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# Control of Green Peach Aphid (*Myzus Persicae*) by Combination of Plant Oil Formulations and Low-dosed Imidacloprid

You Ri Yang[1], Seon Hwa Kim[1], Myung Ryeol Park[1], Iksoo Kim[2] and In Seon Kim[1*]

[1]Division of Applied Bioscience and Biotechnology, [2]Division of Plant Biotechnology, Institute of Agricultural Science and Technology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, South Korea

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**Aphids are one of the major pests in agricultural crops. A number of synthetic pesticides have been used for control of aphids in agriculture, but increasing public concerns over their adverse effects on the environment have required more environmentally-friendly methods for pest management. In this study, we examined plant oil formulations for the control of green peach aphid (*Myzus persicae*). Oil formulations were prepared by hydrolyzing the plant oils in ethanolic KOH solution and diluted at the rate of 1:500 for aphid control. The oil formulations showed aphid mortalities ranging from 24.44 to 43.33% *in vitro*. Significantly increased aphid mortalities were observed by the treatment of oil formulations combined with low-dosed imidacloprid. No significant difference in the aphid mortality was observed between the oil formulations. Mass spectrometry analyses of aphids treated with the low dosed-imidacloprid plus the plant oil formulations detected similar concentrations of imidacloprid between the treatments. In field trial bioassays against aphids, significantly decreased aphid population were observed in the pepper plants treated with soybean oil formulation combined with the low-dosed imidacloprid, while aphid population dramatically increased in the pepper plants treated with the low-dosed imidacloprid alone. These results suggested that the plant oil formulations can be used as an environmentally-friendly method for enhancing the insecticidal effectiveness, which may play a role in reducing the use of synthetic pesticide in agriculture.**

**Key Words:** Aphid, Imidacloprid, Insecticide, Organic farming, Plant oil

## INTRODUCTION

Aphids are one of the major insect pests in agriculture. About 100 aphid species are known to cause damages to over 4,000 species of crop plants throughout the world (Dixon, 1987). Aphids feed on plant nutrients, resulting in curled and deformed leaves, and cause a variety of plant pathogens that grow on sugars secreted by aphids (Moran and Thomson, 2001). Aphids are also the causes of many viral diseases, including the cucumber mosaic virus and pot virus, in several crops (Sylvester, 1989; James and Perry, 2004). Although a number of synthetic insecticides have been widely used to control aphids, their intensive use is of the concerns due to the potential side effects on the environment. Additionally it is a worry for pest management scientists to control pesticide-resistant insects (Foster *et al*., 2000, 2003). Thus, researches have focused on the development of alternative control methods in an effort to reduce the use of synthetic insecticides in agriculture.

Organic farming toward the use of more environmentally safe approach in agriculture has encouraged many scientists to develop new pesticide formulations in order to reduce the use of synthetic pesticides (Kim and Kim, 2009). New trends in crop protection have been triggering the search for not only harmless pesticides or pesticides with very low toxicity and for also ways to reducing the use of

*연락저자:
Tel: +82-62-530-2131 Fax: +82-62-530-2139
E-mail: mindzero@chonnam.ac.kr

agrochemicals (Martín-López *et al*., 2006). One of the ways to reduce the use of synthetic chemicals is to use low-dosed pesticides combined with synergistic agents for crop protection. Application of commercial plant-derived oils is an example approach as a synergistic agent of chemical insecticides (Papachristos *et al*., 2004). Commercial plant oils have been accepted as a recommended product for pest management program in agricultural field (Ayvaz *et al*., 2010). One of main advantages in using plant oils for pest control is that they discourage insects from developing resistance to them (Kaloshians and Walling, 2005). In addition, they are non-toxic to vertebrates and degradable easily in the environment. Thus, the use of plant oils is expected to be an environmentally-friendly approach to pest management program in Korea. However, little study on the evaluation of plant oils-derived formulation for control of aphids has been conducted *in vitro* and *in vivo*, although limited botanical insecticide is available in organic farming in Korea. In the present study we carried out the aphicidal activities of commercial plant oil formulations combined with a low-dosed imidacloprid.

## MATERIALS AND METHODS

### Chemicals

Imidacloprid analytical standard was purchased from Dr. Ehrenstorfer (Ausburg, Germany). Conido$^R$ (SC 8%), an imidacloprid formulation, was kindly provided by Bayer CropScience Korea. The solvents used in this study were of HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA). Plant oils (soybean, canola, grape-seed and olive) were commercially available elsewhere in Korea. All chemicals used were of analytical grade, unless otherwise stated.

