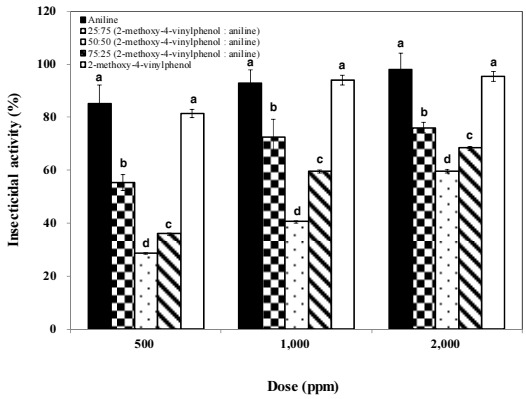


**Fig. 3.** Insecticidal activity of M. persicae (Sulzer) treated with aniline and 2-methoxy-4-vinylphenol by filter paper contact bioassay. Values followed by different letters in the same column differ significantly at $P < 0.05$.

The insecticidal activity of *M. persicae* was shown to be linearly correlated with aniline content (correlation coefficient being 0.9089) (Fig. 4). It is believed that the insecticidal activity is due mainly to the presence of aniline (Fig. 5). Further investigations examining the synergistic effect of aniline and other minor components on the insecticidal activity of *C. chinensis* are desirable.

## 4. CONCLUSION

In this study, the crude extract (70% ethanol) of *C. chinensis* and its three fractions (hexane, chloroform and ethyl acetate fraction) which were separated based on polarity indexes were examined for their insecticidal activities against

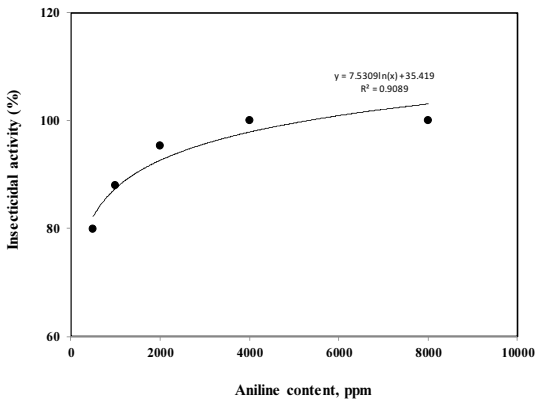


**Fig. 4.** Correlation of the insecticidal activity with aniline.

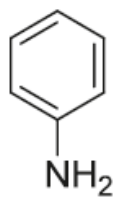


**Fig. 5.** Structure of aniline.

peach-potato aphid (*M. persicae*). The major chemical compounds of different fraction (hexane, chloroform and ethyl acetate fraction) were identified by head space-GC-MS analysis. The chloroform fraction showed the highest insecticidal activity (62.9% in 2000 ppm). The major components of chloroform fraction were aniline (14.4%), 2-methoxy-4-vinylphenol (13.0%). The insecticidal activity was greater for the aniline than 2-methoxy-4-vinylphenol. The insecticidal activity is accordingly believed to be attributable to the aniline component.

From the study, we conclude that, the extract of *C. chinensis* contains an array phytochemicals in it. The chloroform fraction of *C. chinensis* showed insecticidal providing evidence for its usage in botanical insecticides. This may provide a useful starting point for the development of botanical insecticides.

## ACKNOWLEDGEMENTS

This study was carried out with the support of “Forest Science & Technology Projects (project No. S211314L010140)” provide by Korea Forest Service, Republic of Korea and Gyeongsang National University Fund for professors on sabbatical leave, 2013.

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