Evaluation of Efficacy of Fumigants and Natural Product Extracts for Management of Springtail \textit{Hypogastrura vernalis} (Collembola: \textit{Hypogastruridae}) and Green Peach Aphid \textit{Myzus persicae} (Hemiptera: \textit{Aphididae})

by

Qasim Hussein Ahmed

A thesis presented to Murdoch University for the degree of Doctor of Philosophy

November, 2018
I hereby declare that this Ph.D. thesis was carried out by me and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Qasim Ahmed
Acknowledgment

In the name of Allah

I thank Allah very much, who provided me the patience to finish my PhD. Then, I would like to acknowledgment the support, guidance and their profound inspiration of my supervisors, Professor Yonglin Ren and Dr. Manjree Agarwal at Murdoch University and Dr. Robert Emery at the Department of Primary Industries and Regional Development. Their patience and help at every point throughout my period of study. Thanks are also due to Dr. Mikael Calver (Supervisory Committee Chair) for his time and advice. I would like to thank David Cousins from the Department of Primary Industries and Regional Development for his help and supplying aphids.

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Evaluation of Ethyl Formate, Phosphine, and Their Combination to Disinfect Harvested Celery against Purple Scum Springtails

Qasim Ahmed1,2, Yonglin Ren1,4, Robert Emery3, James Newman1, and Manjiree Agarwal1,4

Additional Index Words: commodity treatment, horticultural export, insect, Hypogastria vernalis, Apium Graveolens, fumigation, phytotoxicity

Summary. Export celery (Apium graveolens var. dulce) from Australia has been affected by a natural infestation of purple scam springtails (Hypogastria vernalis). These insects live inside the celery head, contaminating fresh celery, but do not cause any visible damage. As a result, purple scam springtail-infested celery has led to rejection for export with an impact on market value for fresh produce. In this study, fumigation with ethyl formate (EF), phosphine (PH3), and their combination on 200 batches of electrolyzed springtails in naturally infested celery was evaluated. Laboratory experiments were conducted using concentrations of 50, 60, and 90 mg L−1 of EF for 1, 2, and 4 hours; 1, 1.5, 2, and 2.5 mg L−1 of PH3 for 2, 4, and 6 hours; and 30, 40, and 60 mg L−1 of EF combined with 1 mg L−1 of PH3, for 2 and 4 hours at the laboratory temperature 25°C. Complete control was achieved at 50 mg L−1 of EF for 2 hours; however, phytotoxicity was observed in celery treated by EF at all concentrations. PH3 at 2.5 mg L−1 achieved 100% mortality within 6 hours, and no phytotoxicity was evident. Mortality of 100% was achieved also at 30 and 40 mg L−1 EF combined with 1 mg L−1 of PH3 for 2 and 4 hours exposure time; however, phytotoxicity occurred with EF alone treatments and with the combination. From these data, we conclude that PH3 alone has potential as a fumigant for the preshipment treatment of celery infested with purple scam springtails.

Celery is an intensively cultivated and valuable horticultural export product from Australia. Celery is grown in most states with the main production areas in Victoria, Western Australia, and Queensland. There are cultivated areas of ≥255 ha per year (Horticulture Australia, 2009). Australian celery production in 2015 was more than 60,000 t, with a value of 50.1 million Australian dollars. In 2017, celery production for export increased to 3557 t (Horticulture Innovation Australia, 2017). However, this growing market has been threatened by a natural infestation of purple scam springtails, which significantly affected market access as purple scam springtails are considered a quarantine pest in many Middle East countries. Australian Quarantine does not allow export of plant products containing living organisms.

Purple scam springtails are tiny wingless arthropods with a bulbous, rounded form. The natural densities of springtails in general in Australia range between 2000 and 30,000 m−2, depending on climate, season, and habitat (Greenslade, 2007). Springtails in general feed on decaying plant material and other organisms, such as fungi, algae, and occasionally dead animals. A few species of springtails have been recognized as pests that can damage mushrooms and other crops (Greenslade and Ireson, 1986; Greenslade et al., 2014; Poponen, 1917). Regardless of species, the preferred conditions for the growth of purple scam springtails are warmth, moisture, and high organic content (Greenslade and Kitching, 2011). Two species of springtails from the Hypogastriidae family, mushroom springtail (Ceratophyllum densicollis) and purple scam springtail, have been reported on celery in Western Australia (Majer et al., 2014). These species are not considered to be primary pests, but are present because they feed on microorganisms and decaying products (Chahartaghi et al., 2005). Several species of the Hypogastriidae family can cause damage to cultivated mushrooms and are confirmed as pests of edible mushrooms in Australia, of which the most common ones are mushroom springtail, Hypogastria manubrialis, Hypogastria purpurea, and purple scam springtail (Greenslade and Cliff, 2004). Fumigation of the growing environment used in a mushroom culture can prevent invading purple scam springtails and other species from damaging crops (Greenslade et al., 2014).
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Evaluation of Ethyl Formate, Phosphine, and Their Combination to Disinfest Harvested Celery against Purple Scum Springtails

Qasim Ahmed,1,2 Yonglin Ren,1,4 Robert Emery,3 James Newman,1 and Manjereet Aragwal1,4

ADDITIONAL INDEX WORDS: commodity treatment, horticultural export, insect, Hypogastrura vernalis, Apium graveolens, fumigation, phytotoxicity

SUMMARY. Export celery (Apium graveolens var. dulce) from Australia has been affected by a natural infestation of purple scum springtails (Hypogastrura vernalis). These insects live inside the celery head, contaminating fresh celery, but do not cause any visible damage. As a result, purple scum springtail-infested celery has led to rejection for export with an impact on market value for fresh produce. In this study, fumigation with ethyl formate (EF), phosphine (PH3), and their combination on mature Apium graveolens was evaluated. Laboratory experiments were conducted using concentrations of 50, 60, and 90 mg.L-1 of EF for 1, 2, and 4 hours; 1, 1.5, 2, and 2.5 mg.L-1 of PH3 for 2, 4, and 6 hours; and 20, 30, and 40 mg.L-1 of EF combined with 1 mg.L-1 of PH3 for 2, 4 and 6 hours at the laboratory temperature 25 °C. Complete control was achieved at 90 mg.L-1 of EF for 2 hours; however, phytotoxicity was observed in celery treated by EF at all concentrations. PH3 at 2.5 mg.L-1 achieved 100% mortality within 6 hours, and no phytotoxicity was evident. Mortality of 100% was achieved also at 30 and 40 mg.L-1 of EF combined with 1 mg.L-1 of PH3 for 2 and 4 hours exposure time; however, phytotoxicity occurred with EF alone treatments and with the combination. From these data, we conclude that PH3 alone has potential as a fumigant for the preshipment treatment of celery infested with purple scum springtails.

Celery is an intensively cultivated and valuable horticultural export product from Australia. Celery is grown in most states with the main production areas in Victoria, Western Australia, and Queensland. There are cultivated areas of 2.255 ha per year (Horticulture Australia, 2009). Australian celery production in 2015 was more than 60,000 t, with a value of $50.1 million Australian dollars. In 2017, celery production for export increased to 3557 t (Horticulture Innovation Australia, 2017). However, this growing market has been threatened by a natural infestation of purple scum springtails, which significantly affected market access as purple scum springtails are considered a quarantine pest in many Middle East countries. Australian Quarantine does not allow export of plant products containing living organisms.

Purple scum springtails are tiny wingless arthropods with a bulbous, rounded form. The natural densities of springtails in general in Australia range between 2000 and 30,000 m2, depending on climate, season, and habitat (Greenslade, 2007). Springtails in general feed on decaying plant material and other organisms, such as fungi, algae, and occasionally dead animals. A few species of springtails have been recorded as pests that can damage mushrooms and other crops (Greenslade and Ireson, 1986; Greenslade et al., 2014; Popemore, 1977). Regardless of species, the preferred conditions for the growth of purple scum springtails are warm, moist, and high organic content (Greenslade and Kitching, 2011). Two species of springtails from the Hypogastruridae family, mushroom springtail (Ceratophyda densicollis) and purple scum springtail, have been reported on celery in Western Australia (Majer et al., 2014). These species are not considered to be primary pests, but are present because they feed on microorganisms and decaying products (Chahartagh et al., 2005). Several species in the Hypogastruridae family can cause damage to cultivated mushrooms and are confirmed as pests of edible mushrooms in Australia, of which the most common ones are mushroom springtail, Hypogastrura manubrialis, Hypogastrura purpurea, and purple scum springtail (Greenslade and Clift, 2004). Fumigation of the growing environment used in a mushroom culture can prevent invading purple scum springtails and other species from damaging crops (Greenslade

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# List of abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMDIS</td>
<td>Automated mass spectral deconvolution and identification system</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>B</td>
<td>Black pepper</td>
</tr>
<tr>
<td>BE</td>
<td>Combination of black pepper and eucalyptus</td>
</tr>
<tr>
<td>BR</td>
<td>Combination of black pepper and rosemary</td>
</tr>
<tr>
<td>BT</td>
<td>Combination of black pepper and tea tree</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>E</td>
<td>Eucalyptus</td>
</tr>
<tr>
<td>EF</td>
<td>Ethyl formate</td>
</tr>
<tr>
<td>EO</td>
<td>Essential oil</td>
</tr>
<tr>
<td>ER</td>
<td>Combination of eucalyptus and rosemary</td>
</tr>
<tr>
<td>ET</td>
<td>Combination of eucalyptus and tea tree</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GC-FID</td>
<td>The gas chromatography-flame ionization detector</td>
</tr>
<tr>
<td>GC-FPD</td>
<td>Gas chromatography-flame photometric detector</td>
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<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometer</td>
</tr>
<tr>
<td>GPA</td>
<td>Green peach aphid</td>
</tr>
<tr>
<td>HS</td>
<td>Headspace</td>
</tr>
<tr>
<td>HS-SPME</td>
<td>Headspace solid phase micro extraction</td>
</tr>
<tr>
<td>IPM</td>
<td>Integrated pest management</td>
</tr>
<tr>
<td>LD</td>
<td>Lethal dose</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>NIST</td>
<td>The US national institute of standards and technology</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
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<tr>
<td>PH₃</td>
<td>Phosphine</td>
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<td>PPM</td>
<td>Parts per million</td>
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<tr>
<td>PPT</td>
<td>Parts per trillion</td>
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</tbody>
</table>
R  Rosemary
RI  Retention index
RT  Retention time
SD  Standard deviation
SE  Standard error
SPME  Solid phase microextraction
T  Tea tree
TR  Combination of tea tree and rosemary
VOCs  Volatile organic compounds
$\chi^2$  Chi-Square
## Statement of Contribution of Chapter Two

<table>
<thead>
<tr>
<th>Title of Paper</th>
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Conference presentation

Abstract

Vegetables are one of the most important crops in the world and especially in Australia. Under this research, three study cases were investigated for controlling vegetable pests, purple scum springtail *Hypogastrura vernalis* in export celery and green peach aphid *Myzus persicae* in *Brassica* crops. They are a serious quarantine and production pests of economically important celery and crucifer crops and hence were selected for the current study. This research investigates and assesses the impact of the use of various fumigants on infested celery with *H. vernalis* along with the use of plant volatiles and natural plant-based essential oils for control of *M. persicae*. Celery bunches become host to the Australian native springtail (*Hypogastrura vernalis*) (Collembola: Hypogastruridae). Springtails live inside the celery bunch and do not cause damage to the product. Springtails are, however, considered a quarantine pest and have had a significant impact on celery exports. On the other side, using chemical pesticides against aphids has led to resistant against chemical pesticides so that require further experiments on the prospective role of factors affecting *M. persicae* pest status, including plant volatile compound and essential oils are urgently needed.

Australia has widely grown celery *Apium graveolens* var. *dulce* for domestic and export markets. In the field, celery bunches become host to the Australian native springtail. These insects live inside the celery head, contaminating fresh celery but do not cause any visible damage. In chapter study, evaluation of ethyl formate (EF), phosphine (PH$_3$) and their combination were used for celery fumigation against purple scum springtails that naturally infest celery. The selection of EF and PH$_3$ was because both provide fast kill of insect within a few hours and are registered as fumigants in Australia. The material of EF breakdown is ethanol and formic acid, while the PH$_3$ is slightly soluble in water and be broken down quickly into other products in the atmosphere. These are the first experiments that have used EF and PH$_3$ for harvest celery fumigation.

In the laboratory experiments, three concentrations of EF 50, 60 and 90 mg L$^{-1}$; four concentrations of PH$_3$ 1, 1.5, 2 and 2.5 mg L$^{-1}$ and three concentrations of EF 20, 30 and 40 mg L$^{-1}$ combined with 1 mg L$^{-1}$ of PH$_3$ were used for various exposure times at the laboratory temperature 25°C. The result indicated that 100% mortality was achieved at 90 mg L$^{-1}$ of EF for 2 h and 100% mortality was also achieved at 30 and 40 mg L$^{-1}$ EF combined with 1 mg L$^{-1}$ of PH$_3$ for 2 and 4 h
exposure time, however, phytotoxicity was observed in celery treated with EF at all concentrations both in combination and alone. PH$_3$ at 2.5 mg L$^{-1}$ achieved 100% mortality within 6 h, and no phytotoxicity was evident. From these data, we conclude that PH$_3$ alone has potential as a fumigant for the pre-shipment treatment of celery infested with purple scum springtails.

In order to further develop natural or biological methods to manage the interaction between insect pests and host plants (three replicates per treatment) was studied using volatile organic compounds (VOCs). The VOCs from uninfested and infested *Brassica* plants with *M. persicae* were investigated by headspace solid microextraction (HS-SPME) combined with gas chromatography mass spectrometry (GC-MS). There is a need for chemical pesticides replacement with environmentally friendly alternatives because of chemical pesticides have been widely used against various pests including aphids have been shown a negative side of environments and an effect on non-target organisms and to understand the communication between aphid and the host plant. Understanding the biological and chemical basis of volatiles could lead to new approaches to the biocontrol of aphids. In Chapter two, the study was evaluated on VOCs from uninfested and infested *Brassica* plants with *M. persicae*. The results show that 29 compounds were detected in both infested and uninfested cabbage *Brassica oleracea* L. var. capitata, and 25 compounds were identified in both infested and uninfested broccoli *Brassica oleracea* L. var. *italica* plant samples. The HS-SPME combined with GC-MS analysis of the volatiles describes the differences between the infested and uninfested Cruciferous plant samples. Based on peak area from the GC-MS analysis, the VOCs from infested cabbage consisted of Propane, 2-methoxy, alpha- and beta-pinene, Myrcene, 1-Hexanone, 5-methyl-1-phenyl, Limonene, Decane, gamma-Terpinen and 2,4,4-trimethyl Heptane, all these volatiles were higher in the infested cabbage compared with their peak area in the uninfested cabbage. Similarly, the VOCs from infested broccoli were significantly greater than that from uninfested broccoli, such as D-limonene, Undecane, 3,4-dimethyl-, Heptane, alpha-Pinene, Oxalic acid, Citronellol, Tridecane, n-Decanoic acid, Cyclopentane, penty1 and n-Hexadecanoic acid compared with volatiles released from uninfested broccoli.

The results presented in this chapter three outline the response of aphids and parasitoids to plant volatiles by using Y-tube olfactometer. The results show that *M. persicae* were significantly
attracted to infested and uninfested cabbage and broccoli plants compared with clean air; the percentage of aphid choice was 80% and 70% toward infested cabbage and broccoli, respectively, and 7% and 10% were attracted to the clean air choice. While 75.5% and 84% of aphids attracted to uninfested plants comparison with clean air 3% and 7%, for the cabbage and broccoli, respectively. Comparing infested and uninfested plants, the aphids were attracted by 63% and 26.6% for infested cabbage and broccoli respectively, versus 57% and 30% for uninfested cabbage and broccoli, respectively. The results indicate that using an olfactometer, tested parasitoids prefer and are attracted to, the cabbage plants infested with \textit{M. persicae} compared with clean air. Parasitoids can discriminate the infested plant and significantly responded to the infested plant odour and attracted by 86.6% and 100% for both parasitoids toward infested \textit{Brassica} plants.

Another way to reduce chemical pesticides usage is with alternatives such as biopesticides for insect pest management. Therefore, chapter four describes the use of different essential oils (black pepper, eucalyptus, rosemary and tea tree), in combination and alone, against \textit{M. persicae}. These essential oils have insecticidal activity and repellency against many insects including aphids and bioassay studies showed significant control of the green peach aphid through higher mortality. The results show that black pepper and tea tree pure essential oils were effective and caused 80% mortality of aphids for the contact treatment. However, the residual toxins were the most effective on aphids with 100% mortality for pure black pepper and tea tree oil and less than 96% for eucalyptus and rosemary. The combination of essential oils was tested with bioassay as contact and residual toxins. For the contact treatment, the mortality was 98.33% for black pepper + tea tree and rosemary + tea tree. While, in the residual treatment, the mortality was 100% for black pepper + eucalyptus, rosemary + eucalyptus and rosemary tea tree. The essential oil combinations exhibited synergistic, additive and antagonistic interactions for insecticidal activity. The combination of binary essential oils black pepper + tea tree oil showed enhanced activity, with a synergistic rate of 2.19. Essential oil formulation showed effective mortality of aphids, but phytotoxicity appeared on cabbage plants. The Fourier Transform Infrared Spectroscopy (FTIR) analysis of stability of a mixture of essential oils showed that it was not affected by store temperature (15, 25 and 35°C) and all functional groups were not changed during the storage for three months. Based on the results, essential oils can be used as a commercial insecticide against \textit{M. persicae} thereby reducing the use of chemical pesticides and their negative impact on the
In summary, the use of EF fumigant in combination with PH₃ and alone achieved high mortality on purple scum springtails, however, phytotoxicity on treated celery is a negative. Alternatively, PH₃ alone achieved 100% mortality after 6 h without any observed phytotoxicity, therefore, PH₃ has potential as a fumigant for the pre-shipment treatment of celery infested with purple scum springtails. Plant volatile organic compounds that release from the infested cabbage and broccoli can use as an indicator tool for the field infestation related to the differences between the infested and uninfested plant. Base on Y-tube olfactometer, *Myzus persicae* response to both infested and uninfested plants and parasitoids response to the infested plants. From the laboratory experiments, essential oils show high mortality on green peach aphids and could be used as an alternative to chemical pesticides. According to the FTIR analysis, essential oils can be stored at between 15 and 35°C with no effect on the properties of the oil. Therefore, I suggest that tested essential oil constituents both pure and in combination could be screened as a potential natural insecticides. Further they could be involved in the chemical synthesis of new types of pesticides, based on essential oils and their constituents.
Chapter 1: Literature Review

1.1 Introduction

This literature review highlights the issue of protecting plants from springtails and green peach aphids (GPA) using three case studies as different methods of insect control: fumigation for harvested celery; plant volatile organic compounds (VOCs) and their role in attracting natural enemies; and the use of plant essential oils as green pesticides. The issue was investigated because the use of chemical pesticides on infested plants to prevent damage from pests has led to pest resistance, consumer sensitivity to pesticide residues, and chemical pesticides’ effect on the environment as chemical contaminants and transference into the food chain.

Vegetables (e.g. celery *Apium graveolens* var. *dulce* and *Brassica* plants such as cabbage *Brassica oleracea* L. var. *capitata* and broccoli *Brassica oleracea* L. var. *italica*) are among the most important crops grown in the world generally, and especially in Australia. Celery and cruciferous crops are grown worldwide for their edible fruits and leaves. Vegetables are an important component of daily diets, providing nutrition, and essential elements for the growth of the human body and support of the immune system. They are also important sources of revenue for the country through international trade.

Celery, *A. graveolens*, belongs to the family Apiaceae, which has been planted as a biannual vegetable crop worldwide. It is consumed around the world as a raw vegetable with its leaves and crisp stalks used in cooking as a spice or flavouring in many cuisines. Further, its extraction has been used medicinally because of its anti-inflammatory properties (Tamokou et al. 2017).

The importance of the family *Brassicaceae* comes from using these plants economically as food sources. *Brassica* plants such as cabbage and broccoli are grown all over the world; however, they continue to face severe damage caused by several insect pests. The number of pests reported on these crops is increasing worldwide. This may be due to changes in environmental conditions, different cropping patterns, plant movement through the international trade supply chain, pesticide
resistance, or a combination of several factors. Vegetables have many pests, including springtails and aphids. Springtails are tiny insects that have rounded shape. Their name come from the hinged spring located on the underside of the abdomen. There are many species of springtails and they live in moist or humid environments. They feed on dead and decay plant, fungi and algae, and a few species are recorded as a pest on vegetable crops that feed on roots or seedlings and affect germination of seeds. Most of the Hypogastruridae species are considered pests on mushrooms; however, the purple scum springtail *Hypogastrura vernalis* lives in large numbers inside the celery bunch and between celery leaves, which affects the quality of celery crop by contamination, resulting in them being recorded as a quarantine pest (Greenslade et al. 2014; Majer et al. 2014). Aphids are small, soft-bodied insects, usually green or pink, with long, slender mouthparts used to suck out plant fluids. This feeding can cause leaves to wilt and curl downward. They excrete honeydew, which makes the leaves sticky and supports the growth of sooty mold. An important species of aphid that attacks several vegetable families is the green peach aphid *Myzus persicae* (Blackman & Eastop, 2000). Infestation with green peach aphids can cause serious yield and market value reduction of the crops (Liu et al. 1994). *Myzus. persicae* can directly damage plants by sucking and also as a vector of several plant viruses that cause further damage to crops (Van Emden & Harrington 2007).

For tens of thousands of years, people have dealt with the agricultural activity of growing crops including vegetables. However, pest outbreaks and damage to pre-harvest and postharvest crops due to infestation of pests has been a constant battle eventually resulting in development of chemical pesticides. Of course this led to damage caused by chemicals and the search for biological control alternatives. VOCs are an alternative chemical pesticide and recent studies have been undertaken. Therefore, it is important to develop new techniques for controlling springtails and aphids as part of an environmentally friendly, integrated pest management (IPM) system on vegetables. Many natural product insecticides, such as azadirachtin and rotenone, have enticed research interest because of their lower toxicity to mammals; lower environmental residues (eco-friendly); pests do not readily develop resistance against them and they have multiple properties or modes of action. This study involved a review of past literature on pre- and postharvest treatments, use of fumigants along with the use of VOC as a repellent or way of attracting natural enemies; and use of essential oils in pest control.
1.2 Host plants

1.2.1 Celery

Celery (Apium graveolens L. var dulce) is a member of the family Apiaceae and considered to be an important vegetable in family Apiaceae. It is cultivated in different parts of the world and utilised as food because it is an excellent source of many vitamins and nutrients. Celery is a biannual plant and bunches should be harvested once they reach marketable size as it is ready for consumption in the vegetative stage. Celery is grown in areas with rich fertilising soils and a cool climate (Husain et al. 1988). As celery contains around 95% water, keeping them cool and well hydrated is important to maintain quality (Australia Horticulture 2017). Celery is one of the important high yielding vegetables cultivated in Western Australia, with celery production valued at A$59.6 million in 2016/2017 over a production area of 1,220 hectares and production volume of 62,100 tonnes. However, the production of celery has been affected by the infestation of pests (Australian Horticulture 2017) (See Fig. 1.1 and Table 1.1).

Celery plants are affected by several pests and diseases. These include the green peach aphid M. persicae, cotton aphid Aphis gossypii, onion thrips Thrips tabaci and western flower thrips Frankliniella occidentalis; bugs such as the green vegetable bug Nezara viridula; and caterpillars including Helicoverpa spp. and Agrotis species. However, with celery infested with springtail (Collembola) H. vernalis, the negative impact on celery bunches is represented by contamination of the plant without feeding on celery plant parts. This issue caused celery exports being rejected for some countries under the quarantine system (Majer et al. 2014).

1.2.2 Cruciferous plants

Cruciferous crops are a diverse group belong to the family Brassicaceae (Cruciferae) and are consumed as fresh vegetables. The family Brassicaceae consists of cabbage, cauliflower, broccoli, brussels sprouts, radish and turnip. These crops are an important source of vitamins, fibres and minerals, and play an important role in the daily diet. Crucifers are widely grown in many countries in the world. Cabbage is one of the important cruciferous crops cultivated in Western Australia.
and the other Australian states, with a cabbage production value of A$45 million in 2016/2017. Cabbage production occurred in most Australian states during 2016/2017, with a yield of 71,126 tonnes. Cabbage is grown across the Australian states with the largest producing areas in Queensland, New South Wales and Victoria (Australian Horticulture 2017) (see Figure 1.2 and Table 1.2). The production of cabbage has been affected by the infestation of various pests including insects and pathogens. The cruciferous crops are attacked by insect pests, such as different species of aphids like green peach aphid, cabbage aphid (*Brevicoryne brassicae*), turnip aphid (*Lipaphis erysimi*); and different species of cabbage moth like diamondback moth (*Plutella xylostella*), cabbage butterfly (*Pieris brassicae*) and cabbage webworm (*Hellula undalia*).
Table 1.1. The production of celery in Australia (Australian Horticulture Statistics Handbook 2017).

<table>
<thead>
<tr>
<th>Year Ending June</th>
<th>2015</th>
<th>2016</th>
<th>% YoY</th>
<th>2017</th>
<th>% YoY</th>
</tr>
</thead>
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<tr>
<td>Production (t)</td>
<td>59,484</td>
<td>60,873</td>
<td>+2%</td>
<td>62,100</td>
<td>+2%</td>
</tr>
<tr>
<td>Production ($m)</td>
<td>$50.2</td>
<td>$50.1</td>
<td>&gt;-1%</td>
<td>$59.6</td>
<td>+19%</td>
</tr>
<tr>
<td>Production area (Ha)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh Export Volume (t)</td>
<td>2,023</td>
<td>3,557</td>
<td>+76%</td>
<td>3,872</td>
<td>+9%</td>
</tr>
<tr>
<td>Fresh Export Value ($m)</td>
<td>$2.6</td>
<td>$5.2</td>
<td>+100%</td>
<td>$6.2</td>
<td>+19%</td>
</tr>
<tr>
<td>Fresh Import Volume (t)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>Fresh Import Value ($m)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;$0.1</td>
<td>-</td>
</tr>
<tr>
<td>Fresh Supply (t)</td>
<td>56,582</td>
<td>56,417</td>
<td>&gt;-1%</td>
<td>57,310</td>
<td>+2%</td>
</tr>
<tr>
<td>Fresh Supply Wholesale Value ($m)</td>
<td>$56.3</td>
<td>$53.6</td>
<td>-5%</td>
<td>$63.8</td>
<td>+19%</td>
</tr>
<tr>
<td>Supply per Capita (kg)</td>
<td>2.38</td>
<td>2.34</td>
<td>-2%</td>
<td>2.34</td>
<td>&gt;-1%</td>
</tr>
</tbody>
</table>

Sources: AC; AUSVEG; CFVIWA; GTA; MP & DD (Freshlogic Analysis)

Figure 1.2. Cabbages are grown across most states of Australia, with the majority of cabbage grown in Queensland, Sydney and Melbourne (Australian Horticulture Statistics Handbook 2017).
Table 1.2. The production value of cabbages (Australian Horticulture Statistics Handbook 2017).