### Plant oil formulation

Plant oil formulations were prepared by hydrolyzing them under basic conditions. For this, each of the plant oils (92.6 g/L) was hydrolyzed with three equivalent of KOH in ethanol at room temperature. After completely and vigorously mixed for 2 h, the oil solutions were adjusted to approximately pH 10.8 by addition of 5.8 M HCl in ethanol, and the resulting solutions were used as the plant oil formulation.

### Laboratory bioassay for aphid mortality

Laboratory bioassays of the plant oil formulations against green preach aphid (*M. persicae*) were performed in 2-week-old Chinese cabbage as described previously (Kim *et al*., 2008). Chinese cabbage seeds were disinfected with 70% ethanol for 1 min and then rinsed several times with sterile distilled water. The seeds were then planted in commercial compost in square plastic insect-breeding dishes (80 mm × 80 mm × 100 mm) and grown in a growth chamber at 25±2℃ and 60±5% relative humidity with a 16 h light:8 h dark photoperiod for 2 weeks. Ten *M. persicae* were reared on the Chinese cabbage leaves and allowed to produce progeny for 48 h in a greenhouse. The adult aphids were then removed from the leaves, and each dish contained 18 to 24 2nd-instar nymphs. The oil formulations were diluted with water at the rate of 1:500 (hereafter 'OF500') and sprayed onto the nymphs on the Chinese cabbage leaves using a small handheld sprayer. For the bioassay of imidacloprid, Conido$^R$ was sprayed onto the nymphs at a recommended field application rate (40 mg/L) for vegetables. For the bioassays of low-dosed imidacloprid, Conido$^R$ was diluted in OF500 from the recommended dose to reach the application doses of 4.0, 1.6 and 0.8 mg/L and sprayed onto the nymphs as described above. Aphid mortality was calculated using Abbott's formula 24 h after application as previously (Abbott, 1925), where mortality (%) = [(number of live insects on control leaves - number of live insects on treated leaves)] × 100 / (number of live insects on control leaves). All experiments were repeated three times, unless otherwise stated. Statistical analyses was subjected to Duncan's multiple range test ($p<0.05$) using SAS program.

### Field trial bioassay for aphid mortality

Field trial bioassays of the oil formulations were performed against naturally occurring *M. persicae* in a pepper greenhouse in Nampyung, Chonnam Province, Republic of Korea, in July 2009. The greenhouse has cultivated Korean hot pepper plants for at least 7 years. The greenhouse suffered serious *M. persicae* damage during the season. Ten plants with high aphid populations were randomly selected in the greenhouse and subjected to aphid mortality assays. The plants were 2.0 m × 2.0 m apart from each other not to have aerosol migration at the sides of each pepper plant while spraying. Soybean oil formulation was selected as a typical application for aphid mortality assay since it is typical common plant oil commercially available in Korea. Imidacloprid (Conido$^R$) was diluted in soybean oil OF500 solution from the recommended dose to reach the application dose of 0.8 mg/L, as described above, and sprayed onto pepper plants using a farmer's handheld sprayer. The number of aphids was counted before and 24 h after application by sampling from three plants for control samples and ten plants for treated samples. Aphid mortality was investigated as follows: mortality (%) = [1-(number of live aphids after application/ number of live aphids before application)] × 100.

### Detection of imidacloprid in aphids

Imidacloprid was determined by using quadruple time-of-flight mass spectrometry. Thirty dead-aphids treated with low-dosed imidacloprid combined with OF500 were gently washed with 70% (v/v) ethanol solution to remove imidacloprid on the aphid surface and dried in the air. The aphids were then frozen in liquid nitrogen and macerated using a table homogenizer. The homogenates were suspended in 5 mL of 50% (v/v) aqueous methanol and extracted by an ultra-sonicator for 30 min. The extracts were centrifuged at 12,000 × g and the resulting supernatants were filtered through a 0.2 μm membrane filter. The filtrates were used for the detection of imidacloprid in aphids.

### Instrumentals

Gas chromatography mass spectrometry (GC/MS) was used for fatty acid analysis of OF500. GC/MS was a Shimadzu model QP2010 equipped with a DB-5 capillary column (0.25 i.d. × 30 m, 1.0 μm film thickness). The carrier gas was helium and the flow rate was 1.0 mL/min. Injection was done in a split mode of 10:1. The column temperature was set for 4 min at 120ºC, followed by a ramp rate of 5℃/min to 300℃, holding for 10 min. The injector temperature was 280℃. GC/MS was performed in electron impact mode at 70 ev. For the detection of imidacloprid in aphids, quadruple time-of-flight mass spectrometry (Q-TOF MS) was used at extracted ion chromatogram (XIC) mode, using the following operation parameters: capillary voltage: 4100 V; nebulizer pressure: 11.6 psig; collision cell RF: 200 Vpp; dry heater: 200℃; dry gas flow rate: 8.0 L/min. Q-TOF MS was a Bruker Daltonics TOF mass spectrometer connected with a Dionex model P680 HPLC. The HPLC column was a Nova-Pak C18 stainless steel column (2.0 ℃ 150 mm). The mobile phase consisted of 50% aqueous acetonitrile acidified with trifluoroacetic acid, and the flow rate was 0.5 mL/min. The HPLC system was interfaced to Q-TOF MS equipped with electrospray ionization interface in positive ion mode. Accurate mass spectra were scanned in a range of 50 to 800.

## RESULTS

GC/MS analysis of OF500 detected palmitic (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) as the main fatty acids of plant oils (Fig. 1). Oleic acid and linoleic acid were found to be the highest composition among them, accounting for more than 80% of total fatty acid. The ratio of saturated fatty acid to unsaturated fatty acid ranged from 0.10 to 0.16, which was not significantly different between the plant oils. The aphicidal activity of OF500 against *M. persicae* were 36.67% for soybean oil, 43.33% for grape-seed oil, 34.45% for canola oil and 24.44% for olive oil (Table 1), exhibiting the highest mortality for grape-seed oil. Increased aphid populations were observed in the samples treated with water as a negative control.

Imidacloprid exhibited 100% mortality of *M. persicae* at the recommended field application rate, but significantly decreased mortality was observed by the low doses of imidacloprid, exhibiting about 60 and 47% mortality at 1/10 and 1/40 doses of the recommended rate, respectively (Table 2). About 14% mortality of *M. persicae* was observed in imidacloprid treatment at 1/80 dose of the recommended rate. The dose-dependent mortality of *M. persicae* suggested that the mortality was due to the toxicity of imidacloprid.

The aphid mortality of the low-dosed imidacloprid was largely increased by adding OF500 treatment. The mortality of *M. persicae* by the low dosed-imidacloprid combined with soybean oil OF500 ranged from about 81 to 100% (Table 3), which was significantly higher than the mortality of 13.7 to 60.0% by the low-dosed imidacloprid alone. The aphid mortality of the