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2016</th>
<th>% YoY</th>
<th>2017</th>
<th>% YoY</th>
</tr>
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<tbody>
<tr>
<td><strong>Production (t)</strong></td>
<td>67,463</td>
<td>69,454</td>
<td>+3%</td>
<td>71,126</td>
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<td><strong>Production ($m)</strong></td>
<td>$44.1</td>
<td>$42.6</td>
<td>-3%</td>
<td>$45.0</td>
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<tr>
<td><strong>Production area (Ha)</strong></td>
<td>-</td>
<td>2,343</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Fresh Export Volume (t)</strong></td>
<td>1,080</td>
<td>2,118</td>
<td>+96%</td>
<td>2,624</td>
<td>+24%</td>
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<tr>
<td><strong>Fresh Export Value ($m)</strong></td>
<td>$1.7</td>
<td>$3.4</td>
<td>&gt;100%</td>
<td>$4.6</td>
<td>+37%</td>
</tr>
<tr>
<td><strong>Fresh Import Volume (t)</strong></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>-20%</td>
<td>&lt;1</td>
<td>+63%</td>
</tr>
<tr>
<td><strong>Fresh Import Value ($m)</strong></td>
<td>&lt;$0.1</td>
<td>&lt;$0.1</td>
<td>-11%</td>
<td>&lt;$0.1</td>
<td>+7%</td>
</tr>
<tr>
<td><strong>Fresh Supply (t)</strong></td>
<td>56,093</td>
<td>56,742</td>
<td>+1%</td>
<td>57,653</td>
<td>+2%</td>
</tr>
<tr>
<td><strong>Fresh Supply Wholesale Value ($m)</strong></td>
<td>$49.4</td>
<td>$46.0</td>
<td>-7%</td>
<td>$47.5</td>
<td>+3%</td>
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<tr>
<td><strong>Supply per Capita (kg)</strong></td>
<td>2.36</td>
<td>2.35</td>
<td>-1%</td>
<td>2.39</td>
<td>+2%</td>
</tr>
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Sources: ABS; AC; AUSVEG; CFVIWA; GTA; MP & DD (Freshlogic Analysis)

1.3 Insect pest biology

1.3.1 Purple scum springtail *Hypogastrura vernalis*

Springtails (commonly known Collembola) are one of the oldest insects among arthropods. The species purple scum springtail *H. vernalis* belongs to the family Hypogastruridae and order Collembola. Springtails are diverse arthropods with more than 6,000 species described, and the family Hypogastruridae is widespread, with nine genera in Australia and New Zealand and an abundance of *Hypogastrura* in the agricultural farming and urban areas (D’haese 2013; Greenslade et al. 2014). Purple scum springtails are small wingless insects and their name comes from the hinged spring located on the underside of their abdomen which they use to move or escape predators. The size of *H. vernalis* is up to 1.2 mm in length and the colour is purple becoming black when specimens are kept in alcohol (Greenslade et al. 2013) (see Figure 1.3). *Hypogastrura vernalis* is distinguished from other springtail species by the small size of the mucro (a short piece at the end of furcula), its shape and the distinct posterior notch (lamella). Springtails live in humid, moist areas near food sources. They prefer decaying mulches and high humidity environments, and some species can be herbivores or carnivores (Capinera 2001; Verma & Paliwal 2010). During dry conditions, springtails may search for moist areas. Springtails consume fungi, algae, bacteria and decaying plant material (Betsch 1990; Verma & Paliwal 2010). Springtails are particularly
abundant in soil and leaf litter and are especially common in organically rich agricultural soils (Verma & Paliwal 2010). In terms of distribution, *H. vernalis* are found in most Australian states, including Western Australia, Tasmania, Victoria, New South Wales, and South Australia (Greenslade et al. 2014). Verma and Paliwal (2010) reported that *H. vernalis* is well known across Eurasia and in the meadows of Norway.

![Image of Hypogastrura vernalis](image)

**Figure 1.3.** The purple scum springtail *Hypogastrura vernalis* is floating on the surface of water (Greenslade et al. 2014).

### 1.3.1.1 Life cycle of springtail *Hypogastrura vernalis*

The life cycle of springtails presents as ametabolous and develops through three stages (egg, nymph and adult). Eggs are laid singly or in small clusters and hatch after approximately 10 days at 25°C. The female springtail can lay more than 400 eggs during her lifetime. Nymphs have the same shape as adults but are smaller in size and do not have reproductive organs. They have several mouls during their development into adults. The life cycle of springtails is quickly completed
when the food source is high in organic matter and soil moisture (Christiansen 1992; Cranshaw 2004; Hopkin 1997; Lyon 2015).

1.3.2 Green peach aphid *Myzus persicae*

The aphid *M. persicae* belongs to the family Aphididae and the order of Hemiptera; it is commonly known as the green peach aphid. The adult wingless *M. persicae* are yellowish green in appearance, and the winged aphids have a black central patch on the dorsal side of the abdomen. *M. persicae* is a small to medium sized aphid, approximately 1.2–2.1 mm long (Blackman & Eastop 2000). Green peach aphids have different types of hosts with sexual reproduction during their life cycle between primary and secondary host plants in temperate or boreal regions as they have to be able to survive over winter. If the primary host is not present, *M. persicae* follows an anholocyclic life cycle on a secondary hosts (Blackman & Eastop 2000). Over 50 different types of plant families can be secondary hosts for green peach aphids. These include many vegetable crops like squash *Cucumis rapallito*, cabbage *Brassica oleracea var. capitata*, radish *Raphanus raphanistrum*, mustard *Brassica nigra*, tomato *Solanum lycopersicum*, eggplant *Solanum melongena*, celery *Apium graveolens*, lettuce *Lactuca sativa* and potato *Solanum tuberosum* (Blackman & Eastop 2000). Therefore, green peach aphid, which has an enormous host variety exceeding 50 families of plants, is considered one of the most important polyphagous insect pests (Blackman & Eastop 2000).

1.3.2.1 Life cycle of green peach aphid

The life cycle of *M. persicae* is complicated and similar to the other species of aphids. In contrast, several insects can reproduce a new generation and give birth to live young (viviparous reproduction), which further facilitates accelerated development to reproductive maturity (Goggin 2007). Further, the aphid use asexual and sexual reproduction, with asexual reproduction, the aphid’s female can either give birth or lay eggs (Goggin 2007). Harsh environments such as high or cold temperatures lack food resources and a heavy density of aphids on the host especially towards the end of summer and start of autumn, induce the production of sexual aphids (Shingleton et al. 2003). After mating, the females lay eggs that can resist hard winter temperatures (see Figure 1.4).
Asexual reproduction allows aphid populations to grow on the food source of a host plant very quickly given the right environmental conditions and absence of natural enemies. When the host plant is crowded by aphids, some species produce alates (winged individuals) that can distribute or spread over a wide area to other plant hosts (Blackman 2000). Even though there are slight differences in the life cycle, it is similar across many aphid species (Shingleton et al. 2003).

Figure 1.4. The life cycle of aphid *M. persicae* (Shingleton et al. 2003).

1.4 Damage caused by insect pests

1.4.1 *Hypogastrura vernalis* damage

In general, springtails are among the most primitive insects and require humid environments. Most of the springtails are considered beneficial insects and harmless, eating the decaying plants, fungi, algae and improving soil structure, but a few species are recorded as pests that can damage some
crops. Joseph et al. (2015) reported that springtails *Protaphorura fimata* as a pest on lettuce seedlings causing feeding injury to the germination of lettuce seeds. A study by Scott (1964) showed that *Protaphorura armata* was recorded as a pest on a wide range of vegetable crops, such as celery, broccoli, lettuce, cauliflower, sugar beet and spinach in California. Greenslade and Ireson (1986) indicated that large numbers of Hypogastruridae feed on the plant material and damage to the plant had been observed. Several studies reported some of the species belonging to the family Hypogastruridae had been recorded as pests (Sawahata et al. 2000; Womersley 1939). While Chahartaghi et al. (2005) showed that some species of Hypogastruridae are mainly secondary invaders feeding on the decay of plant materials. The species of *Hypogastrura vernalis*, *H. purpurescens* and *H. manubrialis* were reported as a pest on mushrooms (Greenslade & Clift 2004; Greenslade & Ireson 1986).

For celery, Majer et al. (2014) noted that two species of springtails from the family Hypogastruridae, the mushroom springtail *Ceratophysella denticulata* and the purple scum springtail *H. vernalis* had been reported on celery in Western Australia (see Figure 1.5). There was not any injury caused by *H. vernalis* observed on celery bunches; however, *H. vernalis* contaminated the celery because they live in large numbers inside the celery bunches (Majer et al. 2014). However it is not a primary insect pest of crops, other than mushrooms, instead it is a secondary attacker, only invading the crop after primary decay organisms, such as fungi and bacteria. Therefore, the pest status of the species is restricted to edible fungi of various species (Greenslade & Ireson 1986; Greenslade et al. 2002). According to Majer et al.(2014) report, the species of *H. vernalis* has been recorded as a quarantine pest and restrictions placed on exporting celery to the United Arab Emirates.
1.4.2 *Myzus persicae* as a pest

Aphids are considered the most important pest in agriculture worldwide (Pickett et al. 1992). Aphids feed on vegetable crops, and the damage comes from feeding on the phloem sap of plant parts such as stems, leaves, flowers and fruits, which causes a loss in plant nutrients and productivity (Blackman & Eastop 2000). Further, phloem-feeding aphids have been noted to cause limited plant cell damage to cruciferous crops such as cabbage and broccoli (Liu & Sparks 2001).
However, the main impact on plant health is caused by them acting as vectors for plant viruses (Medina et al. 2012).

*Myzus persicae* aphids feed on cruciferous crops directly and indirectly. This causes economic damage to the plant by transmission of plant diseases, contamination of the harvested crop and secretion of honeydew. Aphids are known to secrete honeydew, which is rich in sugar content and deposits on the plant leaves support the growth of fungus, which infests the plant matter. This, in turn, results in the stunted growth of plants, yellowing of plant leaves and wilting of plants and flower buds. Thus, plants infested with aphids are not eligible for sale in the market and this affects the value of the vegetable market (Blackman & Eastop 2000; Liu & Sparks 2001; Kundoo et al. 2018). *Myzus persicae* has been considered as a virus vector transmitting more than 120 plant viruses such as leaf roll virus and mosaic virus (Blackman & Eastop 2007). According to Natwick (2009), cabbage and cole crops are known to undergo much damage due to several such species of aphids, which caused heavily infested leaves to curl and stunt plants, or kill cabbage seedlings. This results in as much as 84% damage of the total plant leading it to hamper the growth and health of the whole plant itself (see Figure 1.6).

![Cabbage leaves damaged by green peach aphids](https://www.growseed.co.uk/blog/identifying-and-controlling-aphids-in-gardens)
1.5 Pest management practices

With rising human population, demand for agricultural crops and food has increased. Chemical pesticides have been widely used and play a significant role in increasing agricultural production. Pesticides have been shown to negatively impact the environment and non-target organisms, such as the effect of pesticides on natural enemy populations (Tietjen & Cady 2007). Overuse of chemical pesticides can result in several ecological issues, including pest resistance, secondary pest outbreaks and reduction of parasitoids and predators (Cloyd 2012). The detrimental effect of chemicals on natural enemies has been a concern for several years and subjected to a large number of research studies (Messelin et al. 2013). Protection of agriculture crops from insect pests has been an issue since agriculture was first developed. Pest management plays an important role in plant protection from pests, and the goal of using IPM is to reduce the use of chemical pesticides by using natural control alternatives.

The use of different control applications is to develop an efficient, safe and aesthetic product against springtail and green peach aphid, which extensively damage vegetables. Ethyl formate (EF), as a chemical that occurs naturally in fruit and vegetables and is one of the alternatives that has been registered and developed as vegetable and fruit fumigant in Australia (Agarwal et al. 2015). It was used on harvest celery alone and combined with phosphine (PH₃), as well as PH₃ alone, to manage pre-shipping treatment against springtails, along with the use of natural products to control green peach aphids on cruciferous crops. Plant VOCs have been used as an infestation indicator and the role of VOCs such as limonene was to attract natural enemies, alongside the use of essential oils and their mixture to manage aphids in the laboratory and glasshouse. As part of a plant’s defence against aphids, volatile plant compounds that are produced from an insect pest’s infestation, constitute indirect defences that involve the interaction between the infested plant, aphids and natural enemies (Lattanzio et al. 2000). As a result, aphid feeding on the plant can induce volatile compounds as a defence mechanism to attract several natural enemies, including parasitoid wasps (Hatano et al. 2008). Natural enemies can be an important component of IPM programs. Natural enemies of insect pests, known as biological control agents, include parasitoids, predators and pathogens, and are safer for the environment in terms of reducing the use of chemicals.
1.5.1 Chemical pesticides

Chemical pesticides are used to kill, influence or deter insect pest by controlling the harmful insects cause. The peach-potato aphid *M. persicae* is a major pest of several plant families with over 50 plant hosts (Askarianzadeh et al. 2005). Many pesticide manufacturers have been developing new insecticides against aphids. Organophosphate, carbamate and pyrethroid groups were used for aphid control; however, neonicotinoids, a new group of chemical insecticide, was approved in many countries to control aphids (Kundoo et al. 2018). The International Code of Conduct on Pesticide Management represented by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) reported that pesticides play an important role in plant protection; however, the overuse of these chemicals can lead to serious issues, such as the development of pesticide resistance and the effect on natural control mechanisms (WHO 2016). Chemical pesticide usage has increased in several countries to control different pests. The main problem is that the amount of pesticide applied on infested plants that reaches the target pests can be low compared to the contamination of the plant leading to side effects on the beneficial insects (WHO 2016). Pesticide residue levels in treated plants and exported products still pose a challenge to the agricultural industry (Lohr 1996). Food safety problems have attracted worldwide attention because the use of pesticides has contaminated foods and affected human health. The use of synthetic insecticides in pest management is a strategy employed by vegetable growers around the world. The commonly used methods to control vegetable pests are chemical pesticides, and some pest can have the ability to develop resistance to these chemicals such as green peach aphids (Foster et al. 2007). The use of biological control and botanical pesticides can be alternative to some groups of chemical pesticides such as organophosphates, synthetic pyrethrins, carbamates and neonicotinoids (Uefune et al. 2013). Despite the fact that the use of synthetic pesticides against pests is generally strategies, pesticides are costly and effect the environments as well as cause pest resistance issues. The use of chemical pesticides impacts animals that exist together with the targeted pests and be affected by the chemical application. The reduction in these other organisms has resulted in changes in the biodiversity of an area and affected the natural biological balances.
1.5.2 Fumigation practice

Ethyl formate and PH3 are potential fumigants to replace methyl bromide (MB) as pre-shipping fumigation. Methyl bromide is extensively used as a fumigant to kill wide range of species of insect pests in commodities (Wagstaffe et al. 2012). It is a non-flammable colourless fumigant that is odourless at low concentrations and heavier than air. The hazards of MB is from the effect on the central nervous system and genetic defects. In addition MB is a dangerous cumulative poison gas and a significant ozone depleter restricted under the Montreal Protocol. The Australian pesticides and Veterinary Medicines Authority (2007) reported that MB fumigant is authorised by the Commonwealth Department of Agriculture for managing quarantine pests to ensure that no exotic insects are allowed to arrive in Australia (Williams et al. 2000). Due to the phase out of MB in alternative treatments are urgently needed.

1.5.2.1 Ethyl formate fumigant

Ethyl formate (EF, Figure 1.7) is considered a food additive and is a naturally occurring compound in wheat, fruits and vegetables. EF is found to kill insect pests in a short time and quickly breaks down into natural products (formic acid and ethanol); it has a low human toxicity; and is registered as a fumigant in Australia (Desmarchelier 1999; Hiroyasu et al. 1972). Ethyl formate has been evaluated commercially as a fumigant by CSIRO Australia for insect pest control for stored grain, fruit and vegetable crops. EF has been approved as a stored product pest control because of its valuable characteristics and consumer safety (Agarwal et al. 2015; Kim et al. 2013; Ren et al. 2008). EF is an efficient and environmentally friendly new fumigant. The EF fumigant is a colourless liquid at a temperature of 54.1°C and has an aromatic odour (Ren & Mahon 2003). The Food and Drug Administration (FDA 1984) in the US reviewed EF for use as a flavouring agent and characterised it as safe. However, the level of absorption of EF fumigants depends on various factors, such as the temperature, type of stored product, type of insect pests, moisture content in the commodity, particle size and composition, exposure time and fumigant dose. EF is highly absorbed in products such as strawberries (Fragaria ananassa), apples (Malus domestica) and some types of cut flowers due to their high moisture content and because EF is highly soluble in water, and the high dose of EF can cause slight phytotoxicity in fresh commodities (Agarwal et al. 2015; Lee et al. 2013; Simpson et al. 2004). Previous experiments on stored products (Tarr et al.
2007) indicated that EF, combined with carbon dioxide (CO₂), was effective for control of sawtoothed grain beetle (*Oryzaephilus surinamensis*) and confused flour beetle (*Tribolium confusum*) on dry vine fruit. Also, EF combined with CO₂ caused high mortality in various insect pests on table grapes after one-hour exposure time for quarantine treatment (Simpson 2007). Different levels of EF combined with a low concentration of PH₃ 0.5 mg L⁻¹ for two hours has resulted in high mortality of three species of aphids; cotton aphid (*Aphis gossypii*), green peach aphid (*M. persicae*) and turnip aphid (*Lipaphis erysimi*) and there was a significant difference in the lethal concentration LC₅₀ and LC₉₀ values in comparison with EF and PH₃ alone (Lee et al. 2014). Also, EF can cause high mortality in adult and nymph onion thrips, (*Thrips tabaci*), on onions (*Allium cepa*) after two-hour exposure without observation of phytotoxicity (Stewart & Mon 1984; Van Epenhuijsen et al. 2007). Therefore, EF is considered an effective alternative fumigant for a number of reasons: first, EF can quickly kill insect pests; second, it can be used on a variety of stored commodities including vegetables, fruits, grain and animal products; fumigated plant seeds maintain a high quality and germination rate is not affected; and at room temperature, it quickly degrades to non toxic residues. Yang et al. (2016) reported that the new application of EF with nitrogen as a carrier gas can reduce fumigation cost and is safe for workers and the environment.

![Ethyl formate structure](image)

**Figure 1.7. Ethyl formate structure.**

Ethyl formate is an ester formed after ethanol reacts with formic acid. Ethyl formate has the characteristic smell of rum and is partly responsible for the flavour of raspberries. It has the Generally Recognized as Safe (GRAS) status by the US Food and Drug Administration. Synonyms: Ethyl methanoate; formic acid ethyl ester; ethyl formic ester; formic ether. Identifiers: CAS No.: 109-94-4; RTECS No.: LQ8400000; DOT UN: 1190 26 DOT label: Flammable Liquid (highly flammable and poses a dangerous fire and explosion risk). Molecular weight: 74.1; Boiling point: 54.1°C; Specific gravity (water = 1): 0.92 at 20°C (Ryan & De Lima 2014).
1.5.2.2 Phosphine fumigant

Phosphine is considered an excellent insecticidal fumigant and has a low residual application history on the control of insect pests in stored products. Phosphine fumigation is easy to apply, and has been used in fresh agricultural quarantine insect pest elimination. Phosphine is a commercially available fumigant for fresh fruit (Horn & Horn 2004; Klementz et al. 2005), which has been used for decades to control insects in various commodities such as stored grain, fruits and vegetables. Unlike methyl bromide, it is generally not phytotoxic; PH$_3$ does not affect the environment because it transforms into harmless amounts of phosphates and phosphoric acid after its release into the atmosphere (Brash et al. 2009). The fumigation with pure PH$_3$ suggested by Horn et al. (2004) caused no phytotoxicity on fresh fruits with high mortality on insect pests, and the fumigated fruit was found to be similar to untreated fruit because there were no phytotoxicity symptoms and no change in fruit colour, flavour or the taste. Thus, the results of the study by Horn et al. (2004) paved the way for use of PH$_3$ fumigant as a postharvest treatment for fresh commodities. Finkelman et al. (2012) provided a protocol for the pre-shipment treatment of herbs by using pure PH$_3$. The tested herbs were dilled _Anethum graveolens_, parsley _Petroselinum crispum_, tarragon _Artemisia dracunculus_, basil _Ocimum basilicum_, mint _Mentha arvensis_, sage _Salvia officinalis_, oregano _Origanum vulgare_, thyme _Thymus vulgaris_ and rosemary _Rosmarinus officinalis_ infested by the Lepidopteran armyworm species _Laphigma_ spp. and _Prodenia_ spp. as well as two-spotted spider mites _Tetranychus urticae_, thrips _Thrips tabaci_ and whitefly _Bemisia tabaci_. The results indicated that PH$_3$ treatment was effective on these insect pests and caused high mortality at concentrations of 900–1000 ppm for 24 hours exposure time. The combination of PH$_3$ with CO$_2$ has been used for postharvest horticultural crops to disinfest insect pests (Jamieson et al. 2012; Williams et al. 2000). The use of PH$_3$ fumigation at low temperature has been shown to have high efficacy against western flower thrips _Frankliniella occidentalis_ for postharvest treatments on different vegetables such as lettuce _Lactuca sativa_, broccoli _Brassica oleracea_, asparagus _Asparagus officinalis_ and strawberries (Liu 2008). However, fumigation with PH$_3$ has been known to cause a significant reduction in shelf and vase life of cut flowers, with both 5.5 and 11 mg L$^{-1}$ for six-hour exposure time on four types of cut flowers for controlling greenhouse thrips _Heliothrips haemorrhoidalis_ and green peach aphids _M. persicae_ (Karunaratne et al. 1997). PH$_3$ was selected because it is currently registered as a postharvest fumigant in Australia for grains, vegetable crops and fruits, and it is an easy-to-use, readily available, low cost fumigant. A review
of the literature on PH$_3$ shows that it is an effective fumigant on various insect pests in all development stages and is a potential fumigant for fresh agricultural products.

1.5.2.3 Combination of ethyl formate and phosphine

Fumigants as synergistic mixtures is a promising application technique and area for research, such as the mixture of EF with phosphine. An effect of the combination of these fumigants is the significant synergistic ratio over using the fumigants separately. Previous studies have shown no phytotoxicity effects on the quality of commodities when used in a safe range. I have evaluated a mixed fumigant formulation, then the use of a single fumigant that has a synergistic effect of killing insect pests. The combination was done to avoid the disadvantages of phosphine’s long exposure time, as well as the use of EF in low doses to maintain the quality of fresh agricultural products such as fruits, vegetables and other fresh vegetable seedlings and cut flowers as postharvest pest elimination products.

Lee et al. (2016) reported that PH$_3$ in combination with low concentration of EF have led to high mortality in cotton aphid (*Aphis gossypii*), they also evaluated the synergistic effect of mixing PH$_3$ + EF to reduce the phytotoxicity in some perishable commodities. In mixing PH$_3$ and EF for quarantine treatment to control citrus mealybug (*Planococcus citri*) on pineapples, the results showed the combination of PH$_3$ with EF has high efficacy against citrus mealybug without phytotoxicity (Yang 2016). According to Kim et al. (2015), PH$_3$ and EF fumigants were effective on adults and larvae of the potato tuber moth (*Phthorimaea operculetta*). The efficacy and synergism between PH$_3$ and EF were determined by Lee et al. (2018) and the results showed that it was not effective against eggs and adults of two-spotted spider mites (*Tetranychus urticae*) on sweet pumpkin after four and six hour exposure times, and there was no observed synergism between PH$_3$ and EF at four and six hour exposure time. The study also suggested the use of EF alone to control (*T. urticae*) on sweet pumpkin.

1.5.3 Plant volatile organic compounds

Today, organic agriculture is increasing worldwide in the hope of reducing chemical pesticides that harm the ecosystem and affect the environment and human health. The big challenge in
organic farming is keeping crops free from insect pests by using the main key, which is an integrated pest management program. One of the methods is to use the plant’s natural defence against pests, one of which is the release of VOCs to attract parasitoids and predators. The VOCs have ecological roles in plant interaction with insect pests, including attracting natural enemies like parasitoids as a direct defence; also, the aphid can be attracted by plant VOCs to infested and uninfested plants (Guerrieri & Digilio 2008; Shrivastava et al. 2010). The target in this study is using the plant VOCs and plant-derived pesticides to control green peach aphids in pest management techniques. Because natural products are often recognised by consumers as healthier and more environmentally friendly.

Insect pests can be attracted to the plant visually (plant colours) and chemically (plant VOCs). Aphids can detect the plant by using a plant chemical signal through volatiles such as green leaf volatiles, benzaldehydes, isothiocyanates and monoterpenes (Johnson & Agrawal 2005; Anstead et al. 2005). Verheggen et al. (2013) pointed out that in their experiment, volatile compounds were released by turnip plants (Brassica rapa) for the control of the aphid, green peach aphid infestation as a way of plant defence. The strong odour can affect insects, and had a negative impact on the aphid, limiting the growth and development of aphids on the turnip plant. Shrivastava et al. (2010) highlighted the importance of developing new pest management methods based on plant’s natural defence by understanding the interactions of the host plant, insect and the environment. VOCs play an important role in plant/insect interaction. According to Giorgi et al. (2012), Achillea Collina plant VOCs are found to be crucial in the interaction of the plant and environment by hampering essential life processes like reproduction, communication and defence. Plant VOCs are generally thought to be produced in metabolic processes as a response to effective factors that can be biotic or abiotic, such as the infestation with green peach aphid M. persicae. Chen et al. (2009) explain that the chemical defences in plants are characterised by the constitutive or production of chemicals of secondary metabolites. Vegetable infestation by pests was often impacted by released VOCs as the main cue for finding the host plant (Omura et al. 2000; Takemoto & Takabayashi 2015). Green peach aphid M. persicae is a serious pest of many crops and fruit trees and causes losses to cabbage and Solanaceae crop production worldwide. Rajabaskar et al. (2013) conducted an experiment that used four varieties of potato to compare the volatiles emitted from the different varieties and the influence on selection, by aphids. Volatiles were collected from two plants
enclosed in a glass chamber for two hours (Eigenbrode et al. 2002). The VOCs, such as limonene and linalool produced by infested plants, were shown as a direct natural defence against various pests by attracting parasitoids and predators to the damaged plants (D'alessandro & Turlings 2005; Bukovinszky et al. 2005; Kessler & Baldwin 2001). The response of the plant to aphid infestation was evaluated against the application of VOCs from plant essential oils, such as 1,8-cineole and menthol (Prinsloo et al. 2007). The differences in the volatile compounds between infested and uninfested plants can be used by researchers and agricultural companies to develop new methods with volatile profiles that work as repellents for herbivores, are attractive to parasitoids and predators, or a combination between them. According to Kergunteuil et al. (2015), the results obtained from the olfactometer revealed that the five plants belonging to the same family or same species exhibit various levels of attractiveness of Delia radicum to these plants based on VOCs. Therefore, natural products for pest control based on plant volatiles and their biological activities can be developed as green pesticides. Enan (2005) indicated that essential oils consist of a mixture of several volatile compounds, and that the active compound in the essential oil will help develop effective and environmentally friendly natural products.

An olfactometer can be used as a bioassay technique to determine the level of secretion of VOC in a particular plant in terms of the response to a repellent by a harmful enemy. Chemical factors represented by VOCs are responsible for affecting the impact of aphids and their parasitoids on infested and uninfested plants. An understanding of chemical ecology can be applied to achieve a pest control goal in the longer term; that is, developing an understanding of the interaction between aphid, plant and environment and applying it for the purpose of reducing the impact of aphid on crops. Plant infestations by aphids are supposed to generate a response against pests that may cause the plant to release volatile compounds in nature. These responses help inform nearby plants of the presence of aggressors, thereby reducing the attack effectiveness by attracting natural enemies through release of repellent compounds as a defence method (Will & Van Bel 2006).

1.5.4 Biological control

The use of living organisms to manipulate the population of insect pests is called biological control. Biological control has increased in recent decades for several reasons: reduced chemical pesticides,
a large number of pests have developed resistance against pesticide action and consumers sensitivity against pesticide residues (Bailey et al., 2009; Frank, 2010). In this control, many organisms are used such as entomopathogenic, parasitoids and predators. Parasitoids are mainly from the order Hymenoptera and the family Aphidiidae are key natural enemies of the peach-potato aphid (Kundoo et al. 2018). The parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) are oligophagus endoparasitoids of *M. persicae* and many other species of aphid and are used as alternative pest control options (Tomanovic et al. 2012). These parasitoids originate in South East but are now broadly commercially available in different parts of the world (Boivin et al. 2012). *Aphelinus abdominalis* is especially effective against *M. persicae* and has been used to control aphids in fields and greenhouses and the use of biological control against aphids has been extensively studied as well as many other parasitoids are known to parasitise *M. persicae* (Tomanovic et al. 2012).