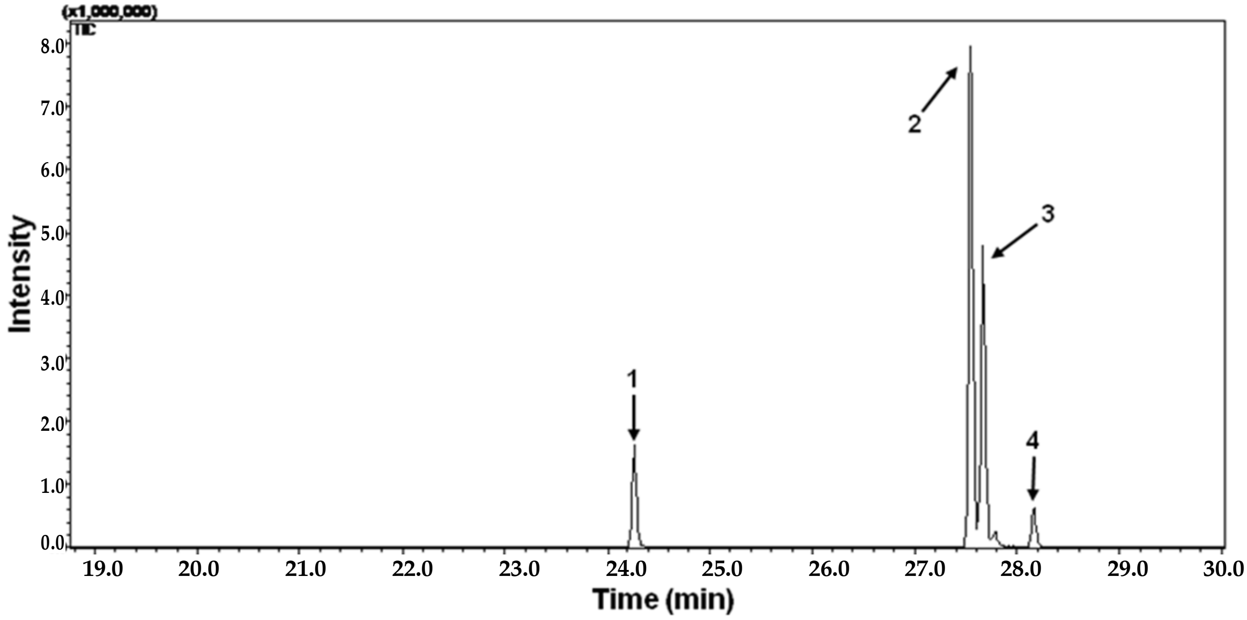


**Fig. 1. Typical GC/ MS spectrum of fatty acids of the soybean oil OF500. Peaks: 1. Palmitic acid methyl ester, 2. Linoleic acid methyl ester, 3. Oleic acid methyl ester, 4. Stearic acid methyl ester.**

**Table 1. Aphid mortalities of plant oil formulations**

| - | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| Soybean | 36.67 ± 11.55 |
| Grape-seed | 43.33 ± 15.28 |
| Canola | 34.45 ± 25.01 |
| Olive | 24.44 ± 12.01 |

* The data are means ± SD of triplicate.

**Table 2. Aphid mortalities of imidacloprid at different concentrations**

| Treated dose (mg/kg) | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| 40.0** | 100.00 ± 0.00 |
| 4.0 | 60.00 ± 17.32 |
| 1.6 | 47.02 ± 17.16 |
| 0.8 | 13.70 ±5.48 |

* The data are means ± SD of triplicate.
** Recommended field application rate

**Table 3. Aphid mortalities of imidacloprid combined with soybean oil OF500**

| Imidacloprid (mg/kg) + soybean oil | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| 40.0** | 100.00 ± 0.00 |
| 4.0 | 100.00 ± 0.00 |
| 1.6 | 100.00 ± 0.00 |
| 0.8 | 81.07 ± 9.63 |

* The data are means ± SD of triplicate.
** Recommended field application rate.

**Table 4. Aphid mortalities of imidacloprid combined with canola oil OF500**

| Imidacloprid (mg/kg) + canola oil | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| 40.0** | 100.00 ± 0.00 |
| 4.0 | 93.33 ± 11.55 |
| 1.6 | 86.30 ± 15.17 |
| 0.8 | 81.67 ±10.10 |

* The data are means ± SD of triplicate.
** Recommended field application rate.

**Table 5. Aphid mortalities of imidacloprid combined with grape-seed oil OF500**

| Imidacloprid (mg/kg) + grape-seed oil | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| 40.0** | 100.00 ± 0.00 |
| 4.0 | 100.00 ± 0.00 |
| 1.6 | 96.67 ± 5.77 |
| 0.8 | 66.67 ±4.41 |

* The data are means ± SD of triplicate.
** Recommended field application rate.

**Table 6. Aphid mortalities of imidacloprid combined with olive oil OF500**

| Imidacloprid (mg/kg) + grape-seed oil | Aphid mortality (%)* |
|---|---|
| Control | 0.00 ± 0.00 |
| 40.0** | 100.00 ± 0.00 |
| 4.0 | 100.00 ± 0.00 |
| 1.6 | 96.67 ± 5.77 |
| 0.8 | 66.67 ±4.41 |

* The data are means ± SD of triplicate.
** Recommended field application rate.

low dosed-imidacloprid combined with canola oil OF500 ranged from about 81.7 to 93.3% (Table 4), comparable to the mortality by the low-dosed imidacloprid alone. The low dosed-imidacloprid combined with grape-seed oil OF500 or olive oil OF500 exhibited aphid mortalities ranging from about 67 to 100% (Table 5 and 6).

In greenhouse experiment, aphids were sprayed with imidacloprid at 1/40 dose of the recommended rate combined with soybean oil OF500, which gave about 50.6 to 87.7% mortalities (Fig. 2). Lower mortalities of aphids in the field trial than those in the laboratory evaluations may be due to aphid reside under plant leaves at full maturity. The control

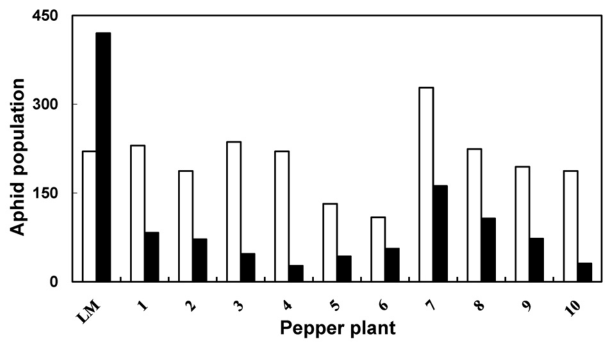


**Fig. 2. Changes in aphid population before (□) after (■) spraying a low dose of imidacloprid combined with or without the soybean oil OF500 in field bioassays against green peach aphids. LM represents the low dose of imidacloprid alone.**

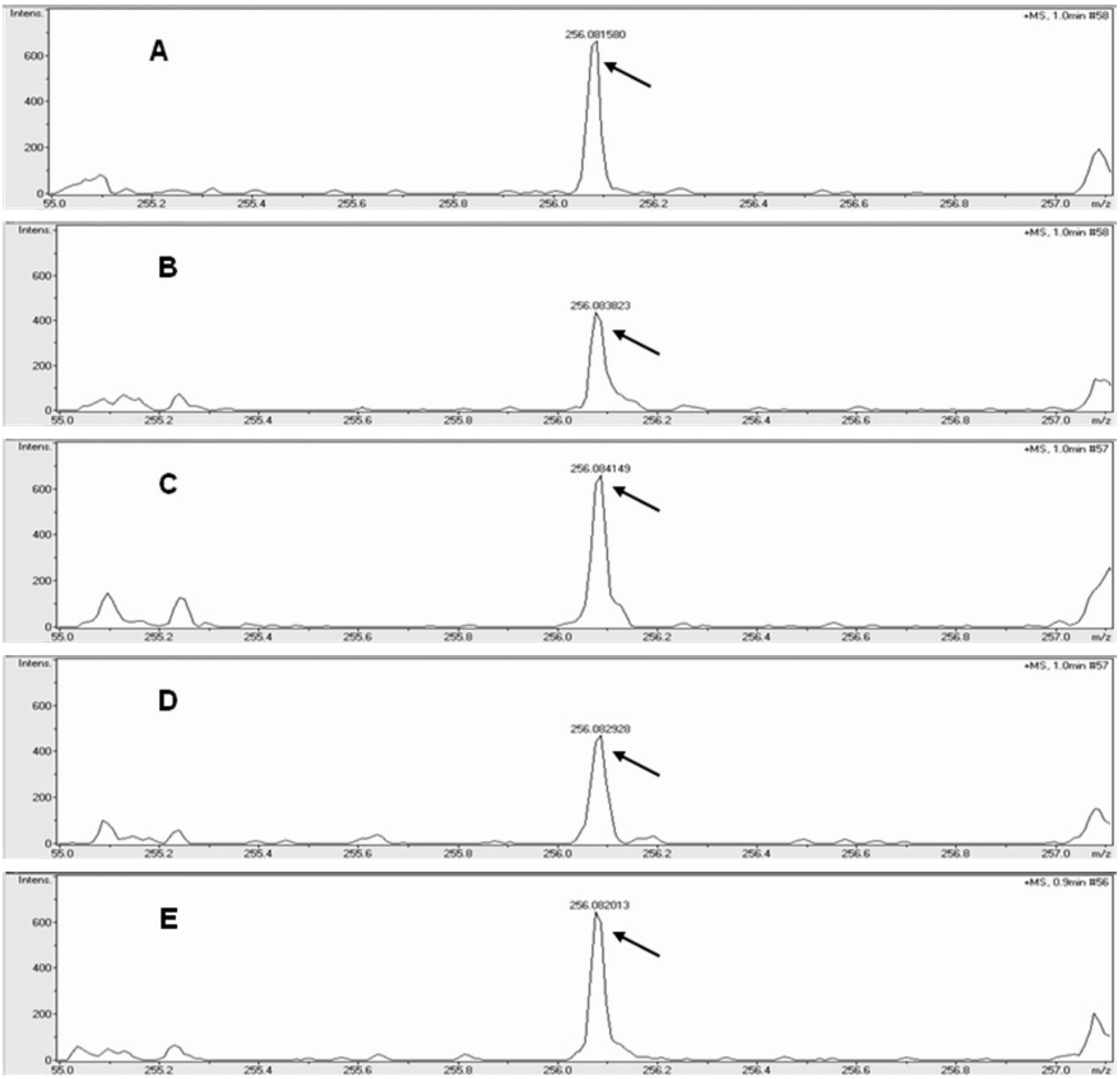


**Fig. 3. Typical TOF-MS spectra of imidacloprid extracted from aphids treated with a low dose of imidacloprid alone (A) and the low-dosed imidacloprid combined with soybean oil OF500 (B), canola oil OF500 (C), grape-seed oil PF500 (D) and olive oil OF500 (E). The arrow symbols represent the peaks of imidacloprid detected by TOF-MS.**

efficiencies of the combined mixture lasted 5 to 7 days. No phytotoxic symptoms were observed by application of OF500.

In order to investigate if the increased aphid mortality of low-dosed imidacloprid was due to more penetration and accumulation of imidacloprid in aphid, aphids were extracted with organic solvents and analyzed by mass spectrometry (Fig. 3). TOF-MS analysis at XIC mode detected about 0.6 mg/L of imidacloprid in aphids treated with the low-dosed imidacloprid combined with soybean oil OF500. About 1.0 mg/L of imidacloprid was detected in aphids treated with the low-dosed imidacloprid combined with canola oil OF500 or olive oil OF500, which was close to the concentration found in aphids treated with the low-dosed imidacloprid alone. TOF-MS detected about 0.7 mg/L of imidacloprid in aphids treated with low-dosed imidacloprid combined with grape-seed oil OF500.

## DISCUSSION

The intensive use of synthetic pesticides in agriculture has become an environmental concern due to their toxicities and persistence in the environment. Thus, Korea government has driven environmentally-friendly agriculture (EFA) program since 1995. The EFA program has been established with the goal of decreasing the use of synthetic pesticides by 40%. This large reduction in the use of synthetic pesticides could be of negative impacts on agriculture, since crop yields may be significantly decreased due to plant pathogens and insect pests that have been managed successfully by synthetic pesticides (Oerke and Dehane, 2004). Thus, alternative approaches are required for pest management, particularly in organic farming. In the present study, we examined plant oil-derived formulations in order to develop a method as more environmentally safe approach for the control of green peach aphids.