### 1.5.4.1 Aphid parasitoids

Aphids are known as a serious pest to the cruciferous crops, as well as other plant families, and cause damages either directly or indirectly. The aphids feed on the plants by sucking plant sap and causes direct damage by turning the leaves yellow with curl and further distorts growth as well as excretion honeydew, which often turns black with the growth of a sooty mold fungus along with indirect damage due to transmission of certain diseases from one plant to others by transporting and feeding on them eventually contaminating whole crops. Parasitoids are one of the most important natural enemies found in the environment and are specific to insect pests. Parasitoids are small, slender, black or brown and have a pinched (wasp) waist. They are a hairless flying insect that has two pairs of wings and flies actively searching for aphids. Aphid parasitoids are found among almost all aphids colonies. The adult female wasps sting aphid bodies and deposit single eggs into nymph or adult aphids. Eggs hatch into larvae and develop inside the aphid bodies by feeding on the internal organs of the aphid and slowly killing them. After the larva completes its development, it pupates and turns the aphid body into a mummy. The mummy is swollen, brown or gold in colour, and the adult wasp comes out by making a hole at the end of the back side of the mummy body. Quicke (1997) showed that parasitoids generally need a second insect to insert their eggs into insects’ bodies to complete the parasitoid life cycle and reach to the adult, free living
stage. The parasitoid’s larva uses the second host insect as a food source after the parasitoid’s egg hatches.

Aphidiid parasitoids (Braconidae and Aphididae) are from the order Hymenoptera, and they are endoparasitoids of aphids. Parasitoid reproduction in the Aphidiidae is sexual and asexual; the fertilised eggs can give females; conversely, the unfertilised eggs can hatch to males (Martínez 2008). *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae, see Figure 1.8) is small, approximately 3 mm in length and is a parasitoid to a wide aphid host range of the family Aphididae, and for that reason, is considered a specialist parasitoid for the family Aphididae level (Soglia et al. 2006). The distribution of the species *Aphidius* is widespread in Australia, North and South America and different parts of Europe. This species is used in biological control programs as a commercial product and sold as mummies (Sampaio et al. 2001; Vásquez et al. 2006).

Figure 1.8. Female and male of *Aphidius colemani*. The male is slightly smaller than the female and the abdomen ends in a rounded abdomen-tip in the male, whereas the female has an acute point (Photograph by Simon Fellous [Martínez 2008]).
The larval stage of *Aphidius colemani* that live inside the host of the *M. persicae* body has four larval instars. The host aphid killed during the last larval stage of the parasitoid, leaves only the cuticle from the body untouched, has a gold colour and is called ‘mummy’ (see Figure 1.9), which is then stuck on to the plant leaf or other plant parts. The pupal stages start inside the mummy, after which the adult emerges, making a circular hole cut on the dorsal side of the mummy where the adult goes out and feeds on aphid honeydew, ready to mate within a few hours of emergence (Hågvar & Hofsvang 1991; Zamani et al. 2007). The benefit of using parasitoids is that it is safe for the environment and the aphid cannot develop resistance against them.

![Figure 1.9. The mummies of the green peach aphid *Myzus persicae* parasitism by the parasitoid *Aphidius colemani* on cabbage plants.](image-url)
Another parasitoid that can be effective against green peach aphids *M. persicae* is *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). This parasitoid is an endoparasitoid that can attack over 200 species of aphids, including potato aphid *Macrosiphum euphorbiae* and foxglove aphid *Aulacorthum solani*. The parasitoid of *A. abdominalis* is small in size, about 3 mm in length and, as with *A. colemani*, the female inserts an egg into the body of aphid, usually using the mid-age of aphid nymphal stages. The egg hatches inside aphid’s body and the larvae of parasitoid begin to feed on the aphid without quickly killing it, then the parasitised aphid turns into the black mummy (Hågvar & Hofsvang 1991; Shrestha et al. 2015). Aphid parasitoids are an important factor and reliable biocontrol agents for aphid species (see Figure 1.10).

![Figure 1.10. The parasitoid *Aphelinus abdominalis* parasitising the nymph of an aphid](http://biologicalservices.com.au/content/products/Aphelinus-info-sheet.pdf)
1.5.5 Botanic pesticides

Botanic pesticides have been used to control many insect pests worldwide (Isman 2008). The reason for using the essential oils as botanic insecticides in pest management is that they have a mixture of active constituents that show a great mortality in different insects. In addition they have different modes of action such as antifeeding, repellence and oviposition deterrence activities (Isman 2008). Several plant families such as Melianaceae, Piperaceae, Myrtaceae and Lamiaceae have many secondary metabolites that have been identified and used against different insect pests (Devanand & Rani 2008). Natural pesticides are safe to the environment, animal and human health (Dubey et al. 2011). Botanical pesticides have been used in plant protection for a long time against pests because they have toxic properties against several insects. Some compounds are considered phytochemicals that are naturally occurring in plants as secondary metabolites; these compounds include glucosides, essential oils, saponins, tannins, alkaloids, flavonoids and phenolic compounds (Isman 2008). The combination of two or more essential oils or their compounds can work synergistically if the combined efficacy is greater than the use of single essential oil or single compound. For example, the binary combination between two essential oils against Spodoptera litura, cause higher mortality than use of single essential oil (Hummelbrunner & Isman 2001). Oparaek et al. (2005) indicated that using a mixture of eucalyptus (Eucalyptus globulus) and neem (Azadirachta indica) extracts can provide good control against plant borers. Isman (2008) showed that botanical pesticides are preferred in organic agriculture. Flint (2012) recommended that integrated pest management programs are the best solution to pest problems. The integrated pest management method will minimize the hazards of pesticides to human health, the environment and nontarget organisms reducing reduce the risk of pesticides. In short, botanical pesticides are safer and have fewer residues than other synthesised pesticides.

1.5.6 Efficacy of essential oils against Myzus persicae

Essential volatile oils obtained by steam distillation of aromatic plants have been used by humans for a long time; as a food flavouring, an important agent for aromatherapy, in the perfume industry, and for traditional pest control in many countries. The worldwide production of essential oils, trade activities and their being non-toxic to mammals (the test of acute oral toxicity on rodents, LD₅₀
values range between 800 to 3000 mg Kg\(^{-1}\) for pure compound and >5000 mg Kg\(^{-1}\) for formulated essential oils), as well as having multiple modes of action that can affect insect pests, are all reasons for encouraging the use of essential oils commercially in pest control (Isman et al. 2010).

Plant-based biopesticides have been reviewed through a number of studies and it was reported that more than 2,000 species of plants possess insecticidal activities belonging to 60 families, which show promise as new botanical pesticides (Dev 2017; Isman 2006; Lydon & Duke 1989; MacKinnon et al. 1997). Most of the botanical pesticides have low to reasonable environmental toxicity, but there are exceptions such as nicotine, and essential oils may degrade in the environment more rapidly than synthetic chemicals (Buss & Park-Brown 2002; Isman et al. 2010; Moretti et al. 2002). Many essential oils have caused high mortality of pests, and have shown their effectiveness through different applications such as fumigation, as well as having antifeedant and repellent properties (Regnault-Roger 1997). Some research has reported that essential oils have a use in controlling aphids as a repellent and feeding preventive activity (Hori 1998; Isman 2000; Tripathi et al. 2009). Moreover, some essential oils showed aphicidal activity such as cumin, anise, oregano and eucalyptus essential oils (Tunc & Şahinkaya 1998).

A number of the source plants have been used for traditional plant protection in stored commodities and fields, especially in the Mediterranean and southern Asia regions. Most of the essential oils were evolved that demonstration of their fumigant and contact insecticidal activities to several species of pests (Dev 2017). The rapid action of essential oils against some pests is an indication of a new generation of natural pesticides that have less effect on the environment (El-Hosary 2011). Aphid control is usually accomplished with chemical pesticides, which, when misused, can lead to environmental pollution and aphid resistance, especially \textit{M. persicae} (Ben-Issa et al. 2017; Devonshire et al. 1998). Green peach aphids have developed resistance to many insecticides due to the frequent application of chemicals such as pyrethroids (Kuhar et al. 2009). Plant essential oils have an important role in pest control because they contain some compounds that may have pesticide, repellent or antifeedant effects against insects (Bernays & Chapman 2007; Digilio et al. 2008).
1.5.6.1 Black pepper essential oil

Black pepper *Piper nigrum* L. extracts showed insecticidal activities because it contains isobutyl amides that are responsible for the toxicity to insects (Bernard et al. 1995; Miyakado et al. 1980). Black pepper is belong to the family Piperaceae that has pesticidal properties (Arnason et al. 2008). Scott et al. (2008) concluded from their studies on the family Piperaceae that piper extracts are valuable source of biopesticides for controlling small insects and reducing the development of pest resistance when mixed as a synergist with other botanical pesticides, such as pyrethrum (*Chrysanthemum cinerariifolium*). In addition, black pepper extracts have several modes of action including contact toxicity, repellent and antifeedant properties (Scott et al. 2004, 2005; Jensen et al. 2006). *P. nigrum* essential oil formulations were found to defend stored wheat from *Sitophilus oryzae* (L.) efficiently, and *Rhyzopertha dominica* (F.) at concentrations more than 100 mg L\(^{-1}\) applied for up 30 days (Sighamony et al. 1986). Kēīta et al. (2000) has shown that black pepper essential oil reduced the adult emergence of cowpea weevil *Callosobruchus maculatus* by 100% after 30 days of treatment. The repellent effect of *p. nigrum* was observed to protect the plant for up to four days post treatment; however, the residual effect of the pepper extract was much less under the direct sunlight and insect damage resumed shortly after treatment (Scott et al. 2003).

1.5.6.2 Rosemary essential oil

Rosemary *Rosmarinus officinalis* is an important essential oil that has been used conventionally as medication in many countries because it is non-toxic to humans and environments (Miresmailli et al. 2006). Also, Miresmailli et al. (2006) reported that the effect of 1% rosemary oil on two-spotted mites *Tetranychus urticae* through contact toxicity on tomato caused high mortality by using a tomato leaf disc test for 12 and 48 hours. *Rosmarinus officinalis* essential oil has been commercialised as pesticides for its effectiveness against several insect and mite pests (Choi et al. 2004; Hummelbrunner & Isman 2001; Isman 2000; Isman et al. 2011). Moreover, it has been shown that rosemary oil vapour has ovicidal and larvicidal activity on several stored product insects (Papachristos & Stamopoulos 2004). Isman et al. (2008) have shown the chemical composition of commercial rosemary oil and determined the LD\(_{50}\) values of the oil when applied to cabbage loopers *Trichoplusia ni* and fall armyworms *Pseudaletia unipuncta* was significantly
toxic. Essential oil of rosemary has antifeedant or repellent properties (Regnault-Roger 1997; Regnault-Roger et al. 2012).

1.5.6.3 Eucalyptus blue gum essential oil

Eucalyptus blue gum *Eucalyptus globulus* essential oil possesses a wide range of pesticide activities including insecticidal, insect repellent, herbicidal, acaricidal, fungicidal and antimicrobial, and eucalyptus essential oil is considered non-polluting and environmentally friendly with lesser or no toxicological effect (Batish et al. 2008; Ben-Issa et al. 2017). As per a report, eucalyptus essential oil is used for many insect controls, such as repellents in the action of mosquitos and human body lice; it controlled some storage pests, for instance, confused flour beetle, Mediterranean flour moth, maize weevil and rice weevil; and the eucalyptus oil extract caused high mortality on the mushroom fly (Choi et al. 2006; Erler et al. 2006; Negahban & Moharramipour 2007; Tunc et al. 2000). *Eucalyptus globulus* essential oil has insecticidal activities that use various forms, such as contact, antifeeding, inhibit oviposition, repellence and fumigant. Eucalyptus leaf extract has been used against many pests included aphids and cotton leafhopper by using different ways such as direct use and antifeeding method, the effect of the oil against cotton stainer by inhibit oviposition; in the other hand, eucalyptus oil has been used as fumigant against housefly. The use of dry powder extract of eucalyptus and mix with potatoes to protect potatoes from tuber moth in the store. (Ahmed & Eapen 1986; Batish et al. 2008; Jaipal et al. 1983; Koul et al. 2008; Lal 1987; Mathews 1981). Pavela (2006) has shown the high mortality results by using four types of essential oils (catnip *Nepeta cataria*, lavender *Lavandula angustifolia*, marjoram *Origanum majorana* and rosemary) against cabbage aphids.

1.5.6.4 Tea tree essential oil

Tea tree *Melaleuca alternifolia* has been used as insect control agents because it contains bioactive chemicals (Choi et al. 2003). The oil of tea tree has been used by indigenous Australians as a traditional remedy for insect bites (Budhiraja et al. 1999; Trumble 2002). Hammer et al. (2006) have indicated that tea tree essential oil is toxic to several insect species, and suggested that doing more studies are needed. Carson et al. (2006) have described the composition of tea tree oil as
being composed of terpene hydrocarbons, monoterpenes and sesquiterpenes with associated alcohol. Liao et al. (2017) have indicated that *M. alternifolia* may provide a new and safe alternative to chemical pesticides. Many essential oils including tea tree oil have been examined on several hemipterans (aphids, thrips, whiteflies and mealybug) (Cloyd et al. 2009; Mann et al. 2012).

Some chemical-based pesticides that are used in pest management programs are likely to remain so as long as effective and inexpensive chemicals are available, however they can cause environmental pollution and pest resistance for agriculture chemicals (Farajzadeh et al. 2014). The botanical pesticides could be divided into two generations: the first generation includes nicotine, rotenone, sabadilla, ryania, pyrethrum and plant essential oils; while the second generation includes synthetic pyrethroids and azadirachtin, as well as potential new botanicals (Regnault-Roger & Philogène 2008). Essential oils are plant-based, produced commercially from several types of plant sources, many of species of the mint family Lamiaceae, and are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes (Isman 2000).

### 1.5.6.5 Synergism between essential oils

The synergy among essential oils is important in enhancing the efficacy of natural formulations as natural pesticides. Plants usually present their defences by using different ways like increasing the wax layer in their leaves, releasing some compounds as a repellent; yet, these compounds are not individual ones but may be a mixture that acts as synergists (Akhtar & Isman 2013). Synergistic effects of the combinations are certainly important in natural plant defence and natural pesticide formula against insect pests. The identification of synergies between essential oil combinations may provide knowledge about the development of effective pest control agents and how to use low concentrations within mixtures to be effective and achieve a high level of efficacy (Akhtar et al. 2012). The interaction between mixing essential oils can be quite different as synergistic, antagonistic or additive depend on essential oil structure and its constituents (Singh et al. 2009). Hummelbrunner and Isman (2001) reported that synergy can occur in the binary mixture against *Spodoptera litura*, and they suggested that the effect of a binary mixture of pure essential oils is
more significant than the use of individual ones in most studies. Akhtar et al. (2012) relayed that in a comparison of the deterrent activity of the full combination with the artificial blends containing some constituents, the results showed the active feeding deterrent was more effective than the artificial one, and that may act as a synergistic effect. In addition, Akhtar and Isman (2003) believed that the compound mixture could occur in natural insect–plant interactions, and such mixtures were synergistic in past experiments in terms of their feeding deterrence to *Spodoptera* larvae. Based on combinations of essential oils the synergist can play an important role in developing insecticides and use in pest management programs.

Hence, it has been seen in the literature that springtails and green peach aphids are beginning to have increasing importance in the field of pest control, especially with respect to the insect pests of plant protection. I have further reviewed various key concepts related to the research topic. It is evident from the above review of relevant literature that an important requirement to evaluate natural products for control of springtails and aphids on vegetables without harming consumers and the environment. This was followed by the literature gap, where I have noted the need to use older literature as compared to more recent studies. Further, the introduction of different fumigants along with the use of plant volatiles in biological control and organic compounds based on essential oils both before and after harvest, could help in the control of target insects on studied plants.

### 1.6 Objectives of the current three case studies

#### 1.6.1 Research framework

This thesis covers three case studies of various fumigants in combination with natural plant-based essential oils investigated for control of vegetable pests. Among the vegetable pests, springtails in export celery and green peach aphids in *Brassica* vegetables are the major pests; hence, they were selected for the case studies (see Figure 1.11). Currently, there are no studies on the use of EF, PH₃ and their combination on exported celery to control springtails and the use of VOCs to diagnose infested plants by green peach aphids, and their role in attracting parasitoids to control aphid on cabbage and broccoli alongside the use of essential oils and their combinations as botanic pesticides.
The use of biological control agents can reduce aphid infestations and decrease the need for chemical pesticide use. Most fields and greenhouses require at least one application against *M. persicae*; however, using green pesticides and parasitoids as biological control applications can result in saving the expense of additional applications. Further, agricultural practices for pest control will keep insect pests below economically damaging levels. The use of more than one option for springtail and aphid control is called integrated pest management (IPM). IPM is a method to manage pests on a principle systems approach that controls pests, and is regulated by using natural products with biological control practices, which includes understanding how the pests interact with their plant hosts and the environment. Many control methods have been used in an attempt to control springtails and green peach aphids, which include EF as a safe fumigant, along with PH₃ for celery treatment, a biological method that focuses on using VOCs attractiveness to parasitoids and natural products based on plant essential oils used in this study.

Figure 1. Research framework three cases studies to control springtails *Hypogastrura vernalis* and green peach aphids *Myzus persicae* on vegetables, as well as chemical ecology between insect and host.
1.6.2 Aims of research

- Evaluate highly efficient fumigant to eradicate Springtail *Hypogastrura vernalis* (Collembola: Hypogastruridae) and phytotoxicity on export celery.
- Evaluate VOCs from host vegetable because of the infestation by green peach aphids *Mysuz persicae* (Hemiptera: Aphididae) and the role of VOCs to attractive natural enemy as well as understanding inter-communication between insect and host plant for further guiding development of diagnostic tool (unique VOCs signals), lure and repellants.
- Evaluate natural products, either using the extract or a pure compound for rapid killing of green peach aphids *M. persicae* on targeted vegetable crops, and the synergistic effect of their combinations.
Chapter 2: Evaluation of Ethyl Formate, Phosphine and Their Combination to Disinfest Harvested Celery of Purple Scum Springtails

2.1. Abstract

Export of celery *Apium graveolens* from Australia has been affected by a natural infestation of purple scum springtails *Hypogastrura vernalis* (Collembola: Hypogastruridae). These insects live inside the celery head, contaminating fresh celery but do not cause any visible damage. As a result, purple scum springtail-infested celery has led to rejection for export with an impact on market value for fresh produce. In this study, fumigation with ethyl formate (EF), phosphine (PH$_3$) and their combination on mortality of purple scum springtails in naturally infested celery was evaluated. Laboratory experiments were conducted using concentrations of 50, 60 and 90 mg L$^{-1}$ of EF for 1, 2, and 4 hour (h); 1, 1.5, 2 and 2.5 mg L$^{-1}$ of PH$_3$ for 2, 4, and 6 h; as well as 20, 30, and 40 mg L$^{-1}$ of EF combined with 1 mg L$^{-1}$ of PH$_3$, for 2 and 4 h in the laboratory temperature 25°C. Complete control was achieved at 90 mg L$^{-1}$ of EF for 2 h; however, phytotoxicity was observed in celery treated by EF at all concentrations. PH$_3$ at 2.5 mg L$^{-1}$ achieved 100% mortality within 6 h, and no phytotoxicity was evident. Mortality of 100% was also achieved at 30 and 40 mg L$^{-1}$ EF combined with 1 mg L$^{-1}$ of PH$_3$ for 2 and 4 h exposure time, however, phytotoxicity occurred with EF alone treatments as well as with the combination. From these data, we conclude that PH$_3$ alone has potential as a fumigant for the pre-shipment treatment of celery infested with purple scum springtails.

2.2. Introduction

Celery *Apium graveolens* var. *dulce* is an intensively cultivated and valuable horticultural export product from Australia. Celery is grown in most states with the main production areas in Victoria, Western Australia and Queensland. There are cultivated areas of about 235 ha per year (Horticulture Australia 2009). Australian celery production in 2015 was more than 60000 t with a value of 50.1 million Australian dollars. In 2017, celery production for export increased to 3557 t (Horticulture Innovation Australia 2017). However, this growing market has been threatened by a natural infestation of purple scum springtails, which significantly affected market access as purple
scum springtails are considered a quarantine pest in many Middle East countries. Australian Quarantine does not allow export of plant products containing living organisms.

Purple scum springtails are tiny wingless arthropods with a bulbous, rounded form. The natural densities of springtails in general in Australia range between 2000 and 30000 per square meter depending on climate, season and habitat (Greenslade 2007). Springtails in general feed on decaying plant material, and other organisms such as fungi, algae, and occasionally dead animals. A few species of springtails have been recorded as pests that can damage mushrooms and other crops (Greenslade & Ireson 1986; Greenslade et al. 2014; Popenoe 1917). Regardless of species, the preferred conditions for the growth of purple scum springtails are warmth, moisture and high organic content (Greenslade & Kitching 2011). Two species of springtails from the Hypogastruridae family, mushroom springtail Ceratophysella denticulata and purple scum springtail H. vernalis have been reported on celery in Western Australia (Majer et al. 2014). These species are not considered to be primary pests but are present because they feed on microorganisms and decaying products (Chahartaghi et al. 2005). Several species in the Hypogastruridae family can cause damage to cultivated mushrooms and are confirmed as pests of edible mushrooms in Australia, of which the most common ones are mushroom springtail C. denticulate, Hypogastrura manubrialis, H. purpurescens and purple scum springtail H. vernalis (Greenslade & Clift 2004). Fumigation of the growing equipment used in a mushroom culture can prevent and kill springtails and other species from damaging crops (Greenslade & Clift 2004; Greenslade & Ireson 1986; Greenslade et al. 2014).

Ethyl formate is a naturally occurring compound that has been evaluated as a fumigant for stored grain, fruit and vegetable applications and has been commercially adopted because of its valuable characteristics of rapid kill and consumer safety (Agarwal et al. 2015; Kim et al. 2013; Ren et al. 2008; Ren & Mahon 2006). However, EF is highly absorbed in products such as strawberries Fragaria ananassa, apples Malus domestica and several types of cut flowers due to their high moisture content and because EF is highly soluble in the water (Agarwal et al. 2015; Lee et al. 2013; Simpson et al. 2004). Some studies (Tarr et al. 2007) indicated that EF, as well as EF in combination with 10% carbon dioxide (CO2), could be used for control of sawtoothed grain beetle Oryzaephilus surinamensis and confused flour beetle Tribolium confusum on dry vine fruit.
Different levels of EF combined with low concentration of PH₃ 0.5 mg L⁻¹ for 2 h have resulted in high mortality of three species of aphids which are cotton aphid *Aphis gossypii*, green peach aphid *Myzus persicae* and turnip aphid *Lipaphis erysimi* and there was significantly difference in the lethal concentration LCT₅₀% and LCT₉₀% values in comparison with EF and PH₃ alone (Lee et al. 2014). Also, EF can cause high mortality in adults and nymphs of onion thrips, *Thrips tabaci*, on onions *Allium cepa* after 2 h exposure (Van Epenhuijsen et al. 2007).

Phosphine is a commercially available fumigant, which has been used for decades to control insects in stored grain. PH₃ in combination with CO₂, such as ECO2FUME (2% PH₃ +98% CO₂) supplied by Cytec Industries, Dongbu Hannong Co., Seoul, Korea; has been used for disinfestation of postharvest horticultural products (Jamieson et al. 2012; Williams et al. 2000). PH₃ fumigation at low temperature has been shown to have high efficacy for postharvest control of western flower thrips *Frankliniella occidentalis* on lettuce *Lactuca sativa*, broccoli *Brassica oleracea*, asparagus *Asparagus officinalis*, and strawberries (Liu 2008). However, fumigation with PH₃ has been known to cause a significant reduction in shelf and vase life of cut flowers with both 5.5 and 11 mg L⁻¹ for 6 h exposure time to four types of cut flowers for controlling greenhouse thrips *Heliothrips haemorrhoidalis* and green peach aphids *M. persicae* (Karunaratne et al. 1997).

There has been no published work on control of purple scum springtails on harvested celery. Under our current study, we researched the efficacy of EF, PH₃ and their combinations for control of purple scum springtails on celery. In this preliminary study, optimal fumigant concentration, exposure period, and evaluation of phytotoxic damage to celery bunches are reported.

### 2.2. Materials and Methods

#### 2.2.1. Celery and Target Pest

Celery infested with purple scum springtails was supplied by the Mandogalup Celery Farm (Sumich Group) located 23 km south of Perth, Western Australia (lat. 32.20°S, long. 115.84°E). Celery bunches were weighed with a digital balance, and their weight ranged between 686-1020 g per bunch.
The purple scum springtail species found in Western Australia were identified taxonomically by Majer et al. (2014) and current species were confirmed by the Department of Primary Industries and Regional Development (DPIRD) by mounting a number of specimens and following the Greenslade et al. (2014) classification key. Celery samples were stored in a cold room at 15°C at Murdoch University (Murdoch, Western Australia, Australia) for 1-2 days.

2.2.2. Reagents and Apparatus

Ethyl formate 97% purity was supplied by Sigma-Aldrich Company, Castle Hill, Australia. A cylinderized PH$_3$ gas mixed with nitrogen (N$_2$) (1.4% PH$_3$ with 98.6% N$_2$) was supplied by BOC Australia. Gas sampling bags 10 L (SKC Tedlar®; Air-Met Scientific Pty Ltd, Perth, Australia) were used to collect PH$_3$ from the gas cylinder. One liter gas sampling bags were used to take the liquid EF samples and made into vapor. One liter Erlenmeyer flasks (FE 1 L/3; Bibby Sterilin, Staffordshire, UK) were used for the preparation of gas standards. An air vacuum pump (AP-02B Vacuum pump, supplied by Tianjin Automatic Science Instrument Company, Tianjin, China) was used to suck air from the fumigation chambers to create negative pressure in preparation for fumigation.

A 100-μL syringe (005250, SGE, Melbourne, Victoria, Australia) was used for the injection of gas samples into the gas chromatograph (GC) and transfer liquid EF into fumigation chambers. A 5-μL syringe (5R-GT, SGE) was used to transfer liquid EF to prepare gas standards.

2.2.3. Fumigation Chamber

A cylindrical stainless-steel chamber (60 × 36 cm i.d.) with a capacity of 61 L was used. Each chamber was equipped with Swagelok compression (Swagelok Company, Perth, Australia) fittings for attachment to a diaphragm pump between the top and bottom rim of the chamber. The removable metal lids were fitted with a centrally located septum for the introduction of gas during fumigation. The fumigation chamber had two gas sampling ports located on the side of the fumigation chamber. Each lid was fitted with gas-tight seals, which were pressure-tested before use.
For testing pressure halving time of the fumigation chambers, a digital manometer was connected to one of the Swagelok fittings after securing the metal drum with lids. An air pump was used to apply an increased pressure of 250 Pa to the sealed drum via another Swagelok fitting. Once the digital manometer displayed value of 250 Pa, the air pump was turned off and the ball valve tap closed. The air pressure was then timed until the value displayed was 125 Pa giving the drum’s “pressure half-life”. A half-life of more than 10 min indicated a well-sealed chamber suitable for fumigation.

2.2.4. Determination of Ethyl Formate and Phosphine Concentration

Ethyl formate was analyzed using a portable GC (Companion 600; DPS Instruments, Rancho Cucamonga, CA) installed with a flame ionization detector (FID). Zebron capillary column 30 m \(\times\) 0.53 mm (i.d.) 0.5 \(\mu\)m model ZB-WAX (B13844, Part no. 7HK-G007-17, Phenomenex Company, Castle Hill, Australia) was used at an oven temperature of 95\(^\circ\)C. \(\text{N}_2\) carrier gas was used at a flow rate of 6 mL min\(^{-1}\) at 103 Pa. The concentrations of fumigants were monitored at different time intervals (depending on exposure time for each experiment) throughout the fumigation period.