Mortality of aphid treated with low doses of imidacloprid was significantly enhanced *in vitro* by the plant oil formulations as compared to the mortality by low-dosed imidacloprid alone. Results of greenhouse experiments indicated that the plant oil formulations may provide high mortality levels of aphids under field conditions. TOF-MS analyses demonstrated that the increased aphid mortality would not be due to the increased penetration or accumulation of imidacloprid, since the concentration of imidacloprid found in aphids treated with the combined mixture was similar to the concentration found in aphids treated with low-dosed imidacloprid alone. The synergistic effects of the plant oil formulations on aphid mortality may be an explanation for the increased mortality, as demonstrated earlier (Puri *et al*., 1994; Horowitz *et al*., 1997; Rao and Dhingra, 1997; Liu and Stansely, 2000). The plant oil formulations are expected to play the roles of wetting, spreading and sticking agents on aphids. One of possible actions of plant oil formulations was suggested to be their asphyxial effects on aphid surface, as described previously a synergistic effect of oils on insect control (Dhingra, 1996). Aphids would be covered with a thin film of oily materials such as fatty acids produced from plant oils. Botanical insecticides, such as nicotine from tobacco leaves, rotenone from derris tree roots, pyrethrum from chrysanthemum flowers and azadirachitin from neem tree have accepted as alternatives to conventional synthetic insecticides (Isman, 1997; Edelson, 2002; Casanova *et al*., 2005, Kim *et al*., 2007, 2009). The plant oil formulations can be also used in order to increase the insecticidal efficacy of botanical insecticides. The plant oil formulations are, therefore, suggested to be an alternative approach for pest management in an effort to reduce the use of synthetic pesticide in agriculture. Further works to examine the oil formulations for aphid control under different agricultural conditions are required. Our results showed clearly that the plant oil formulations are applicable for control of aphid under laboratory and greenhouse conditions.

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