Phosphine concentrations were determined at timed intervals using an HP 5890, Series II gas chromatograph (Hewlett-Packard Company, Wilmington, Delaware, USA) equipped with a flame photometric detector (FPD) with a phosphorus filter following isothermal separation on a 30 m \(\times\) 0.25 mm (i.d.), 0.25 \(\mu\)m, Varian capillary column part no. CP8944 (Sigma-Aldrich Company, Castle Hill, Australia) at oven temperature 50\(^\circ\)C and inlet temperature 105\(^\circ\)C. For FPD the flow of hydrogen and air was 50 and 100 mL min\(^{-1}\) respectively. Hydrogen was used as the carrier gas with a constant flow of 2 mL min\(^{-1}\). After fumigation processes, two replicate injections were administered through each gas sampling port and injected into GC (two injections from the top and also two other injections from the bottom port of the fumigation chamber). Both EF and PH\(_3\) concentrations were calculated based on peak areas against external EF and PH\(_3\) gas standards.
2.2.5. **Fumigation of Purple Scum Springtail Infested Celery with Ethyl Formate**

Three treatments consisting of 50, 60, and 90 mg L\(^{-1}\) of EF plus an untreated check were investigated for 1, 2, and 4 h the duration of exposure. Celery bunches were transferred after storage for 1-2 d from the 15°C cold room to the 23-25°C laboratory for up to 4 h before fumigation. Two replicate fumigation chambers were used to treat the celery. Each chamber contained between 8545-9261 g for 10 bunches of celery held together with rubber bands and the bottom part of celery facing down (see Figure 2.1), with each chamber having around 8% loading ratio of celery. Treatments of fumigation were carried out sequentially, starting with the first, second and the third concentration of fumigants. The remaining celery bunches from the first experiment were stored at 15°C cold room for next experiments. Ethyl formate experiments with two replicate containers were repeated a second time for confirmation of phytotoxicity only by using fresh celery bunches that were stored for less than 24 h at 15°C cold room. The untreated controls were prepared the same way in duplicate as EF treatments, except that no fumigant was applied. Before fumigation, the chambers were sealed tightly with locking rings attached to the lid.

Gas sampling bags were used to prepare fumigant at experimental concentrations. Calculated volumes of liquid EF were injected into 1 L gas sampling bags and immersed in a hot water bath at 90°C for 10 min for complete vaporization of EF. Vaporized EF inflated the gas sampling bags. To facilitate injection of vaporized EF into experimental chambers, around 3 L of air was sucked out using an air pump connected to the sealed chamber. The EF concentrations prepared in the gas sample bag were sucked into the chamber under negative pressure to balance the air pressure with ambient. Ethyl formate gas standard was prepared in a 1 L Erlenmeyer flask by taking out the estimated amount of air from the flask and injecting a calculated amount of liquid EF to the small piece of filter paper attached under the tight lid fitted with a rubber septum of the Erlenmeyer flask. Headspace samples were taken from both gas sampling ports (top and bottom) of the fumigation chamber immediately; then 2 and 4 h later for each concentration (50, 60, and 90 mg L\(^{-1}\)) along with gas standards and injected in duplicate into GC-FID. The fumigation was conducted at a laboratory temperature between 23 and 25°C.
2.2.6. Fumigation of Infested Celery with Phosphine

Celery samples were brought from the 15°C cold room and left for about 4 h to bring to ambient laboratory temperature of 23-25°C before fumigation. Ten celery bunches were placed in each chamber with a loading ratio of 9.2%. Around 3 L of air were removed from each chamber before PH₃ injection and balanced with ambient air pressure. The same procedure and the number of celery bunches were applied for untreated check treatments.

Four concentrations of PH₃ were used; 1, 1.5, 2, and 2.5 mg L⁻¹ plus an untreated check treatment with three replicates for each treatment. The GC reading was recorded immediately followed by 2, 3, 4, 5, and 6 h after treatment commenced. Gas standards were prepared in an Erlenmeyer flask (1 L) by removing the calculated amount of air and injecting a calculated amount of PH₃ into the Erlenmeyer flask. All headspace samples were collected from the top and bottom ports in the each of fumigation chamber and injected in duplicate into the GC along with PH₃ standards.
2.2.7. Fumigation of Infested Celery with the Mixture of Ethyl Formate and Phosphine

To test the effect of combined EF and PH$_3$ on purple scum springtails; 10 bunches of celery were placed into two replicate chambers similar to previous (EF treatment) section. All experiments of the mixture of EF and PH$_3$ were repeated twice by using fresh celery bunches that were stored less than 24 h at 15°C cold room to confirm phytotoxicity only. The liquid EF was vaporized by using the gas sampling bag in hot water bath around 90°C for 10 min until the bags were inflated by EF vaporization. Phosphine of required concentration was stored in separate gas sampling bags. Both EF and PH$_3$ was injected into the chamber with EF as described above. Three treatments of EF+PH$_3$ concentrations 20, 30, and 40 mg L$^{-1}$ EF, with 1 mg L$^{-1}$ PH$_3$ were used along with an untreated check treatment. All samples were taken from each (top and bottom) port of chamber immediately, 2 and 4 h later along with standards injected into the GC in duplicate.

2.2.8. Mortality Assessment of Purple Scum Springtails

The bioassay samples were retrieved at the end of the fumigation treatment by opening and aerating the fumigation chambers inside the fume-hood for about 30 min. The fumigated celery samples were divided into two lots from each drum. Half of the sample lot from each drum was used for counting purple scum springtail mortality. The second half of the sample lot from each drum were stored at 15°C and 65% to 70% relative humidity (RH) for product quality and phytotoxicity studies.

After aeration, the mortality of purple scum springtails was evaluated by transferring five celery bunches from each replicate to a white plastic tray for mortality assessment. This was carried out by dismantling all leaves of celery from external to internal leaves and carefully transferring all dead and live insects into a 9 cm glass petri dish to ensure that all purple scum springtails were recovered. A magnifying glass was used to determine dead and live purple scum springtails. Similarly, mortality assessment was conducted for untreated check treatments.
2.2.9. Evaluation of Fumigant Phytotoxicity

Phytotoxicity for EF, PH$_3$, and their combination treatments was evaluated by cutting the treated and untreated celery bunches in half and looking for any damage or color change relative to untreated control samples. The observation of fumigant damages were taken every day from the day 2 until day 7.

2.2.10. Sensory and Taste Evaluation

Sensory and taste evaluation were carried out by selected 20 volunteers (10 males and 10 females, between the age 30-60) to check and taste the treated and untreated celeries. Prior to conduct sensory and taste tests, PH$_3$ fumigated celery was aired for two hours in under fume-hood. The fumigated and untreated control celery samples were washed, leaves removed and cut into pieces (15 cm long) for sensory and taste test. Treated and untreated celery were placed in 2 blank coded plates and the panel was asked to randomly pick the celery and rate their visual attributes related to color and taste related to flavor (characteristic flavor and odor), texture (crispness and juiciness) and smells from scale of 1 to 9 (1 = dislike extremely to 9 = like extremely) (Raffo et al. 2006; Yommi et al. 2013; Barrett et al. 2010).

2.2.11. Statistical analysis

Mortality for all treatments was calculated with one-way analysis of variance (ANOVA) using SPSS Advanced Statistics software (version 24.0; IBM Corp, Armonk, NY) at the least significant difference level (5%) and Tukey's 95% confidence intervals. The variation of all treatments and standards were assessed with duplicate injections and gas chromatography data. The average and standard deviation (SD) of EF, PH$_3$, and their combination absorption by celery were calculated on the basis of peak areas against the external gas standards of EF and PH$_3$ using Microsoft Excel 2010 (Microsoft Company, Perth, Australia). The sensory evaluation results were treated by analysis of variance (ANOVA) with significant level 5%.
2.3. Results

2.3.1. Sorption of Fumigants by Celery

The concentration of EF and PH$_3$ in the fumigation chambers was measured during 4 h of fumigation with EF and EF+PH$_3$, and 6 h for PH$_3$. The concentrations of fumigants are shown in Figures 2.2, 2.3 and 2.4. All concentrations of EF decreased rapidly within half an hour (see Figure 2.2), as EF is absorbed by the commodity. The concentration of EF in the 90 mg L$^{-1}$ treatment recorded a decline of 70% from the initial dose and more than 80% for the initial EF concentrations of 50 and 60 mg L$^{-1}$. After 2 h of fumigation, 85 to 90% of EF was absorbed by the commodity for all applied concentrations of EF. For the PH$_3$ fumigation after 2 and 6 h, approximately 40 to 50% and 60 to 70% of PH$_3$ was absorbed by the commodity for applied concentrations of 1, 1.5 and 2 mg L$^{-1}$ respectively. However, for 2.5 mg L$^{-1}$ PH$_3$, about 22% and 32% of the initial applied concentration was lost over 2 and 6 h respectively (see Figure 2.3).

For the EF+PH$_3$ combination fumigation treatments, less than half of EF and approximately 50% PH$_3$ was lost within 2 h (see Figure 2.4). Generally, after 4 h of fumigation, about 90% of EF and about 40% of PH$_3$ was lost from the headspace of fumigation chambers (see Figures. 2.2–2.4).
Figure 2.2. The concentration of ethyl formate (EF) with time of exposure (h) in the headspace of fumigation chambers containing celery bunches at 50 (-▲-), 60 (-●-) and 90 (-♦-) mg L\(^{-1}\) for 1, 2 and 4 h exposure. Vertical bars represent standard deviation (SD). 1 mg L\(^{-1}\) = 335ppm (v/v).
Figure 2.3. The concentration of phosphine (PH$_3$) with time of exposure (h) in the headspace of fumigation chambers containing celery bunches at 1 (-●-), 1.5 (-■-), 2 (-▲-) and 2.5 (-●-) mg L$^{-1}$ for 2, 4, and 6 h exposure. Vertical bars represent standard deviation (SD). 1 mg L$^{-1}$ = 730ppm (v/v).
Figure 2.4. The concentrations of ethyl formate (EF) and phosphine (PH₃) with time of exposure (h) in the headspace of fumigation chambers containing celery bunches during fumigation with the combination of ethyl formate EF at 20 ( ● ), 30 (♦) and 40 (■) mg L⁻¹ plus phosphine (PH₃) (▲) (1 mg L⁻¹) respectively for 2 and 4 h exposure. Vertical bars represent standard deviation (SD). 1 mg L⁻¹ = 335ppm (v/v) for EF and 1 mg L⁻¹ = 730ppm (v/v) for PH₃.
### 2.3.2. Bioassay of Ethyl Formate Alone on Purple Scum Springtails

The total number of purple scum springtails tested varied in all treatments due to natural infestation. The numbers of pests ranged between 132-238 purple scum springtails per treatment. With celery fumigated in chambers with EF at the concentration of 50 mg L\(^{-1}\), the mortality of purple scum springtails was 82.77, 91.58 and 93.28% for 1, 2 and 4 h exposure, respectively (Table 2.1). The second treatment of EF at a concentration of 60 mg L\(^{-1}\) for 1, 2 and 4 h exposure, showed observed mortality of 98.21, 97.46 and 96.04%, respectively. In the third treatment of EF, with a concentration of 90 mg L\(^{-1}\), the observed mortality was 98.73 and 100% for 1 and 2 h exposure, respectively. These bioassay results show that there is a significant difference in the level of mortality between 1, 2 and 4 h exposure with EF alone compared to the untreated check. Also, there is a significant difference in the level of mortality among the concentrations 50, 60 and 90 mg L\(^{-1}\) for all fumigation times. There was no significant difference in mortality between the concentrations of 60 and 90 mg L\(^{-1}\) for any duration of fumigation. The high mortality of 100% was achieved only at 90 mg L\(^{-1}\) after 2 h exposure.

The results of this study indicate that increasing EF concentration plays a more significant role than the duration of fumigation on mortality of purple scum springtails. There was a significant effect of concentration and time on purple scum springtail mortality (F=5.773; P<0.007). The relationship between concentration and time was assessed by variance analysis and accounted for R\(^2\)=0.999 and adjusted R\(^2\)=0.998.

### 2.3.3. Bioassay of Phosphine Alone on Purple Scum Springtails

In the PH\(_3\) only treatment, varying numbers of purple scum springtails (80-217) were tested per treatment on purple scum springtail-infested celery. The relatively low and variable number of purple scum springtails per treatment is due to the celery being naturally infested.

Initially, two treatments of PH\(_3\) concentrations (1 and 1.5 mg L\(^{-1}\)) were tested (Table 2.2). The mortality achieved was 24.33, 24.26 and 26.40% at 1 mg L\(^{-1}\) for 2, 4 and 6 h exposure time respectively and 37.20, 20.90 and 35.80% at 1.5 mg L\(^{-1}\) for 2, 4 and 6 h exposure time respectively.
Other treatments of PH3 concentrations were 2 and 2.5 mg L⁻¹ for 2, 4 and 6 h exposure. The results show mortality of 64.70, 70.73 and 73.23% at 2 mg L⁻¹ of PH3 and 53.86, 73.63 and 100% achieved at 2.5 mg L⁻¹ for 2, 4 and 6 h exposure respectively. Mortality varied significantly by concentration and time of exposure (F=4.663; P<0.001). The relationship between concentration and time variance accounted for R²=0.940 and adjusted R²=0.912.

Table 2.1. The mortality of purple scum springtails after 1, 2 and 4 h exposure to ethyl formate (EF) at different concentrations in fumigation chambers containing celery.

<table>
<thead>
<tr>
<th>EF concn (mg L⁻¹)</th>
<th>Time of exposure (h)</th>
<th>N⁵</th>
<th>Mortality [mean ± SE (%)]⁶</th>
<th>95% CI (range)⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>147</td>
<td>0±0 a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>168</td>
<td>0±0 a</td>
<td>0</td>
</tr>
<tr>
<td>Untreated check</td>
<td>4</td>
<td>135</td>
<td>0±0 a</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>132</td>
<td>82.77±1.17 b</td>
<td>79.95–85.59</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>166</td>
<td>91.58±0.90 c</td>
<td>89.42–93.74</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>164</td>
<td>93.28±0.10 cd</td>
<td>93.03–93.53</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>238</td>
<td>98.21±1.45 de</td>
<td>94.71–101.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>230</td>
<td>97.46±2.07 cde</td>
<td>92.47–102.44</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>156</td>
<td>96.04±1.87 cde</td>
<td>91.55–100.53</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>140</td>
<td>98.73±1.03 de</td>
<td>96.25–101.22</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>193</td>
<td>100.0±0 e</td>
<td>100–100</td>
</tr>
</tbody>
</table>

LSD₀.₀⁵ = 3.512

z = 1 mg L⁻¹ = 335ppm (v/v); y = N refers to the total number of purple scum springtails; x = Mean mortality followed by the same letters in each group were not significantly different base on Tukey differences multiple range test P>0.05; w = 95% CI refers to the confidence interval; v = LSD₀.₀⁵ refers to the least significant difference 0.05.
Table 2.2. The mortality of purple scum springtails after 2, 4 and 6 h exposure to phosphine (PH$_3$) at different concentrations in fumigation chambers containing celery.

<table>
<thead>
<tr>
<th>Concn of PH$_3$ (mg L$^{-1}$)$^z$</th>
<th>Time of exposure (h)</th>
<th>N$^y$</th>
<th>Mortality [mean ± SE (%)]$^x$</th>
<th>95% CI (range)$^w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Untreated</td>
<td>2</td>
<td>133</td>
<td>7.50±1.46 a</td>
<td>3.23–18.25</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>217</td>
<td>3.09±2.64 a</td>
<td>0.62–6.82</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>156</td>
<td>2.05±3.06 a</td>
<td>1.96–6.07</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>127</td>
<td>24.32±3.62 ab</td>
<td>6.94–41.71</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>101</td>
<td>24.25±1.00 ab</td>
<td>14.05–34.45</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>151</td>
<td>26.38±0.58 abc</td>
<td>19.37–33.39</td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>81</td>
<td>37.18±2.63 bcd</td>
<td>30.72–43.65</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>198</td>
<td>20.86±2.48 ab</td>
<td>11.92–29.80</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>130</td>
<td>35.79±3.70 bc</td>
<td>28.50–43.08</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>80</td>
<td>64.69±3.74 de</td>
<td>49.06–80.32</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>134</td>
<td>70.72±0.16 ef</td>
<td>61.79–79.64</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>121</td>
<td>73.20±2.72 ef</td>
<td>64.28–82.13</td>
</tr>
<tr>
<td>2.5</td>
<td>2</td>
<td>155</td>
<td>53.88±3.02 cde</td>
<td>38.78–68.99</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>97</td>
<td>73.59±3.74 ef</td>
<td>62.58–84.59</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>130</td>
<td>100±0 f</td>
<td>100–100</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$$^v$ 15.384

$z = 1$ mg L$^{-1} = 730$ppm (v/v); $y = N$ refers to the total number of purple scum springtails; $x = $ Mean mortality followed by the same letters in each group were not significantly different based on Tukey differences multiple range test $P>0.05$; $w = 95\%$ CI refers to the confidence interval; $v = $ LSD$_{0.05}$ refers to the least significant difference 0.05.
2.3.4. Bioassay of Phosphine plus Ethyl Formate in Combination on Purple Scum Springtails

In order to reduce phytotoxicity on EF treated celery, the dose of EF was decreased to 20, 30 and 40 mg L$^{-1}$ and combined with 1 mg L$^{-1}$ PH$_3$. The combination of PH$_3$ and EF was tested on purple scum springtail-infested celery with three concentrations of EF tested: 20, 30 and 40 mg L$^{-1}$ each combined with 1 mg L$^{-1}$ of PH$_3$. The number of target pests ranged between 101 and 211 insects per treatment.

The mortality of purple scum springtails was 100% for 2 and 4 h exposure at 40 mg L$^{-1}$ of EF mixed with 1 mg L$^{-1}$ PH$_3$ compared with the untreated check treatment. Mortality of 98.8% and 100% mortality was achieved at 30 mg L$^{-1}$ of EF mixed with 1 mg L$^{-1}$ PH$_3$ for 2 and 4 h exposure respectively (Table 2.3). Mortality at 20 mg L$^{-1}$ of EF with 1 mg L$^{-1}$ of PH$_3$ was 89.5% and 95.5% for 2 and 4 h exposure respectively. The interaction between concentration and time on purple scum springtail mortality was significant ($F=16.56; \ P<0.001$). The relationship between concentration and time was assessed by variance analysis and accounted for $R^2=1.000$ and adjusted $R^2=1.000$. 
Table 2.3. The mortality of purple scum springtails exposed to various concentrations of the combination of ethyl formate (EF) plus phosphine (PH₃) for 2 and 4 h in fumigation chambers containing celery.

<table>
<thead>
<tr>
<th>Concentration of EF+PH₃ (mg L⁻¹)</th>
<th>Time of exposure (h)</th>
<th>N&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mortality [mean ± SE (%)]&lt;sup&gt;x&lt;/sup&gt;</th>
<th>95% CI (range)&lt;sup&gt;w&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 + 0</td>
<td>2</td>
<td>168</td>
<td>0±0 a</td>
<td>0</td>
</tr>
<tr>
<td>Untreated check</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 + 1</td>
<td>2</td>
<td>211</td>
<td>89.50±0.57 b</td>
<td>85.41–99.10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>158</td>
<td>95.50±0.17 c</td>
<td>87.69–97.78</td>
</tr>
<tr>
<td>30 + 1</td>
<td>2</td>
<td>184</td>
<td>98.80±0.99 d</td>
<td>96.39–101.17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>192</td>
<td>100.00±0.00 d</td>
<td>100–100</td>
</tr>
<tr>
<td>40 + 1</td>
<td>2</td>
<td>101</td>
<td>100.00±0.00 d</td>
<td>100–100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>122</td>
<td>100.00±0.00 d</td>
<td>100–100</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;&lt;sup&gt;v&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>1.629</td>
</tr>
</tbody>
</table>

<sup>z</sup> = 1 mg L⁻¹ = 335ppm (v/v) for EF and 1 mg L⁻¹ = 730ppm (v/v) for PH₃; <sup>y</sup> = N refers to the total number of purple scum springtails; <sup>x</sup> = Mean mortality followed by the same letters in each group were not significantly different based on Tukey differences multiple range test P≥0.05; <sup>w</sup> = 95% CI refers to the confidence interval; <sup>v</sup> = LSD0.05 refers to the least significant difference 0.05.
2.3.5. Effect of Fumigants on Celery Quality and Phytotoxicity

It was observed that EF fumigation can result in significant phytotoxicity of celery. Symptoms are changes in the color of leaves especially inside layers of young leaves, which turn brown. Our results indicate that all concentrations of EF used in this study affected celery and caused phytotoxicity. Damage was apparent in two ways: firstly, only young leaves are damaged at low EF concentrations and secondly, both old and young leaves of celery were damaged at high concentrations of EF. Importantly, celery subjected to PH₃ treatments alone at various doses showed no evident phytotoxicity.

Damage to celery appeared within 1 d of treatment with 20 mg L⁻¹ EF plus 1 mg L⁻¹ PH₃. The browning of leaves appeared almost immediately at 30 and 40 mg L⁻¹ doses of EF. The quality of celery was reduced by wilting of outer foliage and yellowing of inside layered leaves by EF and the mixture of EF+PH₃ after 2 to 5 d of treatment depending on the concentration of fumigant (see Figure 2.5).

Figure 2.5. Symptoms of phytotoxicity on commodity (a) celery treated with ethyl formate, (b) celery fumigated with the combination of ethyl formate + phosphine, (c) celery treated with phosphine only and (d) untreated check.
The scores of sensory test for both treated and untreated celery were ranged between 7 and 9 for all attributes (Table 4) corresponding to 'like moderately' to 'like extremely'. There were no statistically significantly difference ($P > 0.05$) between untreated control and treated samples.

Table 2.4. Effect of fumigation on quality and sensory scores (1-9 hedonic scale) of celery.

<table>
<thead>
<tr>
<th>Quality attribute</th>
<th>Treatments</th>
<th>N$^z$</th>
<th>Score scale (Mean ± SE)$^y$</th>
<th>95% CI (range)$^x$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crispness</td>
<td>PH$_3$ treated</td>
<td>20</td>
<td>8.05±0.05</td>
<td>7.95–8.15</td>
<td>0.574</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>8.00±0.07</td>
<td>7.85–8.15</td>
<td></td>
</tr>
<tr>
<td>Juiciness</td>
<td>PH$_3$ treated</td>
<td>20</td>
<td>7.50±0.19</td>
<td>7.08–7.92</td>
<td>0.313</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>7.75±0.14</td>
<td>7.45–8.05</td>
<td></td>
</tr>
<tr>
<td>Flavor and taste</td>
<td>PH$_3$ treated</td>
<td>20</td>
<td>7.85±0.19</td>
<td>7.44–8.26</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>7.95±0.17</td>
<td>7.59–8.31</td>
<td></td>
</tr>
<tr>
<td>Color and appearance</td>
<td>PH$_3$ treated</td>
<td>20</td>
<td>8.30±0.16</td>
<td>7.96–8.64</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>8.20±0.15</td>
<td>7.87–8.58</td>
<td></td>
</tr>
<tr>
<td>Aroma and smell</td>
<td>PH$_3$ treated</td>
<td>20</td>
<td>7.45±0.56</td>
<td>6.91–7.99</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>7.75±0.22</td>
<td>7.27–8.23</td>
<td></td>
</tr>
</tbody>
</table>

$z = N$ refers to the number of panelists that ranged the sensory scores; $y = \text{Mean with the standard error for the number of liking on 1 to 9 point hedonic scale}$; $x = 95\% \text{ CI refers to the confidence interval for mean}$. 
2.4. Discussion

Purple scum springtails are a common insect in Australia, occasionally found on fresh celery. This has led to export rejection to Middle East countries where it is a quarantine pest requiring fumigation. Our investigations showed that EF, PH₃ and their combination had a significant effect on purple scum springtail mortality. Experimental concentrations of fumigants EF, PH₃, and their combination were found to decrease significantly over time which may be attributed to the absorption by celery bunches because of the high surface area and very high moisture content of the commodity (see Figures. 2.1-2.4). The sorption of fumigants EF and PH₃ is known to depend on several conditions such as the type of commodity, target pest, temperature, moisture content, particle size and composition, exposure time and fumigant dose (Berck 1968; Dhaliwal 1975). Further Reddy et al. (2007) reported that PH₃ concentration might vary according to food commodity types and found that the PH₃ concentration in the headspace of the commodities varied from 0 to >2000ppm. The higher moisture content of fresh products may be one of the contributing factors for sorption of PH₃, as outlined in their studies where PH₃ was applied to 74 products with high moisture content. This result is consistent with previous experiments that demonstrated EF and PH₃ can be significantly absorbed by the commodity or broken down to by-products because of the high moisture content of the commodity (Agarwal et al. 2015; Reddy et al. 2007). Furthermore, the toxicity to the pest can be increased with longer exposure time (Lee et al. 2015; Liu 2011; Ren et al. 2011; Van Epenhuijsen et al. 2007).

In the current study, mortality of purple scum springtails varied among different EF concentrations with 100% mortality achieved at 90 mg L⁻¹ within 2 h. The results for EF indicate a significant increase in purple scum springtail mortality with increasing exposure to EF. This result is consistent with findings of other experiments which showed that vacuum fumigation of packaged lettuce with 0.5%, 1.0% and 1.5% of EF for 2 h had little impact on the quality of lettuce while achieving high mortality of green peach aphids. Ethyl formate is approved for treatment of an export paprika market, as well as tomatoes in Korea (Kim et al. 2013). Additionally, EF achieved high mortality against cotton aphids (Aphis gossypii), and two-spotted mites (Tetranychus urticae) (Kim et al. 2013; Lee et al. 2013; Stewart & Aharoni 1983).
Table one shows the different percentage mortality of purple scum springtails with EF fumigation alone. A high dose of EF, of 90 mg L\(^{-1}\) achieved 100% mortality, compared with relatively low mortality at a concentration of 50 mg L\(^{-1}\) of only 91.58% over the same exposure period. These results of high mortality on purple scum springtails may be attributable to fumigant penetration into each celery bunch. This result is consistent with results of a past studies that showed 100% mortality against Indian meal moth (*Plodia interpunctella*) and confused flour beetle by using EF alone on dried vine fruit and also high EF concentration caused high mortality on the eucalyptus weevil (*Gonipterus platensis*) in export apples (Agarwal et al. 2015; Tarr et al. 2007).

Fumigation of celery bunches with PH\(_3\) alone was studied, and 100% mortality was achieved at 2.5 mg L\(^{-1}\) after six hours fumigation. Lower doses of 1, 1.5 and 2 mg L\(^{-1}\) also caused mortality of purple scum springtails, but levels of mortality were less (Table 2). These results are consistent with Liu (2009) who reported that PH\(_3\) fumigant caused 100% mortality at 1.4 mg L\(^{-1}\) after 24 h fumigation for western flower thrips on different vegetables such as lettuce, broccoli, and asparagus, which were successfully fumigated at low temperature (2°C) without injury to these vegetables. For controlling lettuce aphids using PH\(_3\) fumigation, 2.4 mg L\(^{-1}\) of PH\(_3\) achieved 100% mortality over 72 h fumigation at low temperature (2°C) (Liu, 2009). Moon (2012) reported that concentrations of PH\(_3\) should be more than 2 g m\(^{-3}\) for >24 h exposure for control of cotton aphid at 8°C on cut flowers and nursery stock.

Current studies show high mortality over short fumigation periods with the toxicity of EF plus PH\(_3\) being much higher compared with PH\(_3\) or EF alone. Low concentration of EF (30 and 40 mg L\(^{-1}\)) mixed with PH\(_3\) (1 mg L\(^{-1}\)) at 2 and 4 h exposure gave 100% mortality compared with the high dose of EF or PH\(_3\) at the same exposure time when used alone (100% mortality achieved at 90 mg L\(^{-1}\) EF alone for 2 h exposure and also with PH\(_3\) at 2.5 mg L\(^{-1}\) alone at 6 h).

Hence, we believe that there is a synergistic effect between EF and PH\(_3\) for control of purple scum springtails and the combination produces a greater effect than either EF or PH\(_3\) alone. At concentrations of 30 and 40 mg L\(^{-1}\) of EF combined with 1 mg L\(^{-1}\) PH\(_3\) 100% mortality was achieved compared with 20 mg L\(^{-1}\) of EF combined with the same amount of PH\(_3\), which gave less mortality over the same exposure time. This result shown in Table three is consistent with Lee et
al. (2015) who demonstrated a synergistic effect between EF and PH₃ for control of cotton aphid when applied in combination. They showed that the fumigant combination can cause high mortality of all stages of citrus mealybug and less damage to the commodities at low temperature (8°C) for 4 h (Yang et al. 2016). There have been a number of research studies looking to reduce the fumigation period and increase PH₃ effectiveness with mixtures of different gases. For example, Liu (2008 and 2011) found that exposure time of PH₃ can be reduced at low temperature and still control several postharvest pests, such as western flower thrips and grape mealybug (*Pseudococcus maritimus*) by using oxygenated PH₃.

All fumigants were effective against purple scum springtails in given short treatment times for exporting fresh produce with respect to disinfesting produce of live insects. However, EF fumigant alone and in combination with PH₃ impacted the quality of celery and caused phytotoxicity symptoms. The observation of celery phytotoxicity from EF and the combination of EF and PH₃ demonstrated that high sorption of EF by the commodity is phytotoxic, even at low concentrations of EF or short fumigation times. Phytotoxicity of the fumigant to the commodities is very much dependent on the type of commodity treated, and the interaction of fumigant or its by-product with the chemical constituents of the commodities. These results are supported by Weller and Graver (1998) and Zhang and Van Epenhuysen (2004) who reported that EF causes high phytotoxicity on cut flowers. In some previous studies, less damage to commodities was shown by using EF/PH₃ in combination achieving high mortality of target pests (Lee et al. 2015; Yang et al. 2016). Lee et al., (2014) indicated high mortality of insect pests by a combination of EF and PH₃ in naturally infested strawberries and cut flowers with less apparent phytotoxicity. Agarwal et al. (2015) have shown no apparent toxicity of EF to apple fumigation.

In the case of PH₃ fumigation alone, there was no difference in celery quality or phytotoxicity observed compared with untreated celery. This is consistent with the result obtained by Liu (2008). Previous studies have shown that PH₃ fumigation of vegetables is effective and safe for postharvest and commercial applications for insect control. Therefore, PH₃ remains one of the best fumigants for vegetable fumigation because it causes less phytotoxicity compared to other gases (Horn et al. 2005; Lee et al. 2012; Liu 2008). Likewise, PH₃ treatments alone at various doses achieved high mortality on purple scum springtails and kept commodity at high quality. The sensory evaluation
of fumigated celery bunches were similar to untreated control. There was no negative effect on quality parameters. Several studies indicated that PH₃ fumigation cause no significant effect on sensory evaluation in broccoli, tomato and green pepper (Ertürk et al. 2018; Liu 2008).

2.5. Conclusion

Based on our results, treatments on purple scum springtails in celery, EF alone and in combination with PH₃ can control the target pest of purple scum springtails; however, the combination is phytotoxic to the commodity. In contrast, PH₃ alone achieved 100% kill of the target pest with no phytotoxic effect on the commodity. There is no significant differences on the sensory evaluation between treated and untreated celery by PH₃.

Based on these findings we conclude that PH₃ has excellent potential for pre-shipping treatment of export celery for the control of purple scum springtails with little or no adverse effect on the taste and celery quality.
Chapter 3: Evaluation of Volatile Organic Compounds from *Brassica* Plants Infested with Green Peach Aphid *Myzus persicae* (Hemiptera: Aphididae) and Parasitoids *Aphidius colemani* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) Attraction

3.1 Abstract

Volatile organic compounds (VOCs) from uninfested and infested *Brassica* plants with green peach aphid *Myzus persicae* were investigated by headspace solid microextraction (HS-SPME) combined with gas chromatography mass spectrometry (GC-MS). Overall, 29 compounds were detected in infested and uninfested cabbage, while 25 compounds were identified in uninfested and infested broccoli plant samples. Some VOCs released from infested cabbage were greater than uninfested plants and increased the quantity of the composition from infested plants. The HS-SPME combined with GC-MS analysis of the volatiles described the differences between the infested and uninfested Cruciferous plant samples. Based on peak area from the GC-MS analysis, the VOCs from infested cabbage consisted of Propane, 2-methoxy, alpha- and beta pinene, Myrcene, 1-Hexanone, 5-methyl-1-phenyl-, Limonene, Decane, gamma-Terpinen and Heptane, 2,4,4-trimethyl all these volatiles were higher in the infested cabbage compared with their peak area in the uninfested cabbage. Whereas, the VOCs from infested broccoli were significantly greater than that from uninfested broccoli, such as D-limonene, Undecane, 3,4-dimethyl-, Heptane, alpha-Pinene, Oxalic acid, Citronellol, Tridecane, n-Decanoic acid, Cyclopentane, penty1- and n-Hexadecanoic acid compared with volatiles released from uninfested broccoli.

The results from study using a Y-shape olfactometer showed that *Myzus persicae* were significantly attracted by the VOCs released from infested cabbage and broccoli plants, more than uninfested plants or clean air. The percentage of aphid choice was 80% and 70% towards infested cabbage and broccoli respectively, 7% and 10% were attracted to the clean air choice and uninfested plants. With the comparison between clean air and uninfested plants, the aphids were attracted by 75.56% and 84% for cabbage and broccoli respectively, while 3% and 7% were attracted towards clean air. Comparing between infested and uninfested, the aphid attracted by 63% and 26.6% for infested cabbage and broccoli respectively, versus 57% and 30% for uninfested
The preferences of *Aphidus colemani* and *Aphelinus abdominalis* to the infested, uninfested plants with *M. persicae* and compared with clean air were measured using Y-shape olfactometer. The results indicated that parasitoids could discriminate the infested cabbage and broccoli and significantly respond to the plant odour and attractive by 86.6% and 100% for both parasitoids towards infested *Brassica* plants.

### 3.2 Introduction

The green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), has a worldwide distribution, including Australia, and is considered a serious pest that has caused damage to hundreds of horticultural species of plants in more than 66 families (Kim & Jander 2007; Valenzuela & Hoffmann 2015). The aphid mainly exists in young plant tissues, causing reduced leaf size, delayed growth of the plant and reduced yield (Yoon et al. 2010). The green peach aphid is an important pest of greenhouse crops and horticultural vegetation and it decreases the commercial value by transferring plant viruses (Kim et al. 2005). The damage can be caused in two ways: feeding on plant sap by sucking, and also acting as a virus transmission on the plant (indirect damage) (de Little & Umina 2017). *M. persicae* is considered a common pest insect of cruciferous crops, especially cabbage and broccoli, and sucks plant sap leading to yellowing and curling of plant leaves. Also, excretion of honeydew by aphids affects plant photosynthesis and encourages fungal growth (Amarawardana et al. 2007).

Cabbage and broccoli are as Cruciferous crops from the Cruciferae (*Brassicaceae*) family. These plants are commonly attacked by several insect pests, such as species of aphids like turnip aphid *Lipaphis erysimi*, cabbage aphid *Brevicoryne brassicae* and green peach aphid *M. persicae*, which economically damage these crops (Liu & Sparks 2001). The green peach aphid is known to damage the cruciferous crops either directly or indirectly. The direct damage of aphids comes from their feeding on plant juice by inserting their mouthparts and sucking the sap, which ultimately causes direct damage to the cruciferous crops. Also, aphids usually feed on the young leaves of the cabbage and are generally found on the upper as well as lower surfaces of the cabbage (Hooks & Johnson 2003; Liu & Sparks 2001). Indirect damage is caused by transmission of plant viruses from one crop to another, as well as damage resulting from the secretion of honeydew. Also, aphids can cause an economic effect on a crop at levels of population densities that would decrease yield.
and affect marketability by physical contaminations with cast skins of aphid and honeydew (Liu & Sparks 2001).

Chemical insecticides play a significant role in controlling insects on crop plants. Insecticides have been extensively used in horticultural systems; however, they can cause the appearance of secondary pests instead of primary pests, pesticide resistance, contamination of environment and affect non-target organisms (Anstead et al. 2005; Nauen & Denholm 2005). Therefore, it is necessary to find alternative methods for pest management. In biological control, aphid parasitoids from families such as Braconidae and Aphelinidae are important and can cause a high percentage of mortality on aphids (Reed et al. 1995; Takemoto & Takabayashi 2015). Natural enemies of aphids can reduce the rate of population increase, and the use of wasp parasitoids in biological control of aphids has been successful (Goh et al. 2001).

Plants VOCs play an important role in plant–insect interactions by influencing insect communication and plant defence (Guerrieri & Digilio 2008). When sucking insect pests such as the green peach aphid feed on the plant, one response from the plant is to release odours in the form of VOCs. The VOCs have an important role in plant–insect interactions because they can be used by parasitoids to locate their host (De Farias & Hopper 1997).

Solid Phase Microextraction (SPME) was presented by Arthur and Pawliszyn (1990), who reported the SPME had become a useful tool in organic analytical chemistry combined with GC-MS because it was easy to use and has a rapid extraction procedure that does not need organic solvents (Bicchi et al. 2000). Therefore, SPME can be used as an alternative tool to hydrodistillation, traditionally used for the collection of VOCs from plant samples. Also, SPME is a simple, fast and useful tool for gas chromatography analysis of volatiles from different samples (Huang et al. 2010; Zhang et al. 2007).

Cabbage and broccoli attacked by aphids may emit volatile compounds that attract parasitoid wasps or predators (Shiojiri et al. 2000; Vuorinen et al. 2004). Previous studies have concluded that natural enemies can recognize the VOCs that release from the infested plants and confirmed
by using the olfactometer; the olfactometer results provided explanation of insects responses to these VOCs (Steinberg et al. 1992; Vet & Dicke 1992).

*Aphidius colemani* (Hymenoptera, Braconidae) and *Aphelinus abdominalis* (Hymenoptera, Aphelinidae) are endoparasitoids of many species of aphids and both attack *M. perisecae* (Najar et al. 2015). These parasitoids lay their eggs in aphid and their young larvae consume the insides of the body of aphid, eventually pupating within its body and turning aphid to mummy. The parasitoid pupa then emerges as an adult to begin the new life cycle (Sampaio et al. 2001; Soglia et al. 2006). The VOCs released from infested *Brassica* plants by aphids can bring parasitoids, which showed the family of *Brassicaceae* possess chemical defence (Girling & Hassall 2008). When the aphids feed on the plant leaves, the plant produces blends of volatiles as a response to the infestation by aphids, releases volatile compounds in different quantities and qualities from damaged *Brassica* plants, and these differences in the VOCs can attract both other pests and natural enemies (Najar et al. 2015). Many natural enemies like parasitoids *Aphidius spp* respond to plant VOCs and can distinguish healthy from infested plants (Powell et al. 1998). Some studies have shown natural enemies, such as parasitoid wasps, use their ability to identify single VOC signs from herbivore–plant complexes to find specific parasitoid or host species. Therefore, there is a three-way interaction between plant, herbivore and parasitoid wasps taking place (Poelman et al. 2011). The parasitoid wasps *A. colemani* and *A. abdominalis* are specific to green peach aphids, and their females use VOC cues to detect and locate where aphids are feeding and lay eggs into hosts (Godfray 1994; Hatano et al. 2008). Also, honeydew excreted by aphids on plants could lead to the release of semiochemicals or VOCs attracting and guiding parasitoids to the aphid (Hågvar & Hofsvang 1989; Leroy et al. 2011).

In Y-tube olfactometer tests, Reed et al. (1995) reported no attraction of the parasitoid *Diaeretiella rapae* to the cabbage leaves. However, the choice of wasps to infest cabbage plants by *B. Brassicaceae* was more significant than other plants infested by different species of aphid, such as Russian wheat aphid *Diuraphis noxia*. These results indicate that the cabbage plant VOCs are more important than other plants in attracting the parasitoid to the aphid location (Reed et al. 1995). The heavy population of *M. persicae* on the plant can accumulate wasps, while the uninfested plant sees few parasitoids come to the plant because wasps failed to locate the healthy plant (de Rijk et al. 2013;
Hågvar & Hofsvang 1991). Moreover, Read et al. (1970) found that when parasitoid females were given a choice between *M. persicae* feeding on Cruciferous or beet plants, the parasitoids preferred to attack aphids feeding on crucifer plants.

The identification of VOCs can be a signal for aphids and their parasitoids’ receivers, and development of methods to analyse VOCs as diagnostic indicators that involve aphid management. Therefore, the study aimed first, to determine the VOCs released from cruciferous plants (cabbage and broccoli) between healthy and infested plants by green peach aphids *M. persicae*; second, to elucidate the responses of Y-tube olfactometer to the sucking pest for green peach aphids *M. persicae* (Hemiptera, Aphididae) and their parasitoids *A. colemani* (Hymenoptera, Braconidae) and *A. abdominalis* (Hymenoptera, Aphelinidae) when cruciferous plants are not infested, and when it is infested by aphids and affected by plant VOCs. Understanding the treatments influencing the attraction of the parasitoids may provide fundamental data for controlling green peach aphids and generating new methods for aphid biological control.

### 3.3 Materials and methods

#### 3.3.1 Experimental plants

Cabbage (*Brassica oleracea* L. var. *capitata*) and broccoli (*Brassica oleracea* L. var. *italica*) seeds were sown in a 90 mm square pot filled with potting soil mixture (Richgro Regular Potting Mix, NSW, Australia) and grown under greenhouse conditions at 23–25°C, 60–70% relative humidity and L16: D8 light cycle. Plants were grown in a glasshouse to the 7–9 leaves stage and used for all experiments. Green peach aphid was reared on both cabbage and broccoli in cages made from anti-insect white mesh with external dimensions of 40 cm x 40 cm x 40 cm.

#### 3.3.2 Insects

*Myzus persicae* for experiments were obtained from the Department of Primary Industries and Regional Development, Entomology Branch (Western Australia) and maintained on potted cabbage and broccoli seedlings in a greenhouse that were placed into large cages (210 cm x 90 cm) covered by anti-aphid mesh and provided with a control light system set at L16: 8D
photoperiod, at the glasshouse temperature 23–25°C, located at Murdoch University (Western Australia).

*aphidus colemani* (Hymenoptera, Braconidae) and *aphelinus abdominalis* (Hymenoptera, Aphelinidae) were commercially obtained from Biological Services (South Australia) as mummies and maintained on potted cabbage and broccoli plant with *M. persicae* as hosts. Mummies of wasps were removed from the plant leave on the 12th day for the *A. colemani* and 15th day for the *A. abdominalis* of the parasitism, and placed in open 9 cm petri dishes inside a small cage 40 cm x 40 cm x 40 cm, in glasshouse conditions (23–25°C, 60–70% RH, 16:8 L:D) until emergence. Then the parasitoids were allowed to mate in the cage for one day with provided 50% honey solution for feeding. After that, the parasitoid was held individually in glass vials (one wasp per vial), a small piece of cotton attached to the vial cap for the drop of 50% honey solution to feed the parasitoid until tested. Female wasps were used for the Y-shape olfactometer choice test (Takemoto & Takabayashi 2015).

### 3.3.3 Volatiles collection and GCMS analysis using HS-SPME

#### 3.3.3.1 Limit of detection

The separation and limit of detection (LOD) were performed based on GC-MS responses of C7-C30 alkane standard (Supelco, Bellefonte, USA). One litre Erlenmeyer flasks (FE 1 L/3; Bibby Sterilin, Staffordshire, UK) were used after cleaning with deionised water and oven drying at 80°C for 12 hours. The stock gas standard C7–C30 was prepared by injecting 2 μl of liquid C7–C30 alkane standard into a sealed Erlenmeyer flask using a 5 μl syringe (5R-GT, SGE, Melbourne, Victoria, Australia) to obtain parts per million (ppm) level of C7–C30 gas standard. For preparation parts per billion (ppb) level of C7–C30 gas standard, 1 mL of (ppm) level of the standard was transferred into an empty 1 L Erlenmeyer flask. One mL of (ppb) level of the standard was injected into a 1 L empty Erlenmeyer flask to form a (ppt) level of standard. Two replicates of each level of gas standard were used and SPME fibre 50/30 μm (PDMS/ CAR/ DVB; Sigma-Aldrich, Australia) extraction for one flask and injection were repeated three times. The fibre was exposed in the gas standard flask for one hour to extract C7–C30 compounds at laboratory temperature (25±1°C). After one hour extraction, the SPME fibre was injected into GC-MS
injection port for 10 min desorption of extracted C7–C30 compounds from the fibre at 270°C (see Section 3.3.3.3). The peak areas and retention time were recorded by the computer installed with Qualitative Analysis B.07.00 and NIST software.

3.3.3.2 VOCs extraction with HS-SPME

The analysis of volatiles was focused on cabbage and broccoli for infested and uninfested plants with the green peach aphid. Cabbage and broccoli were placed individually into 4 L glass jars, and one plant in each jar was analysed. For each glass jar, a 5 mm port was drilled into the side, into which a septa (20633 Thermogreen® LB-2 Septa, plug) was placed and used for collection of infested and uninfested plant VOCs. Aluminium foil 100 m x 44 cm (Vital Packaging Company) was used to carefully cover and wrap the surface of the top of the plant pot, and the glass jar placed upside down on the plant. The reason for selecting glass jars is that it is easy to capture the VOCs emitted and also easy to wash, clean and oven dry them at 100°C for a minimum of 30 min to sterilise. VOCs were extracted from samples, which were infested and uninfested cabbage and broccoli plants with *M. persicae*, respectively. For extracting VOCs from samples, headspace technique analyses were used with three replicates in all experiments, for profiling and characterisation of VOCs from both plants. The identification of VOCs was conducted with the SPME fibre by extracting the compound from the headspace of treatments. Three phase fibres 50/30 μm divinylbenzene/carboxen/polydimethyl siloxane (PDMS/CAR/DVB; Sigma-Aldrich, Australia, catalogue number 57347-U) coating was selected for volatiles released from infested and uninfested plants. The SPME fibre is commonly used and this three phase fibre was selected because it was being used for the analysis of a wide range of analytes (Piotrowicz 2016). The fibres were first conditioned at recommended range of operating temperature by the manufacturer, before analyses were conducted. For optimising various conditions, the sealing time was optimised to 2.30 hours under laboratory temperature 25±1°C, and the SPME fibre was exposed to the headspace of the samples by inserting the SPME into the jar through the septum for two hours to extract the VOCs, which characterised the optimum extraction time. The desorption time of SPME fibre was 5 min in the GC injection port. The SPME was used because it is a fast, simple and modern tool for GC-MS analysis.
3.3.3.3 Samples analysis with GC-MS

The analysis of VOCs obtained by HS-SPME was performed on a gas chromatography mass spectrometer (GC Agilent GCMS 7820A) equipped with MS detector 5977E (Agilent Technologies, USA) and a DB-35ms column (30 m x 250 μm x 0.25 μm) (Santa Clara, CA 95051, USA). The fibre was desorbed in the splitless injector 270°C of GCMS with other operation conditions. The initial temperature of the column was 50°C and held for 2 min, then increased to 250°C at 5°C min⁻¹ and held for 5 min at 250°C. Helium gas (He) was used as a carrier and supplied by (BOC Gas, Sydney, Australia) and the flow rate of the column was 1:1 mL min⁻¹, while the splitless was 20 mL min⁻¹ at 1.5 min and the total GC-MS run time was 45 min. The calibration of the SPME fibre was done by injecting the n-alkanes standard C7–C30.

HS-SPME/GC-MS analysis of the VOCs were identified by using AMDIS software version 2.72 and the US National Institute of Standards and Technology (NIST) 2014 MS database. The VOCs were confirmed by comparing GC retention time data with those of authentic standards or from the published literature (Truong et al. 2014).

3.3.4 Evaluation of olfactory responses of M. persicae and its parasitoids

A glass Y-tube olfactometer was used to determine the responses of M. persicae and its two species parasitoids, A. colemani and A. abdominalis, to each of the following pairs of plant treatments. For the aphid responses, the test was (1) infested (cabbage or broccoli plant infested with M. persicae) versus clean (filter) air; (2) non-infested versus clean air; and (3) infested versus non-infested plants (see Figure 3.1). For the test of parasitoid wasps, A. colemani and A. abdominalis, (1) infested plant versus clean air; (2) non-infested plant versus clean air; and (3) infested versus non-infested plants. Bioassays to compare their olfactory responses to VOCs released from healthy plants versus clean air or infested plants with M. persicae versus healthy plants.

Volatile preference experiments were made using a glass Y-tube olfactometer as previously described (Saad et al. 2015), with a 7 cm arm length and 2 cm internal diameter, ground glass fitting for the air that passed 200 mL min⁻¹ through each arm, controlled by air flow meter (SCFH AIR, Dwyer Instruments, Michigan City, IN 46360, USA) (see Figure 3.1). Each arm tube was
connected to a glass chamber (2 L desiccator). Couples of blend VOCs (released from different plant treatments) were presented in a sealed glass chamber (2 L each) at the end of either arm. The compressed air was filtered by using activated charcoal passed through two glass chambers, before the treatment plant could be introduced, and then the air passed through the olfactometer. After assembly, the olfactometer was left to stabilise for 15 min prior to use (Pope et al. 2012).

The Y-tube olfactometer work was carried out under the same conditions as the glasshouse conditions. The area surrounding the olfactometer (below and around) was covered by white paper and white light was placed directly over the olfactometer. For the bioassay, a single aphid or single parasitoid was introduced into the main arm of olfactometer and pushed 1–2 cm inside the main arm. Each aphid or wasp was given up to 3 min in the olfactometer to respond. Once an individual moved beyond 2 cm and into one of the Y-tube arms, it was considered to have made a choice for the conforming plant treatment in that arm. Non-responders that did not make a choice in 5 min were discarded and excluded from the statistical analysis (non-responsive parasitoids counted in statistical analysis in the experiment of comparison of clean air with the healthy plant).

Three replicates and 30 adults of wingless aphid *M. persicae* were assayed for each replicate, and each aphid was tested only one time. Every 10 aphids were assayed, the volatile treatment resources were removed, and all glass vessels cleaned with ethanol, then washed with water and oven dried at 100°C for a minimum of 30 min. For the comparison, three replicates were carried out on different days using new aphids and fresh infested and non-infested plants. All plant resources were the same age and same size.

The same procedure above was carried out for the parasitoid *A. colemani* and *A. abdominalis*. Also, three replicates were used for the parasitoids with 15 wasps for each replicate and wasps were used only once. Throughout experiments, after all 15 wasps were assayed for each replicate, the apparatus was cleaned with water and ethanol, then dried and heated in the oven at 100°C for more than 30 min. Statistical significance between wasp responses to pairwise combinations of plant treatments was determined using Chi-Square tests at the 5% level.
3.3.5 Statistical analysis

To identify the differences in the emission of volatile compounds between healthy and infested cabbage and broccoli by aphids, all peak area analyses were performed with MetaboAnalyst software for $P$-value, principal component analysis (PCA and PLS-DA) and the hierarchical clustering heatmaps (Chong et al. 2018). Differences in the results were compared by using the least significant difference test ($P \leq 0.05$) for determining the means between infested and healthy plants. The peak area was divided by 100,000 for every single compound that obtained from GC-MS. The data of the Y-tube olfactometer bioassays were analysed for preference (aphid *Myzus persicae* and their parasitoids *Aphidus colemani* and *Aphelinus abdominalis* choice between two treatments tested) using the Chi-Square goodness of fit test by using SPSS software version 24.0.
3.4 Results

3.4.1 Limit of detection

The detection of n-alkane C7–C30 was done using SPME fibre. The GC-MS analysis of SPME trapped C7–C30 standard showed that octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, eicosane, heneicosane, tricosane and pentacosane can be detected at ppm level. Some alkane standard compounds were not detected at the level of ppb (nonane, decane, dodecane and tricosane), could be limitation of GC instrument in detecting them at ppb level; while octane, undecane, tridecane, hexadecane, heptadecane, eicosane and heneicosane can be detected at ppt level (see Table 3.1). Other alkane standard compounds were not detected because they were less than 0.005 at the level of ppt.

Table 3.1. Limit of detection (LOD) of alkanes standard C7–C30 by using three-phase SPME 50/30μm (PDMS/CAR/DVB) fibre.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Formula</th>
<th>RT</th>
<th>RI</th>
<th>ppm</th>
<th>ppb</th>
<th>ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octane</td>
<td>C8H18</td>
<td>4.36</td>
<td>816</td>
<td>56.91</td>
<td>0.54</td>
<td>0.03</td>
</tr>
<tr>
<td>Nonane</td>
<td>C9H20</td>
<td>7.55</td>
<td>916</td>
<td>10.92</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Decane</td>
<td>C10H22</td>
<td>11.12</td>
<td>1015</td>
<td>43.06</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Undecane</td>
<td>C11H24</td>
<td>14.29</td>
<td>1115</td>
<td>63.73</td>
<td>6.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Dodecane</td>
<td>C12H26</td>
<td>17.61</td>
<td>1214</td>
<td>39.45</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Undecane</td>
<td>C11H24</td>
<td>14.29</td>
<td>1115</td>
<td>63.73</td>
<td>6.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Tridecane</td>
<td>C13H28</td>
<td>20.47</td>
<td>1313</td>
<td>31.36</td>
<td>6.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Tetradecane</td>
<td>C14H30</td>
<td>23.15</td>
<td>1413</td>
<td>10.06</td>
<td>0.61</td>
<td>n.d</td>
</tr>
<tr>
<td>Pentadecane</td>
<td>C15H32</td>
<td>25.66</td>
<td>1548</td>
<td>24.08</td>
<td>1.30</td>
<td>n.d</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>C16H34</td>
<td>28.03</td>
<td>1602</td>
<td>0.68</td>
<td>0.35</td>
<td>0.02</td>
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<tr>
<td>Heptadecane</td>
<td>C17H36</td>
<td>30.27</td>
<td>1626</td>
<td>11.26</td>
<td>9.06</td>
<td>1.11</td>
</tr>
<tr>
<td>Eicosane</td>
<td>C20H42</td>
<td>36.19</td>
<td>2009</td>
<td>8.10</td>
<td>1.72</td>
<td>0.04</td>
</tr>
<tr>
<td>Heneicosane</td>
<td>C21H44</td>
<td>38.04</td>
<td>2109</td>
<td>24.39</td>
<td>2.18</td>
<td>0.20</td>
</tr>
<tr>
<td>Tricosane</td>
<td>C23H48</td>
<td>41.52</td>
<td>2416</td>
<td>28.03</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Pentacosane</td>
<td>C24H50</td>
<td>44.73</td>
<td>2699</td>
<td>12.41</td>
<td>2.64</td>
<td>n.d</td>
</tr>
</tbody>
</table>

a = RT referred to the retention time; b = RI referred to the retention index; c = ppm parts per million; d = ppb parts per billion; e = ppt parts per trillion; f = < 0.005 ppb; g = < 0.005 ppt.
3.4.2 VOCs released from *M. persicae* infested and uninfested plant

Analysis of the volatiles of cabbage and broccoli induced by *M. persicae* for the infested and uninfested plant treatments showed significant differences. Several compounds were present in all samples that were trapped by SPME and identified by GC-MS. Plants damaged by *M. persicae* can change in plant odour emission, and the volatiles of samples were significantly higher than uninfested plants. The volume and the variety of VOCs released from infested cabbage were greater than the uninfested plant, and the qualitative differences in the composition of the odour from these treated plants consisted of propane, 2-methoxy that released from both infested and uninfested cabbage. The average peak area in the uninfested plant was 23.10 compared with the peak area in the infested plant of 7.84, while alpha- and beta pinene were much higher in the infested than uninfested plants (see Table 3.2). There was a significantly larger quantity of (E)-3-hexen-1-ol, beta-pinene and decane released from the infested plant. Moreover, the peak area for the following volatile compounds which were detected from infested cabbage; Myrcene, 1-Hexanone, 5-methyl-1-phenyl-, Limonene, Decane, gamma-Terpinen and Heptane, 2,4,4-trimethyl were higher in the infested cabbage compared with their peak area in the uninfested cabbage. However, some of the volatile compounds from uninfested cabbage were released in a high amount based on peak area detected by GC-MS as compared with the infested plant. These compounds were Eucalyptol, Cyclohexasiloxane, 3,4-Dihydroxyphenylglycol, 1,5-Pentanediame, octamethyl and decamethyl. VOCs are leading to odour differences between aphid infested plants and uninfested plants. Figure 3.2 shows the heat map that graphically displays results by hierarchical clustering of the volatile compounds from the infested and uninfested cabbage. This work was conducted to find the closeness of individual compounds in both samples. Distances between samples and assays were calculated for hierarchical clustering based on Pearson’s Correlation Distance. Each volatile compound has a peak area from GC-MS, presented by the colour scale that illustrates the differences between the infested and uninfested cabbage. The value of compound represented by red, orange and dark blue for the maximum (2), average (0) and minimum (–2) (see Figure 3.2). In addition, principal component analysis (PCA) was performed to show the separation of the two samples using the significantly different (*P*<0.05), and the relationship between the VOCs within infested and uninfested is profiled in Figure 3.3).
Table 3.2. Volatile compounds detected in the headspace of infested and uninfested cabbage with *M. persicae* by using solid phase microextraction (SPME)

<table>
<thead>
<tr>
<th>No</th>
<th>Compound name</th>
<th>RTa</th>
<th>Uninfested plant Area ±SDb</th>
<th>Infested plant Area ±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Propane, 2-methoxy</td>
<td>3.12</td>
<td>23.10±3.13</td>
<td>7.84±2.70</td>
<td>0.020*</td>
</tr>
<tr>
<td>2</td>
<td>n-Hexane</td>
<td>3.28</td>
<td>15.38±4.21</td>
<td>8.40±3.83</td>
<td>0.199</td>
</tr>
<tr>
<td>3</td>
<td>Benzene</td>
<td>3.61</td>
<td>72.20±1.55</td>
<td>601.75±28.09</td>
<td>0.305</td>
</tr>
<tr>
<td>4</td>
<td>3-Hexen-1-ol, (E)</td>
<td>6.38</td>
<td>NDc</td>
<td>28.83±1.51</td>
<td>0.223</td>
</tr>
<tr>
<td>5</td>
<td>4,6-Heptadiyn-3-one</td>
<td>9.33</td>
<td>90.28±2.26</td>
<td>601.75±28.09</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Toluene</td>
<td>11.02</td>
<td>12.50±3.48</td>
<td>1.65±0.31</td>
<td>0.653</td>
</tr>
<tr>
<td>7</td>
<td>Oxime-, methoxy-phenyl</td>
<td>12.43</td>
<td>757.69±322.83</td>
<td>680.68±300.96</td>
<td>0.200</td>
</tr>
<tr>
<td>8</td>
<td>2-Pentenal, (E)-</td>
<td>12.49</td>
<td>16.86±0.82</td>
<td>23.36±0.76</td>
<td>0.136</td>
</tr>
<tr>
<td>9</td>
<td>Alpha-Pinene</td>
<td>13.32</td>
<td>24.44±4.96</td>
<td>131.41±16.53</td>
<td>0.003*</td>
</tr>
<tr>
<td>10</td>
<td>Sabinene</td>
<td>13.47</td>
<td>72.54±34.72</td>
<td>55.75±17.03</td>
<td>0.930</td>
</tr>
<tr>
<td>11</td>
<td>Myrcene</td>
<td>15.22</td>
<td>20.15±7.96</td>
<td>68.45±30.99</td>
<td>0.046*</td>
</tr>
<tr>
<td>12</td>
<td>beta-Pinene</td>
<td>16.25</td>
<td>ND</td>
<td>55.75±17.03</td>
<td>0.930</td>
</tr>
<tr>
<td>13</td>
<td>1-Hexanone, 5-methyl-1-phenyl</td>
<td>16.81</td>
<td>21.05±3.78</td>
<td>35.38±7.44</td>
<td>0.004*</td>
</tr>
<tr>
<td>14</td>
<td>p-Cymene</td>
<td>17.28</td>
<td>422.85±144.03</td>
<td>564.67±82.08</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>3-Hexen-1-ol, acetate, (Z)</td>
<td>17.48</td>
<td>394.93±152.39</td>
<td>245.99±62.11</td>
<td>0.277</td>
</tr>
<tr>
<td>16</td>
<td>Eucalyptol</td>
<td>19.97</td>
<td>129.50±5.22</td>
<td>96.14±34.98</td>
<td>0.036*</td>
</tr>
<tr>
<td>17</td>
<td>Limonene</td>
<td>20.38</td>
<td>14.66±1.92</td>
<td>247.26±84.09</td>
<td>0.003*</td>
</tr>
<tr>
<td>18</td>
<td>Decane</td>
<td>23.57</td>
<td>ND</td>
<td>39.31±5.50</td>
<td>0.020*</td>
</tr>
<tr>
<td>19</td>
<td>gamma-Terpinen</td>
<td>24.81</td>
<td>9.03±1.70</td>
<td>56.55±3.68</td>
<td>0.007*</td>
</tr>
<tr>
<td>20</td>
<td>Heptane, 2,4,4-trimethyl</td>
<td>26.24</td>
<td>3.75±1.44</td>
<td>91.50±45.46</td>
<td>0.001*</td>
</tr>
<tr>
<td>21</td>
<td>Cyclopentasiloxane, decamethyl</td>
<td>27.84</td>
<td>1.95±0.23</td>
<td>314.91±12.00</td>
<td>0.212</td>
</tr>
<tr>
<td>22</td>
<td>1-Undecyne</td>
<td>30.22</td>
<td>2.68±0.52</td>
<td>110.55±13.59</td>
<td>0.036*</td>
</tr>
<tr>
<td>23</td>
<td>Heptane, 2,5,5-trimethyl</td>
<td>30.82</td>
<td>2.17±0.43</td>
<td>33.82±4.85</td>
<td>0.630</td>
</tr>
<tr>
<td>24</td>
<td>Cyclohexasiloxane</td>
<td>34.24</td>
<td>123.62±53.60</td>
<td>1.16±0.17</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>3,4-Dihydroxyphenylglycol</td>
<td>37.29</td>
<td>20.15±7.96</td>
<td>1.72±0.41</td>
<td>0.286</td>
</tr>
<tr>
<td>26</td>
<td>1,5-Pentanediol</td>
<td>40.10</td>
<td>249.45±12.70</td>
<td>10.33±0.64</td>
<td>0.127</td>
</tr>
<tr>
<td>27</td>
<td>Octamethyl</td>
<td>42.66</td>
<td>565.00±22.07</td>
<td>7.89±2.42</td>
<td>0.129</td>
</tr>
<tr>
<td>28</td>
<td>Decamethyl</td>
<td>41.43</td>
<td>113.05±55.42</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

a = RT indicated to the retention time of compounds; b = SD referred to the standard deviation of peak area; c = ND referred to not detected; *indicated to the significant different 5%.
Figure 3.2. Clustering result is shown as a heat map of volatile compounds released from uninfested and infested cabbage with green peach aphid *M. persicae*. Each volatile compound’s peak area detected by GC-MS is shown by colours.
Figure 3.3. Principal component analysis (PCA) scatter plots of the volatile compounds detected in uninfested and infested cabbage with *M. persicae*.

Results from the volatile profiling are taken from the headspace of infested or uninfested broccoli *B. oleracea italica* by SPME. Table 3.3 shows the differences between uninfested and broccoli infested with *M. persicae*; with 25 VOCs identified in both plant samples by HS-SPME and GC-MS. VOC emission from all samples and the identity of volatiles confirm that infestation of broccoli with aphids significantly increased the peak area of 10 volatile compounds which were released in high amount, such as D-limonene, Undecane, 3,4-dimethyl, Heptane, alpha-Pinene, Oxalic acid, Citronellol, Tridecane, n-Decanoic acid, Cyclopentane, pentyl- and n-Hexadecanoic acid. The peak area was 7.20, 9.74, 26.38, 42.08, 6.13, 24.59, 8.59, 5.24, 5.28 and 65.76 respectively, compared with the volatile compounds that were not released from uninfested broccoli. These compounds were released highly after the infestation of the plant with *M. persicae*. 
The response of infested broccoli can be reduction in the peak area odour released by plants for some volatiles; 2-Propenamide, Naphthalene, 2-methyl, Benzyl Benzoate, n-Butyric acid, 2-ethylhexyl ester, Ethyl 2,2-diethoxypropionate and Nonyl alcohol. Figure 3.6 shows that the infested and uninfested treatments were well separated, and there was no overlap in the volatile compounds produced between the two treatments of broccoli from the aphid infestation based on spare PLS-DA (see Figure 3.4). The heat map of infested and uninfested broccoli plants determines the differences between two samples by colour and hierarchical clustering performed on both infested and uninfested plants (see Figure 3.5).
Table 3.3. Volatile compounds detected in the headspace for uninfested and broccoli infested with *M. persicae* by using solid phase microextraction (SPME)

<table>
<thead>
<tr>
<th>No</th>
<th>Compound name</th>
<th>RT</th>
<th>Uninfested broccoli Area±SD</th>
<th>Infested broccoli Area±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Camphor</td>
<td>5.13</td>
<td>5.31±3.83</td>
<td>16.90±3.00</td>
<td>0.099</td>
</tr>
<tr>
<td>2</td>
<td>Decanal</td>
<td>5.30</td>
<td>9.08±0.57</td>
<td>34.17±16.97</td>
<td>0.581</td>
</tr>
<tr>
<td>3</td>
<td>Octanedioic acid</td>
<td>6.64</td>
<td>4.60±4.06</td>
<td>6.52±2.84</td>
<td>0.232</td>
</tr>
<tr>
<td>4</td>
<td>D-limonene</td>
<td>9.75</td>
<td>ND c</td>
<td>7.20±1.74</td>
<td>0.015*</td>
</tr>
<tr>
<td>5</td>
<td>Undecane, 3,4-dimethyl-</td>
<td>10.15</td>
<td>ND</td>
<td>9.74±0.18</td>
<td>0.521</td>
</tr>
<tr>
<td>6</td>
<td>Heptane</td>
<td>10.24</td>
<td>ND</td>
<td>26.38±13.24</td>
<td>0.013*</td>
</tr>
<tr>
<td>7</td>
<td>alpha-Pinene</td>
<td>13.05</td>
<td>ND</td>
<td>42.08±26.92</td>
<td>0.012*</td>
</tr>
<tr>
<td>8</td>
<td>n-Butyric acid 2-ethylhexyl ester</td>
<td>14.31</td>
<td>14.00±2.15</td>
<td>18.05±0.78</td>
<td>0.017*</td>
</tr>
<tr>
<td>9</td>
<td>1,14-Tetradecanediol</td>
<td>16.59</td>
<td>1.74±0.17</td>
<td>8.69±0.01</td>
<td>0.076</td>
</tr>
<tr>
<td>10</td>
<td>Oxalic acid, 2-isopropylphenyl octyl ester</td>
<td>18.38</td>
<td>ND</td>
<td>6.13±1.09</td>
<td>0.010*</td>
</tr>
<tr>
<td>11</td>
<td>Citronellol</td>
<td>20.27</td>
<td>ND</td>
<td>24.59±6.41</td>
<td>0.007*</td>
</tr>
<tr>
<td>12</td>
<td>Nonyl alcohol</td>
<td>21.35</td>
<td>71.02±4.96</td>
<td>6.14±4.45</td>
<td>0.013*</td>
</tr>
<tr>
<td>13</td>
<td>Cyclandelate</td>
<td>23.89</td>
<td>14.05±1.77</td>
<td>5.07±3.70</td>
<td>0.018*</td>
</tr>
<tr>
<td>14</td>
<td>3-Oxobutan-2-yl 2-methylbutanoate</td>
<td>24.24</td>
<td>3.29±1.83</td>
<td>4.27±0.36</td>
<td>0.645</td>
</tr>
<tr>
<td>15</td>
<td>Tridecane</td>
<td>24.53</td>
<td>ND</td>
<td>8.59±0.87</td>
<td>0.706</td>
</tr>
<tr>
<td>16</td>
<td>Hexadecanedioic acid</td>
<td>27.97</td>
<td>7.06±0.40</td>
<td>8.50±1.30</td>
<td>0.427</td>
</tr>
<tr>
<td>17</td>
<td>Benzene, 1-butylheptyl-</td>
<td>33.44</td>
<td>10.94±0.67</td>
<td>4.49±1.53</td>
<td>0.011*</td>
</tr>
<tr>
<td>18</td>
<td>n-Decanoic acid</td>
<td>33.78</td>
<td>ND</td>
<td>5.24±2.25</td>
<td>0.050*</td>
</tr>
<tr>
<td>19</td>
<td>Cyclopentane, pentyl</td>
<td>36.01</td>
<td>ND</td>
<td>5.28±0.62</td>
<td>0.007*</td>
</tr>
<tr>
<td>20</td>
<td>n-Hexadecanoic acid</td>
<td>37.35</td>
<td>ND</td>
<td>65.76±21.14</td>
<td>0.022*</td>
</tr>
<tr>
<td>21</td>
<td>Ethyl 2,2-diethoxypropionate</td>
<td>38.24</td>
<td>16.62±0.65</td>
<td>ND</td>
<td>0.005*</td>
</tr>
<tr>
<td>22</td>
<td>n-Butyric acid, 2-ethylhexyl ester</td>
<td>39.40</td>
<td>20.53±3.44</td>
<td>ND</td>
<td>0.019*</td>
</tr>
<tr>
<td>23</td>
<td>Benzyl Benzoate</td>
<td>39.47</td>
<td>1.99±0.81</td>
<td>ND</td>
<td>0.182*</td>
</tr>
<tr>
<td>24</td>
<td>Naphthalene, 2-methyl</td>
<td>39.95</td>
<td>3.21±0.04</td>
<td>ND</td>
<td>0.007*</td>
</tr>
<tr>
<td>25</td>
<td>2-Propenamide</td>
<td>40.42</td>
<td>3.13±0.17</td>
<td>ND</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

*a* = RT indicated to the retention time of compounds; *b* = SD referred to the standard deviation of peak area; *c* = ND referred to not detected; *indicated to the significant different 5%.
Figure 3.4. Separation scores plot between principal component PCs of the volatile compounds detected in uninfested and broccoli infested with *M. persicae*.

### 3.4.3 Effect of VOCs on attractive parasitoid

Results of the laboratory experiments using Y-tube olfactometer bioassays showed the response of the aphids *M. persicae* and their parasitoids *A. colemani* and *A. abdominalis* to the uninfested and infested plants (cabbage or broccoli) by 30 individual aphids and 15 individuals per replicate of parasitoid (See Figure 3.6 and Figure 3.7).

These results indicated that green peach aphids in both cabbage and broccoli were significantly more attracted to the VOCs released from infested plant rather than clean air. The percentage of
aphids attracted to the infested plant was 80% versus 7% clean air for the cabbage plants, and 70% versus 10% for the broccoli (Chi-Square ($\chi^2$) = 18.61, df= 1 and $P<0.0005$ for the cabbage and $\chi^2$ = 14.44, df=1 and $P<0.0005$ for the broccoli) (see Figure 3.6). Results showed that $M. persicae$ were significant different in the preference for both cabbage and broccoli, with more attracted to the healthy plants than clean air. The percentage of attracted aphids was 75.56% versus 3% for the cabbage and 84% versus 7% for the broccoli ($\chi^2$= 20.16, df= 1 and $P<0.0005$ for the cabbage and $\chi^2$ = 19.59, df=1 and $P=0.0005$ for the broccoli). While the results indicated that the aphids were significantly more attracted to the infested cabbage compared with the healthy plant, however, the results showed broccoli volatiles have no significant effect. The percentage of aphid numbers attracted towards infested plants was 63% and 57% for the cabbage and broccoli plants respectively, versus attracted 26.67% and 30% to healthy plant for cabbage and broccoli plants respectively ($\chi^2 = 4.48$, df= 1 and $P<0.034$ for the cabbage and $\chi^2 = 2.46$, df=1, and $P=0.117$ for the broccoli).

For the parasitoids experiments, the attraction of parasitoids $A. colemani$ and $A. abdominalis$ to volatiles released by plants where they were given a choice between healthy and infested plants. Both $A. colemani$ and $A. abdominalis$ were significantly more attracted to volatiles from plants infested with green peach aphids compared with clean (see Figure 3.7). The frequency of parasitoid attraction was 93.33% and 100% towards the infested cabbage plant versus 7% and 20% towards the clean air for both parasitoids $A. colemani$ and $A. abdominalis$ ($\chi^2 = 11.26$, df= 1 and $P=0.001$ for $A. colemani$ and $\chi^2 = 4.57$, df=1 and $P=0.033$ for $A. abdominalis$). The statistical analysis showed that both parasitoids were significantly attracted to the infested plant. However, there was no differences between attracted wasps for the odours released from a healthy plant and clean air, there were no responses for both parasitoids $A. colemani$ and $A. abdominalis$ to the health plant odour versus clean air (both parasitoids showed significantly no responses to the treatments). On percentage, 4.44% of $A. colemani$ wasp and 7% of $A. abdominalis$ were attracted to volatiles released from uninfested plants, versus 7% for both parasitoids headed for clean air treatment, while the percentage of no responses of parasitoids was 88.86% and 86.66% for both $A. colemani$ and $A. abdominalis$ respectively ($\chi^2 = 19.20$, df= 2 and $P=0.001$ for $A. colemani$ and $\chi^2 = 19.20$, df=2 and $P=0.001$ for $A. abdominalis$). When given a choice between uninfested and infested cabbage plants, $A. colemani$ and $A. abdominalis$ parasitoids were significantly more attracted to
volatiles released from infested plant rather than attracted towards healthy cabbage plants. On percentage, 86.67% of the *A. colemani* and 100% of the *A. abdominalis* responded towards infested cabbage versus 9% of the *A. colemani* and 0% of the *A. abdominalis* attracted to healthy plants ($\chi^2 = 10.28$, df= 1 and $P=0.001$ for *A. colemani* and $\chi^2 = 12.25$, df=1 and $P=0.0005$ for *A. abdominalis*).

![Figure 3.5. The heat map clustering of the VOCs released from uninfested and infested broccoli with green peach aphid *M. persicae.*](image)
Figure 3.6. Olfactory response of green peach aphid *M. persicae* in Y-tube olfactometer experiments to volatiles released from infested and uninfested cabbage and broccoli (a) uninfested versus infested plants (b) uninfested versus clean air (c) infested versus clean air. All treatments presented with standard deviation (SD) bar. Asterisks (*) indicates significant difference $P<0.05$ (Chi-square test).
Figure 3.7. Olfactory response of two parasitoids *Aphidius colemani* and *Aphelinus abdominalis* in Y-tube olfactometer experiments to volatiles released from infested and uninfested cabbage *B. oleracea* (a) uninfested versus infested plants (b) uninfested versus clean air (c) infested versus clean air. All treatments presented with standard deviation (SD) bar. * indicates significant difference P<0.05 (Chi-square test).
3.5 Discussion

The VOCs that released from infested two species of *Brassica* plants by *M. persicae* showed many compounds comparing with uninfested plants and reported by previous studies (Bruinsma et al. 2009; Mathur et al. 2013; Taveira et al. 2009). In the current study, volatile compound profiles of uninfested and infested cabbage and broccoli plants with *M. persicae* were compared to show the differences between treated plants and used as identification tools for the infestation. Mathur et al. (2013) reported that a comparison of volatile compounds identified from uninfested and aphid infested plants from several *Brassica* plants. The damage of cruciferous plants caused by aphids can emit many volatile compounds such as glucosinolate metabolites, phenolics and terpenoids (de Vos & Jander 2010; Hien et al. 2014). However, our results showed the *M. persicae* preferred damaged *Brassica* plants because the infested plant released different VOCs, such as alpha- and beta pinene, (E)-3-hexen-1-ol, Myrcene, 1-Hexanone, 5-methyl-1-phenyl, Limonene, Decane, gamma-Terpinen and Heptane, 2,4,4-trimethyl. This finding is consistent with Najar et al. (2015) who reported that alpha- and beta pinene and Limonene could increase in *Brassica* plants infested by aphids. Some VOCs had disappeared from uninfested plants, such as 3-Hexen-1-ol-(E) and beta-Pinene because of some VOCs has been quickly disappeared (Najar et al. 2015; Winde & Wittstock 2011). The increase of (E)-3-hexen-1-ol, beta-pinene and decane in infested plants could be expected because these compounds are well known as green leaf volatiles and are involved in the attraction of natural enemies such as parasitoids and predators (Leroy et al. 2012; Li et al. 2017; Sasso et al. 2007; Wei et al. 2011). The VOCs can be released by an intact and healthy *Brassicaceae*ous plant in large amounts (Mumm et al. 2008); however, the VOCs of Limonene, Undecane, 3,4-dimethyl-, Heptane, alpha-Pinene, Oxalic acid, Citronellol, Tridecane, n-Decanoic acid, Cyclopentane, pentyl- and n-Hexadecanoic acid were detected from infested broccoli. These compound were found in the headspace of infested broccoli and can be involved in attracting beneficial insects as a response to the aphid infestation (Leroy et al. 2012; Li et al. 2017; Pinto-Zevallos et al. 2018). Thus, the selection of SPME in the extraction of volatile compounds from uninfested and infested *Brassica* plants (cabbage and broccoli) with *M. persicae* was based on the peak areas of all compounds identified in the treatments.

The results of Y-tube olfactometer bioassays confirmed the results of aphids *M. persicae* and the parasitoids *A. colemani* and *A. abdominalis* were influenced and attracted to volatiles produced by
Cruciferous plants. These wasps significantly preferred, and were attracted to, volatiles from aphid infested plants over uninfested plants. The use of Y-tube olfactometer to test the response of aphid *M. persicae* to the host plant, *B. oleracea* var. *capitata* and *B. oleracea* var. *italica*, indicated that *M. persicae* was influenced by the volatiles released from *B. oleracea* var. *capitata* and *B. oleracea* var. *italica* and were significantly attracted to both healthy and infested plants when compared with clean air choice.

Aphids can find their host visually and chemically such as by chemical, colour, size and the shape of the host, and this may be a useful guide to attracting aphids. This result confirms past studies (Döring 2014; Hatano et al. 2008; Hori 1999) that show aphids find their host plants by plant odour as well as visual cues. Moreover, the attraction of aphids to the plant volatiles using olfactometer has been reported in experiments testing plant odour against aphids and their host-finding ability (Döring 2014; Hori 1999; Verheggen et al. 2013). Our results showed that aphids tended to be attracted to both damaged and undamaged plants. Our observation that plant compounds can explain the variance in attraction by aphids and also plant volatile compounds can increase in response to feeding (Hopkins et al. 2017; Nottingham et al. 1991; Will & van Bel 2006; Züst & Agrawal 2016).

Results from the olfactometer studies demonstrated that parasitoids respond to the plant volatiles and that *A. colemani* and *A. abdominalis* respond to the odour released from infested plants. Both tested parasitoids are responsive to plant volatiles significantly when compared with a clean air treatment. This finding is consistent with Kalule and Wright (2004). The preference of *A. colemani* and *A. abdominalis* showed no response of parasitoid attraction to clean air and uninfested cabbage; while, the parasitoids noted statistically a significant non-response. Van Emden et al. (2008) explain that the attraction of parasitoids can be significantly higher to the infested plant and attack aphids feeding on the same plant as the origin of the mummy offered. The parasitoids *A. colemani* and *A. abdominalis* showed their responses to the infested *B. oleracea*, preferring aphid-induced volatiles. Both parasitoids have significant responses to infested plants with aphids. The results are consistent with da Silva et al. (2016) who showed parasitoid *A. colemani* could be attracted to volatiles released from *Brassica juncea* and preferred plants damaged by green peach aphids rather than plants damaged by *M. persicae* and *Plutella xylostella* caterpillars.
3.6 Conclusion

The HS-SPME with GC-MS analysis for the volatiles described the differences between the infested and uninfested Cruciferous tested crops and their role in attracting natural enemies of aphids. Collection of volatiles from Cruciferous crops (cabbage and broccoli) occurred by using HS-SPME to detect volatiles compounds between uninfested and plants infested with *M. persicae* and examined the attraction of natural enemies. A total of 29 VOCs were identified in cabbage plant treatments and 25 VOCs were identified in broccoli plant treatments, by using HS-SPME combined with GC-MS. The parasitoids *Aphidus colemani* and *Aphelinus abdominalis* are laid eggs within the body of *M. persicae* and immature stages complete development inside the hosts, eventually killing them by feeding the wasp larva on inside aphids, the parasitoid pupates inside the aphid mummy and they emerges as an adult. To detect and locate hosts it is believed that *A. colemani* and *A. abdominalis*, such as many parasitoids, rely on odours released from infested plants as a response to aphids feeding. The results indicated that the preferences of *A. colemani* and *A. abdominalis* to infested plants with *M. persicae* compared with uninfested plants and clean air by using an olfactometer. The results showed that parasitoids can discriminate the infested cabbage and broccoli and significantly respond to the plant odour. Thus, we believe that aphid parasitoids can find damaged plants and then detect aphids on the plant by plant odour. It is likely that the natural enemies’ search for aphid infestation may start before landing on the uninfested plant, because parasitoids will first find a damaged plant and then begin searching for aphids. For this reason, many aphid parasitoids efficiently search for damaged plants where aphids will be present as explained by Hatano et al. (2008).
Chapter 4: Evaluation of Aphicidal Effect of Essential Oils and their Synergistic Effect against *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in the Laboratory and the Glasshouse on Sweet Pepper *Capsicum annuum* and Cabbage *Brassica oleracea*

4.1. Abstract

The insecticidal activities of essential oils obtained from black pepper, eucalyptus, rosemary and tea tree and their binary combination were investigated against the green peach aphid *Myzus persicae* (Aphididae: Hemiptera), under laboratory and glasshouse conditions. All the tested essential oils significantly reduced and controlled the green peach aphid population and caused high mortality. In the first study, black pepper and tea tree pure essential oils were found to be effective insecticide with 80% mortality when used through contact application. However, the residual toxins were found to be more effective on aphids, with 100% mortality for black pepper and tea tree oils and less than 96% for eucalyptus and rosemary oils. All combinations of essential oils were tested as contact and residual toxins. From the contact treatment, the mortality was 98.33% for black pepper + tea tree and rosemary + tea tree. While, in the residual treatment, the mortality was 100% for black pepper + eucalyptus, rosemary + eucalyptus, and rosemary + tea tree. The essential oil combinations exhibited synergistic, additive and antagonistic interaction for insecticidal activities, particularly among a combination of binary essential oils that were tested. The combination of black pepper + tea tree showed enhanced activity, with a synergy rate of 2.19. While essential oil formulations were effective on the mortality of aphids, there was some phytotoxicity on cabbage plants. The Fourier Transform Infrared Spectroscopy (FTIR) stability analysis of a mixture of essential oils showed it was not affected by store temperature (15, 25 and 35°C) and all functional groups were not changed during storage. Based on our results, the essential oils which are black pepper, eucalyptus, rosemary and tea tree and their binary combination can be used as a commercial insecticide against *M. persicae*. 
4.2. Introduction

Green peach aphid sometimes is known as peach-potato aphid *Myzus persicae* (Sulzer) belongs to the family Aphididae in the order Hemiptera. This species is considered a polyphagous species that can be found globally distributed worldwide. The green peach has been reported to feed on more than 500 species of plants from 40 families and is considered a major pest of many vegetable crops such as cucurbits, legumes, crucifers like cabbage, cauliflower, and broccoli, and solanaceous crops like potato, tomato and capsicum (Blackman & Eastop 2000; Ben-Issa et al. 2017; Kuhar et al. 2009; Omkar & Surabhi 2013). The damage caused by green peach aphids is from their feeding on plant sap that causes yellowing and leaf curling of the plant. In addition, *M. persicae* has been involved in transmitting over 180 plant viruses. Hence, green peach aphids, with a large host range exceeding 40 families of plants, is considered one of the most polyphagous aphids (Blackman & Eastop 2000; Favret & Miller 2012).

Plant-based biopesticides have been reviewed and reported for several species of plants possessing insecticidal activities belonging to 60 families and showing promise as new botanical pesticides (Dev 2017; Isman 2006; Lydon & Duke 1989; MacKinnon et al. 1997). Most of the botanical pesticides have low to reasonable environmental toxicity, but there are exceptions such as nicotine, and essential oils tend to degrade more rapidly in the environment than synthetic chemicals (Buss & Park-Brown 2002; Moretti et al. 2002). Many essential oils can cause high mortality of pests and have been shown to be effective when used in different applications such as fumigation as well as having antifeedant and repellent properties (Regnault-Roger 1997). Moreover, some essential oils showed aphicidal activity, such as cumin *Cuminum cyminum*, anise *Pimpinella anisum*, oregano *Origanum vulgare* and eucalyptus essential oils (Tunc & Şahinkaya 1998).

Black pepper *Piper nigrum* L. extracts showed insecticidal activities because it contained isobutyl amides that are responsible for the toxicity of insects (Bernard et al. 1995; Miyakado et al. 1980). Scott et al. (2008) concluded in their studies on Piperaceae, that piper extracts are a unique and valuable source of biopesticide, effectively controlling small insects and reducing the development of pest resistance when mixed as a synergist with other botanical pesticides such as pyrethrum. Kéïta et al. (2000) showed that black pepper essential oil reduces the adult emergence of cowpea weevil *Callosobruchus maculatus* by 100% after 30 days of treatment in storage.
Rosemary *Rosmarinus officinalis* essential oil has been used conventionally as medications in many countries because it is non-toxic to humans and environments (Miresmailli et al. 2006). Also, Miresmailli et al. (2006) have reported that the effect of 1% rosemary oil contact toxicity against two-spotted mites *Tetranychus urticae* on tomato resulted in high mortality when using a tomato leaf disc test for 12 and 48 hours. Rosemary essential oil has been commercialised as a pesticide for its effective control of several insect and mite pests (Choi et al. 2004; Hummelbrunner & Isman 2001; Isman 2000; Isman et al. 2011). Isman et al. (2008) have shown chemical compositions of commercial rosemary oil and determined the LD$_{50}$ values of the oil when applied to cabbage loopers *Trichoplusia ni* and fall armyworms *Pseudaletia unipuncta*; the toxicity was significant.

Eucalyptus essential oil possesses a wide range of pesticidal activity including insecticidal, insect repellent, herbicidal, acaricidal, fungicidal and anti-microbial, and is considered non-polluting with little or no toxicological effect on the environment (Batish et al. 2008; Ben-Issa et al. 2017). Eucalyptus essential oil has various insecticidal activities such as contact, antifeeding, oviposition inhibition, repellency and fumigant. Eucalyptus leaf extract is effective against aphids by using contact test method and antifeeding; is effective against cotton leafhopper and cotton stainer *Dysdercus suturellus* by inhibit oviposition; the fumigant of eucalyptus oil works against housefly *Musca domestica*; and the oil and dry powder extract is used to protect potatoes against potato tuber moth *Phthorimaea operculella* (Ahmed & Eapen 1986; Batish et al. 2008; Jaipal et al. 1983; Koul et al. 2008; Lal 1987; Mathews 1981).

Tea tree *Melaleuca alternifolia* essential oil has been used as insect control agents because it constitutes bioactive chemicals (Choi et al. 2003). Hammer et al. (2006) have concluded that tea tree essential oil is toxic to several insect species, and suggested more studies are required. Liao et al. (2017) indicated that *M. alternifolia* may provide a new and safe alternative to chemical pesticides. Many essential oils including tea tree oil have been examined on several Hemipterans (aphids, thrips, whiteflies and mealybug) (Cloyd et al. 2009; Mann et al. 2012).

Although pesticides are the basis for many pest management applications, and are likely to remain so as long as effective and inexpensive chemicals are available, they can cause environmental
pollution and pest resistance for agriculture chemicals (Farajzadeh et al. 2014). Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family (Lamiaceae). The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols and sesquiterpenes (Isman 2000).

The rapid action of essential oils against some pests is an indication for a new generation of natural pesticides that have less impact on the environment (El-Hosary 2011). Aphid control is usually accomplished with the use of chemical pesticides, which, when used incorrectly, can lead to environmental pollution as well as aphid resistance, especially in *M. persicae* (Ben-Issa et al. 2017; Devonshire et al. 1998). Green peach aphids already has resistance to many insecticides and also to frequently applied chemicals such as the pyrethroids (Kuhar et al. 2009).

The fact that some pesticides are losing their effectiveness as a result pest resistance encouraged this study to evaluate the insecticidal activity of essential oils against *M. persicae* by studying both contact and residual toxicity, as well as the synergistic potential of black pepper, tea tree, blue eucalyptus and rosemary essential oils. In addition, the study evaluates a synergistic interaction between binary mixtures of essential oils against *M. persicae* and different methods of application. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to check the stability of essential oil mixtures during storage at different temperatures.

### 4.3. Materials and methods

#### 4.3.1. Essential oils and chemicals

Black pepper *Piper nigrum*, rosemary *Rosmarinus officinalis*, eucalyptus blue gum *Eucalyptus globulus* and tea tree *Melaleuca alternifolia* 100% pure essential oils were obtained from Essential Pure Natural Select Ingredient supplies (Range Products Pty Ltd, Perth, Western Australia) and were extracted by steam distillation (see Table 4.1). Methanol 99.9% and hexane 97% were purchased from Sigma-Aldrich (Australia), and acetone 99.5% and ethanol 99% were purchased from Asia Pacific Specialty Chemicals Ltd, NSW, Australia.

Table 4.1. List of essential oils used in this study, including origin, plant part and extraction methods
### 4.3.2. Aphid rearing

Green peach aphids *Myzus persicae* (Sulzer) were obtained from two locations, Agricultural Biotechnology Centre (SABC) at Murdoch University and the Department of Primary Industries and Regional Development, Western Australia. The aphid species was confirmed using online classification (http://idtools.org/id/AphID/polycosmo10.html) (Favret & Miller 2012), and the classification procedure was used to identify the aphid species. The identification of green peach aphids following the procedure of aphid slide mounting was undertaken to produce high quality and permanent slides of aphids. To prepare for slide mounting, fresh adult aphids were collected (primarily wingless and some winged), and specimens that have been stored in 70% ethanol. Aphids were placed in an Eppendorf tube, 95% ethanol added, and then heated at 80°C in a dry block heater for five minutes. Aphids were poured into a cavity glass and the abdominal wall carefully pierced with a fine micro pin to create an extra entry hole for Potassium Hydroxide (KOH). Aphids were then placed back into the empty tube, 10% KOH added, and then tube was heated for 15–20 minutes at 80°C. KOH pipetted off and replaced with distilled water. Specimens were left for about an hour and the water changed once. After that the discarded water was replaced with Glacial Acetic Acid (GAA) and left for 5–10 minutes, to flush the contents with the pipette. The GAA was removed and replaced with 1:1 mix of GAA and Terpineol and left for 5–10 minutes, then the mixture removed and replaced with pure Terpineol. Specimens were transferred to a cavity glass with Terpineol and were ready to be placed on a microscope slide and mounted in Canada balsam. To mount, a micro-tool was used to place a small loop under the desired specimen and lift it from the holding liquid. The micro-tool is inverted and the aphid was carefully placed in the centre of a microscope slide. Small amounts of mounting medium were added and a coverslip carefully lowered onto the slide, with a label placed to record notes. The mounted slides

<table>
<thead>
<tr>
<th>Essential oil name</th>
<th>Plant family</th>
<th>Origin</th>
<th>Plant tissues used</th>
<th>Method of extraction&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>Piperaceae</td>
<td>India</td>
<td>Berries</td>
<td>Steam distillation</td>
</tr>
<tr>
<td>Eucalyptus Blue Gum</td>
<td>Myrtaceae</td>
<td>Australia</td>
<td>Wood and Leaves</td>
<td>Steam distillation</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Lamiaceae</td>
<td>Spain</td>
<td>Herb</td>
<td>Steam distillation</td>
</tr>
<tr>
<td>Tea tree</td>
<td>Myrtaceae</td>
<td>Australia</td>
<td>Leaves</td>
<td>Steam distillation</td>
</tr>
</tbody>
</table>

<sup>a</sup> = essential oils supplied by Range Products Pty Ltd. (Perth, Western Australia)
were put in a warm oven between 40–50°C for three weeks to set the medium. Specimens can be viewed through a microscope directly after mounting (Dooley 2002; Nye 1947; Tagestad 1976).

Green peach aphids were reared in a glasshouse located at Murdoch University, on cabbage *Brassica oleracea* var. *capitata* and sweet capsicum *Capsicum annuum* potted plants. The glasshouse condition for aphid rearing was controlled: the temperature ranged between 18 ± 2°C and 25 ± 2°C during daylight and at night with humidity between 60% ± 2% and 75% ± 2% respectively, and a photoperiod L18:D6. The temperature and humidity were recorded using the HoBoware® (temperature / relative humidity data logger) and its software (Onset Company, One Temp Pty. Ltd., Adelaide, Australia), placed inside the glasshouse. *M. persicae* were transferred by a fine brush and placed on the leaves of cabbage and capsicum plants.

4.3.3. Determination of essential oil components using gas chromatography-mass spectrophotometry (GC-MS)

GC-MS analysis was carried out for 100% pure essential oil of black pepper, eucalyptus blue gum, rosemary and tea tree, and their binary combination by using a Shimadzu GC-MS model QP2010 series, installed with an SGE main category BPX5 column, using 30 m x 0.25 mm film thickness 0.25 μm (Kinesis Australia Pty Ltd, Qld, Australia) and AOC-5000 autosampler (Shimadzu, Kyoto, Japan) as an autosampler. The parameter of GC-MS analysis used the following method: injector temperature 220°C; pressure 63.43 kPa; column flow 1.07 ml min⁻¹; linear velocity 37.8 cm/sec; sample injected volume 1 μL diluted with hexane. Gas chromatography coupled to a mass selective detector (MSD) were recorded with ionisation and interface temperature of 200°C; the solvent cut time was 1.5 min and the carrier gas was helium. Two replicates for each essential oil were injected. The individual constituent of each essential oil was identified by comparing the obtained mass spectra for each component with the values stored in mass spectra libraries, NIST database with data previously reported in the literature. The percentage composition of the oils was calculated in peak areas using the normalisation method.
4.3.4. Determination of insecticidal activity of pure and combined essential oils

4.3.4.1. Contact toxicity bioassay of pure and combination of essential oils

Four concentrations were used to test the contact insecticidal activity of 10 essential oil formulations, four pure essential oils black pepper, eucalyptus blue gum, rosemary and tea tree—and six binary mixtures of essential oils—black pepper + eucalyptus, black pepper + rosemary, black pepper + tea tree, eucalyptus + rosemary, eucalyptus + tea tree and rosemary + tea tree (BE, BR, BT, ER, ET and TR). Three replications were used per treatment of essential oil for both the pure and binary mixture formulations. The control treatment was treated as above but using the solvent only. Between 20 and 30 aphids were placed on Whatman grade 1 filter paper in a 9 cm petri-dish. Each treatment was sprayed by using a micro-spray size 5 mL sprayer and applied 1 mL of essential oil according to each concentration. Petri dishes were covered with mesh and placed in an incubator chamber (25 ± 2˚C, 16:8 L: D, 65 ±5 % RH). Mortality was determined under a microscope after 1, 3, 6, 8 and 24 hours. Three replicates were used for each concentration.

4.3.4.2. Residual toxicity bioassay of pure and combination of essential oils

Aphids were exposed to treated Whatman No. 1 filter papers that were individually sprayed in the small sprayer by spraying approximately 2 mL of essential oil solution as described above. After exposure, treated filter papers were left to dry for 10–15 min and transferred individually to plastic Petri plates (9 cm diameter). Between 20 and 30 untreated green peach aphids were placed on each treated paper. Petri dishes were placed in the incubator chamber (described above). Mortality was determined under a microscope after 1, 3, 6, 8 and 24 hours. Four experiments were conducted in the laboratory to determine the activity of essential oils. The experiments used four types of pure essential oils and six types of essential oil combinations with three replicates for each concentration.

4.3.5. Synergistic interactions between essential oils

To evaluate potential synergism between the essential oils (black pepper, eucalyptus blue gum, rosemary and tea tree), mixtures were prepared maintaining the same concentration of single essential oil, following 1:1 ratio of the oils. Mixtures were applied to aphid adults and their LD$_{50}$ values were estimated after 24 hours. The relationships of the mixtures were determined by using
two statistical models which were Hewlett and Plackett’s model and Wadley’s model to compare expected and observed LD₅₀ values as shown in Equations 4.2.5.1 and 4.2.5.2 (Tak et al. 2016). Depend on Hewlett and Plackett’s calculation; the expected LD₅₀ values (assuming additive interaction) were determined from:

\[ E = (a \times LD_{50}(a)) + (b \times LD_{50}(b)) + (c \times LD_{50}(c)) + \cdots + (n \times LD_{50}(n)) \]  

(Equation 4.2.5.1)

Where a is the proportion of oil A in the mixture, and LD₅₀ (a) is the LD₅₀ of oil A. According to Wadley, theoretical LD₅₀ values were calculated from:

\[ E = \frac{a + b + c + \cdots + n}{LD_{50}(a) + LD_{50}(b) + LD_{50}(c) + \cdots + LD_{50}(n)} \]  

(Equation 4.2.5.2)

The interaction between the observed and theoretical LD₅₀ values was compared:

\[ R = \frac{\text{expected } LD_{50}}{\text{observed } LD_{50}} \]  

(Equation 4.2.5.3)

The relationship between the constituents of the mixture was defined as either synergistic (when \( R > 1.5 \)), additive (\( 1.5 \geq R > 0.5 \)) or antagonistic (\( R \leq 0.5 \)) based on this model.

4.3.6. Botanic insecticides from essential oils

Green insecticides were prepared from the tested essential oils. Six liquid formulations were naturally prepared from mixing binary essential oils as emulsifiable concentration (EC), as
follows: 200 mL of distilled water was added slowly to the 600 mL glass beaker by using burette during 5 min, and then added emulsifiers 1% surfactants span 80, 0.5% potassium oleate and 5% canola oil as carrier oil was added to the 3% and 5% essential oils with continued stirring for 5 min by using magnetic stir (Henríquez 2009). Different formulations were prepared by mixing the binary combination of essential oils (black pepper + eucalyptus), (black pepper + rosemary), (black pepper + tea tree), (eucalyptus + rosemary), (eucalyptus + tea tree) and (rosemary + tea tree) with natural solvent (canola oil and water) with emulsifier (span and potassium oleate) at the quantity mentioned above.

4.3.7. Phytotoxicity studies

Essential oil formulations were tested on cabbage and capsicum potted plants infested with *M. persicae* to study the phytotoxicity caused by essential oils. Cabbage and capsicum were planted in square pots size 90 mm filled by potting soil mixture (Richgro Regular Potting Mix, NSW, Australia) and reared under greenhouse conditions 25±2˚C and 60–70% relative humidity (RH). Based on the preliminary laboratory experiments, two concentration were chosen, 3% and 5% of essential oil combinations. Both cabbage and capsicum were individually sprayed approximately 5 mL with prepared formulations and the treatments were monitoring after 24 hours and any physical change in treated plants recorded. There were three plants as replicates for each formulation for each cabbage and capsicum. For control treatments, three plants of cabbage and three plants of capsicum were sprayed with the same formulation without essential oils and another three plants for each cabbage and capsicum were sprayed with clean water for comparison and the phytotoxicity was visually assessed.

4.3.8. Stability of essential oils and their combination at different time intervals by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the mixture of essential oils was performed on Perkin Elmer Spectrum Version 10.4.2 model Frontier FTIR/NIR in the School of Engineering and Information Technology, Murdoch University. Functional groups were determined with the help of IR correlation charts. IR spectra results showed in the percentage absorbance and the wave number region for FTIR analysis of 4000–400 cm⁻¹. The FTIR software (version 2.3.1.5) and the OMNIC
window with ATR cell were used for the analysis of the states of chemical bonding. The number of scans and resolution was four with resolution of 4 cm⁻¹ and the detector MIR TGS was used. Beamsplitter OptKBr, Apodization strong, spectrum type spectrum, beam type Ratio, phase correction Magnitude, scan speed 0.2, IGram type Double, scan direction Combine JStop 8.94, IR-Laser Wavenumber 15798.00, Description DATR 1 bounce Diamond/KRS5.

The combination of essential oil samples was prepared by mixing different binary types of essential oils with the ratio (1:1) and stored at three different temperatures, which were 15, 25 and 35°C for one, two and three months. The FTIR was conducted over three months to determine the essential oil stability of functional groups for the combination of essential oils. A small drop of around 1 mL of the essential oil was placed on the plate and a spectrum run. The plates were thoroughly cleaned after each scan for an essential oil to prevent contamination of other samples and the diamond wiped with a tissue, then washed several times with methylene chloride to take off the sample, then ethanol. The cleaned surface should be clear and free from scratches and contamination.

4.3.9. Data analysis

Mortality data from the essential oil elimination assay were subjected to analysis of variance (ANOVA) by using SPSS software version 24.0 (IBM Crop, Armonk, NY). Aphids were considered to be dead when no movement was detected by checking with a needle under a magnifying glass. Probit analysis was used to calculate the lethal dose (LD₅₀) values that caused 50% mortality compared with the untreated aphids by using probit analysis MS Chart software version 2016.12.07 (Chi 2017; Finny 1978). Microsoft Office Excel version 2016 was used to analyse FTIR and GC-MS data.

4.4. Results and discussion

4.4.1. Chemical composition of essential oils

GC-MS analysis of black pepper, eucalyptus, rosemary and tea tree essential oils indicated that there are many major constituents in all the types of essential oils (pure and mixture): black pepper α-pinene 12.66%, Sabinene 8.6%, β-pinene 12.17%, 1R- α-Pinene 5.56%, D-Limonene 15.52%,
Eucalyptol 3.21%, and Caryophyllene 24.56%, which were the most abundant compound (see Table 4.2). The percentage of compounds in the eucalyptus oil were p-Cymene 4.82%, D-Limonene 5.72% and Eucalyptol 82.25%; whereas, the main compounds in rosemary oil were α-pinene 15.87%, Camphene 3.89%, β-pinene 8.5%, p-Cymene 2.73%, D-Limonene 3.37%, Eucalyptol 35.27%, (-)-camphor 10.43% and Caryophyllene 4.92%. The proportion of tea tree chemical compositions were (+)-4-Carene 7.31%, p-Cymene 3.8%, Eucalyptol 4.98%, γ-Terpinene 17.74%, Terpinolene 2.78%, (-)-terpinen-4-ol 43.94% and α-Terpineol 3.61%.

The most abundant compounds in six types of essential oil combinations (BE, BR, BT, ER, ET and TR) were α-pinene 6.76, 14.60, 7.95, 8.28, 1.59 and 9.28% found in BE, BR, BT, ER, ET and TR respectively. Camphene 0.23, 2.32, 0.25, 1.97 and 2.00% for BE, BR, BT, ER and TR respectively, with 1R- α-Pinene 2.62, 2.60, 2.85% found in BE, BR and BT respectively. Further, (+)-4-Carene 3.51, 3.55 and 3.96% instituted in BT, ET and TR, while Sabinene 4.22, 4.27 and 4.58% were found in BE, BR and BT respectively. β-pinene 6.12, 10.43, 6.75, 4.35 and 4.6% in BE, BR, BT, ER and TR respectively; whereas, p-Cymene 3.07, 1.94, 2.45, 3.81, 4.45 and 3.34%, and also D-Limonene 10.58, 9.34, 8.80, 4.56, 3.60 and 3.34 were found in all combination types. In addition, Eucalyptol 44.58, 19.63, 4.16, 59.45, 46.31 and 20.56% originated in six mixtures, then γ-Terpinene 8.50, 9.5 and 9.38% in BT, ET and TR. While, the proportion of (-)-terpinen-4-ol was 20.85, 20.94 and 22.02 in BT, ET and TR, as well as the percentage of Caryophyllene in all combination, were 11.50, 14.62, 13.10, 2.36 and 2.73% for BE, BR, BT, ER and TR, respectively.
Table 4.2. Chemical constituents of pure and mixed essential oils, analysis by GC-MS

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<thead>
<tr>
<th>RT(^a) (min)</th>
<th>Composition</th>
<th>Percentage (%) of essential oil composition(^b)</th>
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<td>(\alpha)-Thujene</td>
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</tr>
<tr>
<td>6.54</td>
<td>(\alpha)-Fenchene</td>
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<td>Camphene</td>
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<td>Sabineene</td>
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42.33 Caryophyllene oxide 2.62 - - - 1.17 1.24 1.37 - - -
42.58 Globulol - - - 0.33 - - 0.14 - 0.11 0.11
43.06 Ledol - - - 0.14 - - - - -
45.01 α-Cedrene - - - 0.16 - - - - -
45.12 Spathulenol 0.17 - - - - - - - - -

a = Relative retention time (min)
b = Letters referred to essential oils, for the essential oils: black pepper (B), eucalyptus (E), rosemary (R) and tea tree (T). For the essential oil combinations: black pepper + eucalyptus (BE), black pepper + rosemary (BR), black pepper + tea tree (BT), eucalyptus + rosemary (ER), eucalyptus + tea tree (ET) and tea tree + rosemary (TR).

4.4.2. Contact toxicity of pure essential oils

The use of black pepper, eucalyptus, rosemary and tea tree essential oils showed high mortality against *M. persicae* in the laboratory because they have insecticidal activity when used as contact and residual bioassay methods. The results indicated that black pepper oil exerted contact toxicity against green peach aphids in time and dose-dependent manner, and that the higher dose 5% had the highest mortality of aphids, followed by 3% then 2% and 1% when compared with the untreated aphids for exposure periods of 1, 3, 6, 8 and 24 h (see Table 4.3). The highest mortality in black pepper was 80%; while the results showed that tea tree essential oil had approximately the same effect as black pepper oil in aphid mortality over the same exposure time. Conversely, eucalyptus and rosemary were less effective on the target pest compared with black pepper and tea tree oil. The mortality for eucalyptus was 5, 8.33, 16.67, 33.33 and 53.33% at the concentration of 5% at 1, 3, 6, 8 and 24 h exposure times. The mortality for tea tree oil at doses 5% was 8.33, 8.33, 23.33, 35 and 60% respectively at the same exposure time. R²= 0.801 (Adjusted R²= 0.795). For the use of these oils, it can be concluded that black pepper and tea tree have stronger insecticidal potential than eucalyptus and rosemary.
4.4.3. Residual toxicity of pure essential oils

Most of the essential oils were active as residual toxins against green peach aphids *M. persicae* at the preliminary screening dose, especially at 3% and 5% (see Table 4.4). Black pepper and tea tree produced 100% mortality at the concentration 5% at 24 h exposure time, followed by rosemary and eucalyptus achieved 95.56 and 94.44% mortality at 3% concentration at 24 h treatment. $R^2=0.928$ (Adjusted $R^2=0.925$). All other oil concentrations caused less than 76.6% at 24 h exposure time.

4.4.4. Contact toxicity of essential oil mixtures

All the mixed essential oils tested were active towards aphids (see Table 4.5). In the series of concentrations applied, mortality was concentration dependent. All the essential oils tested caused more than 80% on green peach aphids at the high dose that used, while 1% and 2% concentrations were achieved less mortality in all combinations of essential oils. The contact activities of the three combinations TR, BT and ET of essential oils (EOs) were the highest at the concentration 3% and 5% for 24 h exposure time. $R^2=0.787$ (Adjusted $R^2=0.78$). However, the contact activities of other combinations of EOs were not ideal, causing less than 50–70% mortality during the 24 h exposure.

4.4.5. Residual toxicity of essential oil mixtures

The effect of the different combinations of EOs on *M. persicae* is given in (see Table 4.6). As shown in the table for aphids treated with six different combinations, the highest effect was observed at the concentration of 5% of BE, BR, BT, ER and ET, the combination of BE, ER and TR essential oils showed the highest mortality and the others caused less than 98% at 24 h exposure time. Whereas, the mortality was less than 70% at the concentration of 3% and less than 60% at the dose of 2%. $R^2=0.862$ (Adjusted $R^2=0.859$). Moreover, the mortality at 1% concentration for all EO combinations was less than 20% at all exposure time.
Table 4.3. Mortality of aphids *M. persicae* after contact treatment with four pure essential oils

<table>
<thead>
<tr>
<th>EO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Concentration (µL mL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Mortality (%)± SD&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Letters referred to essential oils (EO): black pepper (B), eucalyptus (E), rosemary (R) and tea tree (T).

<sup>b</sup> Standard division.
Table 4.4. Mortality of aphids *M. persicae* after residual treatment with four pure essential oils

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<th>6h</th>
<th>8h</th>
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<td>12.22±5.0</td>
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*a* = Letters referred to essential oils (EO): black pepper (B), eucalyptus (E), rosemary (R) and tea tree (T).

*b* = Standard division.
Table 4.5. Mortality of aphids *M. persicae* after contact treatment with the mixture of six essential oils

<table>
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<tr>
<th>EO&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Concentration (μL mL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Mortality (%)± SD&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>16.67±5.77</td>
<td>46.67±2.88</td>
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<td>76.67±4.41</td>
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<td>16.67±2.88</td>
<td>51.66±1.67</td>
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<td>18.33±1.67</td>
<td>53.33±4.41</td>
<td>76.67±1.67</td>
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</tr>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td></td>
<td>2</td>
<td>11.67±2.88</td>
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<td>7.0±8.66</td>
<td>75.00±5</td>
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<td>26.66±2.88</td>
<td>78.33±2.89</td>
<td>93.33±5.01</td>
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</tr>
<tr>
<td></td>
<td>5</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.67±2.88</td>
<td>8.33±5.77</td>
<td>13.33±10.41</td>
<td>35.0±8.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.0±5.0</td>
<td>28.33±2.89</td>
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<td>86.67±2.89</td>
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<tr>
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<td>3</td>
<td>15.0±5.78</td>
<td>33.33±4.41</td>
<td>8.0±3.34</td>
<td>96.67±1.67</td>
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<td>5</td>
<td>18.33±1.67</td>
<td>35.0±2.89</td>
<td>81.66±2.88</td>
<td>98.33±1.67</td>
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</tr>
</tbody>
</table>

<sup>a</sup> = For the essential oil (EO) combinations: black pepper + eucalyptus (BE), black pepper + rosemary (BR), black pepper + tea tree (BT), eucalyptus + rosemary (ER), eucalyptus + tea tree (ET) and tea tree + rosemary (TR).  
<sup>b</sup> = Standard division.
Table 4.6. Mortality of aphids *M. persicae* after residual treatment with the mixture of six essential oils

<table>
<thead>
<tr>
<th>EOa</th>
<th>Concentration (μL mL⁻¹)</th>
<th>Mortality (%)± SD b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>3h</td>
</tr>
<tr>
<td>BE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.0±3.33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.89±5.09</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16.67±3.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>33.33±6.67</td>
</tr>
<tr>
<td>BR</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.0±3.33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.89±3.85</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26.67±3.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>37.78±3.85</td>
</tr>
<tr>
<td>BT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8.89±1.92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.33±3.33</td>
</tr>
<tr>
<td></td>
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<td>2.0±3.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>21.11±3.85</td>
</tr>
<tr>
<td>ER</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8.89±1.92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.89±1.92</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25.56±1.92</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>35.56±3.85</td>
</tr>
<tr>
<td>ET</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14.44±1.92</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22.22±5.09</td>
</tr>
<tr>
<td></td>
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<td>3.0±6.67</td>
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<td>5</td>
<td>41.11±8.39</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>37.78±6.94</td>
</tr>
</tbody>
</table>

a = For the essential oil combinations: black pepper + eucalyptus (BE), black pepper + rosemary (BR), black pepper + tea tree (BT), eucalyptus + rosemary (ER), eucalyptus + tea tree (ET) and tea tree + rosemary (TR).
b = Standard division.
4.4.6. **Synergistic activity among essential oil mixtures**

Insecticidal activity among four types of essential oils (black pepper, eucalyptus, rosemary and tea tree) were investigated (see Table 4.7). The following of EO combination showed synergistic interactions; the most significant synergism, based on Wadley’s determination, was the combination of B+E and B+R. the binary combination of two essential oils produces lower LD$_{50}$ than the individual oil. The synergy interaction was found with the mixture of black pepper and tea tree based on Wadley’s calculation, and the synergy ratio was (R>1.5). However, the combination of eucalyptus and rosemary showed antagonistic interaction because the synergism ratio was (R<0.5). All other mixtures of essential oils showed additive interaction.

4.4.7. **Phytotoxicity of essential oil combinations on the plant**

At the maximum concentrations of essential oils used in this study caused different phytotoxic markings on three varieties of capsicum and cabbage plants. All essential oil mixtures showed remarkable insecticidal properties on green peach aphids; however, there was visual evidence of phytotoxicity on the plants. A spray of essential oil formulations on capsicum and cabbage to control *M. persicae* the mixture of oils of BE, BR, BT, ER, ET and RT also showed remarkable insecticidal properties with a similar side effect in terms of phytotoxicity on cabbage but only slightly on capsicum for some of the formulations. Cabbage plants were more sensitive to doses of 3% and 5% of essential oils, although BE oil was very phytotoxic to both capsicum and cabbage. Specifically, *M. persicae*, living on the more sensitive plant species seedlings are more sensitive to the formulation treatments. This means that cabbage plants are sensitive to all essential oils even in a lowest tested doses required to obtain sufficient aphid control. Capsicum, on the other hand, can tolerate higher doses of most formulations after spraying on the plants. Therefore, cabbage plants were more sensitive to all types of essential oils tested in different doses. Furthermore, on capsicum, after the treatment of BE, BR, BT, ER, ET and RT, phytotoxicity can be a delayed outcome, with plants appearing healthy after the exposure to oils, but affected after 1–3 days.
Table 4.7. Comparative insecticidal activity by the mixture of four types of essential oils on green peach aphids *M. persicae*

<table>
<thead>
<tr>
<th>EO</th>
<th>LD$_{50}$ (95% CI)$^a$</th>
<th>Slope (±SE)$^b$</th>
<th>$X^2$</th>
<th>$P$</th>
<th>Expected LD$_{50}$$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H&amp;P$^d$</td>
</tr>
<tr>
<td>B</td>
<td>2.42 (0.88-5.59)</td>
<td>0.4266 (0.40)</td>
<td>8.85</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>3.01 (0.53-6.08)</td>
<td>0.5317 (0.55)</td>
<td>9.41</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>4.11 (0.66-5.05)</td>
<td>0.3161 (0.42)</td>
<td>8.87</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>3.43 (0.77-4.99)</td>
<td>0.2837 (0.38)</td>
<td>7.54</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>BE</td>
<td>0.969 (2.34-3.18)</td>
<td>0.0924 (0.32)</td>
<td>0.36</td>
<td>0.05</td>
<td>3.26</td>
</tr>
<tr>
<td>BR</td>
<td>1.091 (2.23-3.68)</td>
<td>0.163626 (0.32)</td>
<td>1.03</td>
<td>0.05</td>
<td>2.92</td>
</tr>
<tr>
<td>BT</td>
<td>0.452 (0.28-3.86)</td>
<td>0.364217 (0.46)</td>
<td>2.18</td>
<td>0.03</td>
<td>2.71</td>
</tr>
<tr>
<td>ER</td>
<td>1.439 (0.11-3.11)</td>
<td>0.261809 (0.33)</td>
<td>2.06</td>
<td>0.04</td>
<td>3.77</td>
</tr>
<tr>
<td>ET</td>
<td>0.728 (0.29-3.50)</td>
<td>0.337722 (0.38)</td>
<td>2.16</td>
<td>0.03</td>
<td>3.56</td>
</tr>
<tr>
<td>TR</td>
<td>1.127 (0.95-6.41)</td>
<td>0.579426 (0.56)</td>
<td>3.44</td>
<td>0.02</td>
<td>3.22</td>
</tr>
</tbody>
</table>

$^a$ = Lethal dose LD$_{50}$ ± Confidence Interval; $^b$ = Standard error; $^c$ = Expected LD$_{50}$ based on each calculation model; $^d$ = Hewlett and Plackett’s calculation of expected LD$_{50}$; $^e$ = Wadley’s calculation of expected LD$_{50}$; $^f$ = Determination of interaction of the mixture based on Wadley’s determination method: when R>1.5, synergistic (Syn) interaction; when 1.5≥R>0.5, additive (Add) interaction; when R≤0.5, antagonistic (Anta) interaction; $^g$ = Synergy ratio from Wadley’s calculation.

4.4.8. Storage stability of combined essential oils

The essential oil mixtures were stored at three different temperatures (15, 25 and 35°C) for one, two, three, six and twelve months, and all mixtures were analysed with FTIR to determine if the functional groups had degraded during the storage period. The functional groups present in the mixture of essential oils were determined by comparing the vibration frequencies in the wave number. Figures 4.1–4.6 present the spectral absorption of essential oils mixtures obtained from BE, BR, BT, ER, ET and RT measured in the wave number range 4000–400 cm$^{-1}$. The FTIR spectrum of BE was strong broadband at 2922 cm$^{-1}$ and can allocate the Alkanes C-H stretch, a medium band intensity vibration at 1644 cm$^{-1}$ assign NH2, the strong methylene/methyl band at 1464.95 and 1446.16 cm$^{-1}$. The peak of IR at 2931 and 2842 cm$^{-1}$ are due to C-H asymmetric and symmetric stretching. The strong band at 1633 cm and 1631 indicative of C-H bending, at 1306–1361 cm$^{-1}$ represented the –O-H and C-H stretch band. While the number wave at 1015–1270 cm$^{-1}$
showed the strong band of C-O. The position of the C = C stretching regularity that vary slightly as a function of location around the double bond. Carbonyl compounds are often the strongest band in the spectrum and will locate between 1825 and 1575 cm\(^{-1}\). For a double bond, the functional group plays an important role in the observation of the carbonyl group. This includes a connection to an aromatic group to a C = C or C = O. The wave number at 985cm\(^{-1}\) indicated a strong or medium band for C=C-H and at 920.1 cm\(^{-1}\) represented group C-H. All these peaks of function group were not affected by storage temperature tested on essential oils or the time of storage, and all groups kept at the same wavenumber location. We performed the FTIR analysis because it is a fast and relatively cheap technique that allows direct function of groups measurement of components in mixtures.

### 4.5. Discussion

Plant essential oils are potentially valuable for green peach aphids control. They performed in many ways on several types of insects and can be applied to crops or stored products (Isman 2000). Black pepper, eucalyptus, rosemary and tea tree oils are known to possess antifeedant, repellent, ovicidal and insecticidal activities against many insect species (Isman et al. 2011). Additionally, essential oils can be highly effective on insecticide resistant insects (Farajzadeh et al. 2014). In this study, influences of essential oils were measured according to oil type (pure oil and mixture), time and dose on *M. persicae*. The insecticidal activity against green peach aphids was observed with 1, 2, 3 and 5%. These essential oils might be important applicants for natural green peach aphids control agents. All essential oils applied as contact or residual treatments proved toxic to *M. persicae* in the laboratory although they differed in their efficacy. The combination of binary essential oils can cause high mortality compared with the use of a single essential oil. Synergists of essential oils are low in toxicity and have a little or no inherent insecticidal properties as well as have very short residual activity (Buss & Park-Brown 2002).

The GC-MS analysis (see Table 4.2) showed differences in chemical composition and their percentages between tested essential oils in the pure and mixed oils. Black pepper GC-MS analysis has been shown Caryophyllene 24.56% was the most abundant major constituents and then D-Limonene 15.52%, α-pinene 12.66%, β-pinene 12.17%, and Sabinene 8.6%. All of these constituents affected various pests, and these results are consistent with (Bernard et al. 1995; Scott
et al. 2008; Tak et al. 2016). The results indicated that eucalyptol is one of the major chemical compositions in the eucalyptus essential oil that made up 82.25% of chemical compositions total in the oil. Eucalyptol has insecticidal activities against many insects as shown in many previous studies (Batish et al. 2008; Ben-Issa et al. 2017; Pavela 2006). In addition, one of the main constituents in rosemary essential oil was Eucalyptol 35.27%, α-pinene 15.87%, (-)-camphor 10.43 %, and β-pinene 8.5%. These components have pesticidal action that has been used in pest control and presented in many past experiments (Miresmailli et al. 2006; Papachristos & Stamopoulos 2004; Tak et al. 2016). Moreover, the results showed that tea tree essential oil main compounds are (–)-terpinen-4-ol 43.94% and γ-Terpinene 17.74%. These two major constitutions have insecticidal properties and effect on insect enzymes as AChE, GST and CarE (Cloyd et al. 2009; Coronado-Puchau et al. 2013; Liao et al. 2017; Mann et al. 2012). However, the proportion of main compounds in all types of tested essential oils changed after binary mixing. Chemical compositions in the combination of BE were Caryophyllene 11.50%, D-Limonene 10.58%, α-pinene 6.76%, β-pinene 6.12%, and Sabinene 4.22%. The result of GC-MS indicated variations of chemical compound percentage between two pure essential oils, so the mixture reconstituted distribution of chemical compounds. In BE mixture, the main constitutions were reduced to nearly half compared with black pepper and eucalyptus separate (see Table 4.2). Also, the BE combination showed most of the compounds found in the mixture that was not found in the pure oils, and this might give completion of range insecticidal activity between oil constituents. Whereas in the combination of BR, the highest amounts of compounds were α-pinene 14.60%, β-pinene 10.43%, D-Limonene 9.34%, Eucalyptol 19.63%, and Caryophyllene 14.62%; these amounts of compounds decrease compared with their purity before mixing. The combination of BT, the result indicated that (–)-terpinen-4-ol 20.85% and Caryophyllene 13.10% were the highest constituents in the mixture, this finding is consistent with (Liao et al. 2016) that indicated to the high amount of terpinen-4-ol and caryophyllene in tea tree M. alternifolia oil. Moreover, ER, ET and TR have participated in the same components α-pinene (8.28, 1.59 and 9.28%), eucalyptol (59.45, 46.31 and 20.56%), γ-Terpinene (1.36, 9.50, and 9.38) and (-)-camphor (5.10, - and 5.35%). All these compounds have properties against various types of insects as shown in previous studies (Carson & Hammer 2011; Erler et al. 2006; Isman et al. 2008).
The results of this study showed insecticidal effects of black pepper, eucalyptus, rosemary and tea tree essential oils and their binary combinations (BE, BR, BT, ER, ET and TR) in various concentrations on the green peach aphid. However, there were differences in the bioinsecticidal effects of 10 essential oils (four pure and six binary combination oils) despite them all having significant aphidicidal activity on the *M. persicae* in various mortality percentage based on the concentration of essential oils, especially with higher doses tested over 24 hours exposure time. The efficacy of essential oils depends upon their chemical composition and the proportion of each constituent present in the mixture. The essential oil constituents vary from one type to another depending on plant species. Yazdgerdian et al. (2015) and Hollingsworth (2005) reported that α-pinene, limonene and camphene, which are major constituents, have demonstrated aphidicidal activities against the woolly beech aphid *Phyllaphis fagi* and the palm aphid *Cerataphis brasiliensis*. Interestingly, previous studies showed that limonene is able to attract aphid natural enemies, so using essential oils containing a high amount of limonene could act as attractants for parasitoids and predators (Yazdgerdian et al. 2015) while the compound eugenol and 1,8-cineole have been reported against many insects including aphids (Liska et al. 2010; Yazdgerdian et al. 2015). Other major constituents, such as terpinene-4-ol, terpinene, 4-carene and α-phellandrene also have insecticidal effect against several insects and these compounds are found in tested essential oils (black pepper, eucalyptus, rosemary and tea tree) (Abtew et al. 2015; Dambolena et al. 2016; Kasmi et al. 2017). Some previous work related to the aphidicidal effects of rosemary oil are consistent with our findings. For example, rosemary oil has insecticidal activities against several insects and is used in many commercial products as insecticides. A study conducted by Hori (1999) found that rosemary has shown repellency and is a contact toxin to *M. persicae* and has a greater ability to penetrate through the cuticle of aphids than absorption from the gut and intestines. These findings are similar with the results presented by Digilio et al. (2008) and Sampson et al. (2005), indicating an increase in the mortality of aphids when reared on plants that contained a diverse of essential oils. Tomova et al. (2005) confirmed significant effects of essential oil volatiles against three species of aphids demonstrating potential as a control method. Black pepper and tea tree essential oils as contact applications resulted in 80% mortality in the green peach aphids (Tomova et al. 2005). Cloyd et al.’s (2009) report comparable findings where their results of nearly 100% mortality caused by essential oils of the black pepper and tea tree against rose-grain aphid. In addition, the residual application results in 100% mortality by using black
pepper and tea tree essential oils, as well as our findings, indicated that the mortality caused by eucalyptus and rosemary oils were 94.44 and 95.56%, respectively (see Tables 4.3 and 4.4). However, the combination of TR, ET, and BT essential oils can cause higher mortalities of 98.33, 95.00, and 98.33% respectively, when using a contact application. Görür et al. (2008) showed the parallel effect of essential oils against cabbage aphids resulted in more than 85% mortality. Conversely, Görür et al. (2008) concluded that there was an impact of essential oils dosages on the population of aphids on the plant, even when exposed to a low dose. Our findings of insecticidal effects of residual application of combined essential oils resulted in 100% mortality with TR, ET and BT at the concentration 5%, and more than 96% mortality for BR, BT and ET at the concentration 3%. An explanation for these results is the essential oils’ constituents and their volatiles. Our result is consistent with Digilio et al. (2008), who showed high mortality rates on *M. persicae* and *A. pisum* by using different doses of various essential oils (see Tables 4.5 and 4.6).

The toxicity of some tested individual essential oils also vary to the aphids especially with a high dose used. The mixed binary essential oils have also been shown to have additive, synergistic and antagonistic effect. Under this study, toxicity was as high as expected based on many previous studies, especially with short time and use of a low dose. In some cases, the toxicity of the binary mixtures against aphids were higher than the individual oil. This indicates that some combinations have a synergistic effect; however, in one combination there was an antagonistic effect. Synergistic activities have been observed in the BT combination, LD$_{50}$ was 0.45 compared with LD$_{50}$ in their individual 2.42 for black pepper and 3.01 for tea tree (Table 4.7). The additive interaction were observed in some combinations of essential oils and the combination of ER showed antagonistic interaction due to the interaction between essential oils and their components (Faraone et al. 2015). The authors suggested several assumptions that could illuminate the synergistic effect of black pepper and tea tree against *M. persicae*: (1) the insecticidal mechanism of black pepper and tea tree might be different, they performed on different targets of *M. persicae*; (2) the synergistic effect could be due to the parallel effect of their mechanism; and (3) the synergistic effect occurs between combined essential oils because they have insecticidal properties on *M. persicae*. These findings parallel Ngamo et al. (2007) and Pavela (2010) who reported that synergistic insecticidal activities could be observed not only in the combination of essential oils but also in their constituents, as well as between synthetic insecticides with essential oils.
Our data showed that a spray formulation of essential oil tested on capsicum and cabbage seedlings were active against green peach aphids. Essential oil formulations have insecticidal activities and growing interest for aphid control. However there was visual evidence of phytotoxicity on the plant seedlings, especially cabbage plants with the spray application. Digilio et al. (2008) described that many essential oils might effect green peach aphids but with some essential oil phytotoxicity occurred.

The findings of our research indicated that the three temperature degree do not affect essential oil storage based on FTIR analysis of no changes in functional groups in all period of storage. Our results are consistent with Turek and Stintzing (2012) who reported essential oils demonstrated oil stability at various temperature degrees (5–38˚C) and showed no effect from temperature for three months when stored. The purpose of studying storage of essential oils at different temperature was conducted to determine the best temperature that the essential oil can be stored without degrading efficacy.

4.6. Conclusion

In conclusion, the current study demonstrates strong toxic effects against aphids with the essential oils of black pepper, eucalyptus, rosemary and tea tree. Their binary combination also had effective insecticidal activity both as contact and residual treatments against green peach aphids. The contact treatment of pure essential oil indicated that black pepper and eucalyptus were highly effective on aphids. The residual treatments showed the highest mortality on aphids especially with high doses. The identification of essential oil constituents in pure and combination, showed the various amounts of some compounds that might be used in pest control. Essential oils are natural products containing a complex mixture of compounds and thus have multiple insecticidal or aphicidal properties. The essential oil mixtures showed their insecticidal activities on aphid and the interaction between binary oils can lead to synergistic, additive and antagonistic effects. There was a synergistic effect between black pepper and tea tree essential oils. Botanical pesticide formulation made from tested essential oils were effective on aphids, but there was phytotoxicity on cabbage plants but only slight effect on capsicum depending on the type of essential oil. According to the FTIR analysis, essential oils can be stored at up to 35˚C without affecting the
properties of the oil. Therefore, we suggest that tested essential oil constituents in pure and combination should be screened as a potential natural insecticide, or be involved in the chemical synthesis of a new type of pesticides, based on essential oils and their constituents.
Figure 4.1. FTIR analysis of the combination of essential oil black pepper + eucalyptus (BE) over different temperature and storage time.
Figure 4.2. FTIR analysis for the combination of essential oil black pepper + rosemary (BR) over different temperature and storage times.
Figure 4.3. FTIR analysis for the combination of essential oil black pepper + tea tree (BT) over different temperature and storage times.
Figure 4.4. FTIR analysis for the combination of essential oil eucalyptus + rosemary (ER) over different temperature and storage times.
Figure 4.5. FTIR analysis for the combination of essential oil eucalyptus + tea tree (ET) over different temperature storage times.
Figure 4.6. FTIR analysis for the combination of essential oil tea tree + rosemary (TR) over different temperature and storage times.
Chapter 5: General discussion

5.1. Introduction

Springtails and aphids are pests that can cause direct and indirect damage to plants as contaminants or by feeding damage. Three methods were used to manage purple scum springtails on harvested celery and green peach aphids on the cruciferous crops. They were different fumigants on celery to control springtails along with plant volatile organic compounds and plant essential oils as a natural product to control aphids.

In the first experimental chapter of this thesis (represented in chapter two), the evaluation of ethyl formate and phosphine against purple scum springtails \textit{H. vernalis} on celery bunches. The purple scum springtail is considered quarantine pest in celery exportation because they are contaminating fresh celery by living inside the celery bunches (Majer et al. 2014). However, the purple scum springtail \textit{H. vernalis} belongs to the Hypogastruridae family that has many reported species as pests of mushrooms and seedlings of different vegetables (Greenslade & Ireson 1986). Some species of Hypogastruridae are secondary attackers with the pest feeding on vegetable and plant parts (Chahartaghi et al. 2005). The celery bunches were contaminated by \textit{H. vernalis} because they live between celery leaves and stalks. Purple scum springtail is considered a secondary invader on celery and other crops, excluding mushrooms that it is considered a primary pest. The species \textit{H. vernalis} status is restricted to edible fungi of various species but it has been registered as a quarantine pest that can limit export of celery to the Middle East Countries (Majer et al. 2014).

In the second experimental chapter of this dissertation (represented in chapter three), evaluation of plant volatiles released from uninfested and plants infested with green peach aphid \textit{M. persicae} and their role in attracting aphid parasitoids. Plants often emit a wide range of volatile organic compounds (VOCs) to defend themselves against insect invasions. The release of plant volatiles due to the influences of various herbivore species on host plants or the impact of a given herbivore on several host plant species. The infestation of the plant by insects can lead to attraction of beneficial insects such as parasitoids and predators was proven by using Y-tube olfactometer.
In the third experimental chapter in this thesis (represented in chapter four), evaluation of aphicidal activity of different essential oils against green peach aphid *M. persicae*. Green peach aphids can cause extensive damage to vegetables by direct feeding on plant sap, by transmitting plant viruses (Harmel et al. 2008), and by indirect damage as they secrete honeydew that can build up on the surface of leaves and cause secondary pathogenic growths and may reduce photosynthesis (Natwick 2009). *Myzus persicae* can feed on leaves, stems and flowers. The problem of insecticide resistance has grown in Australia agriculture, for instance, green peach aphids have become more resistant to a number of organophosphate insecticides in Australia (Gu et al. 2008). Therefore, there is a need to reduce the chemical pesticides and look toward alternative to synthetic and natural pesticides.

Therefore, the principle of study objective proposed the use of different fumigants and their combination to manage springtails on harvest celery and to test potential alternatives to chemical pesticides for controlling of green peach aphids. This study may lead to the new environmentally friendly control of aphids and the results provided some fundamental knowledge for future management of springtails and aphids.

### 5.2. Management of springtails on harvest celery

Australia exports of fresh celery *Apium graveolens* to some countries has been affected by infestation of purple scum springtails *Hypogastrura vernalis*. This insect belongs to the order Collembola and the family Hypogastruridae. Purple scum springtails live inside the celery bunches, contaminating fresh celery but do not cause any visible damage. Thus, the celery infested with purple scum springtail has led to rejection at shipping with a negative effect on the value of fresh celery at market. In this study, harvest celery bunches were fumigated with three fumigation methods, ethyl formate, phosphine and their combination to test on mortality of purple scum springtails in infested celery was evaluated. A major advantage and reason EF was selected for this study is that EF is considered a food additive, it provides fast kill of insects, has low human toxicity, fast breakdown of residues to natural products and it is registered as a fumigant in Australia. PH$_3$ was also chosen for these investigations because it is a widely used fumigant for
wide range of pest control, at has low cost, is easy to apply and is registered as fumigant in Australia.

The study aimed to test the efficacy of various concentrations from EF, PH₃ and their combination for controlling of purple scum springtails on harvest celery. The laboratory experiment were conducted by using three EF concentrations 50, 60 and 90 mg L⁻¹ for 1, 2 and 4 h exposure time and four concentrations of PH₃ were 1, 1.5, 2 and 2.5 mg L⁻¹ for 2, 4 and 6 exposure time as well as three concentrations of the mixture of EF with PH₃ were 20, 30 and 40 mg L⁻¹ for 2 and 4 h exposure time. Celery samples were prepared for the experimental fumigation by using small stainless steel chambers of 61 L capacity. DPS instrument portable gas chromatography combines with FID detector was used to determine of EF concentration in the headspace of the chamber. Hewlett-Packard 5890 gas chromatography combined with FPD detector to monitor PH₃ concentration in the headspace of fumigation chamber. The optimal fumigant concentration, exposure period, and evaluation of phytotoxic damage to celery bunches as well as sensory evaluation of product quality post-fumigation is reported in this experimental work.

5.2.1. The fumigant concentration inside the chamber

Based on the determination of fumigant concentration by using GC, the results show that the concentrations of EF, PH₃ and their combination declined significantly over time which is attributed to the absorption by celery bunches because of the high surface area and very high moisture content of the celery commodity. The EF concentration in the fumigation chamber was highly absorbed by celery bunches because the celery has highly water content and the EF is greatly soluble in the water. Some previous experiments reported that EF is highly absorbed in stored commodities such as strawberries, apples and cut flowers because these commodities contain high moisture and EF is highly soluble in the water (Agarwal et al. 2015; Lee et al. 2013; Simpson et al. 2004).

Whereas, the results show that sorption of PH₃ by celery was less than EF demonstrated by GC reading. The EF+PH₃ in combination sorption was about 90% of EF and around 40% PH₃ lost from the headspace of fumigation chambers at the end of fumigation time. The previous studies
demonstrated that EF and PH₃ can be significantly absorbed by the stored products or broken down to byproducts because of the high moisture content of the commodity (Ahmed et al. 2018; Agarwal et al. 2015; Reddy et al. 2007). Reddy et al. (2007) reported that PH₃ concentration might be absorbed at varying rates according to type of commodity; they also found that PH₃ is highly sorbed in commodities that have high moisture content, especially fresh products.

5.2.2. The purple scum springtail mortality

The results in this Chapter two show the mortality of purple scum springtails in infested celery varied among different EF concentrations depend on exposure time. High mortality caused by fumigation was found in the concentration of 90 mg L⁻¹ that was 98.73 and 100% mortality for 1 and 2 h exposure, respectively compared to the untreated control. The results for EF and the combination EF+PH₃ indicate a significant increase in purple scum springtail mortality with increasing exposure to EF. A high concentration of EF, of 90 mg L⁻¹ achieved 100% mortality, compared with relatively low mortality at a concentration of 50 mg L⁻¹ of only 91.58% over the same exposure period. There was no significant difference in mortality between the concentrations of 60 and 90 mg L⁻¹ for any duration of fumigation. The results are consistent with findings of other investigations which showed that the fumigation of packaged lettuce with using a vacuum achieved high mortality on green peach aphids and there was little impact on the quality of lettuce. In Korea, EF is approved for treatment of an export paprika market, as well as tomatoes (Kim et al. 2013). The results presented are consistent with Lee et al. (2015 & 2016) and Yang et al. (2016) who demonstrated and reported a synergistic effect between EF and PH₃ for control of cotton aphid and citrus mealybug. The aim of using combination of fumigants was to look for synergistic effects by mixing fumigants and to increase PH₃ effectiveness with mixtures, there is also the potential for breaking down pest resistance and reduction of fumigation periods (Lee et al. 2018). The interaction of the combination of EF and PH₃ can lead to the synergistic effect between EF and PH₃ for control of *H. vernalis* and the mixture of fumigants achieved a greater effect than the use of either EF or PH₃ alone.

Furthermore, the fumigation of celery with PH₃ alone, achieved 100% mortality at 2.5 mg L⁻¹ after 6 h exposure time of fumigation compared with untreated check. These results are consistent with
Finkelman et al. (2012) and Liu (2009) who reported that pure PH$_3$ fumigant caused 100% mortality on two-spotted spider mites whitefly, onion thrips and western flower thrips at 1.2 to 1.4 mg L$^{-1}$ on various vegetables and herbs, which were successfully fumigated at low temperature (2°C) without phytotoxicity to these commodities. PH$_3$ fumigation against lettuce aphids at the concentration 2.4 mg L$^{-1}$ of PH$_3$ was achieved 100% mortality at the temperature 2°C (Liu 2009), this finding supports our results.

5.2.3. The fumigant damage to celery

The use of EF fumigant alone at all concentration and EF combined with 1 mg L$^{-1}$ of PH$_3$ all affected the quality of celery and caused phytotoxicity. The observation of celery phytotoxicity from EF alone and the combination with PH$_3$ demonstrated that high sorption of EF by the commodity causes phytotoxicity, even at low concentrations and short fumigation times. In addition, the high sorption of EF by celery may cause phytotoxicity because the EF breaks down to the formic acid and ethanol and these compounds are known to be phytotoxic on some fumigated products. Zhang and Van Epenhuijsen (2004) reported that EF causes high phytotoxicity on cut flowers. In some previous experiments, less damage to commodities was shown by using EF and PH$_3$ in combination achieving high mortality of target pests (Lee et al. 2015; Lee et al. 2018). Lee et al. (2014) indicated high mortality of insect pests by the combination of EF and PH$_3$ in naturally infested strawberries and cut flowers with less apparent phytotoxicity and these findings are supported by our results.

The results of the celery fumigation with PH$_3$ alone showed no indication of phytotoxicity in fumigated celery compared with untreated check. This is consistent with the result obtained by previous studies that have shown that PH$_3$ fumigation of vegetables is effective and safe for postharvest and commercial applications for insect control, therefore, PH$_3$ remains one of the best fumigants for vegetable fumigation because it causes less phytotoxicity compared to other gases (Horn et al. 2005; Lee et al. 2012). Likewise, PH$_3$ treatments alone at various doses achieved high mortality on purple scum springtails and kept the commodity at high quality. The results show that sensory evaluation (taste, crunchy, visual and flavour) of fumigated celery bunches were similar
to untreated control. Ertürk et al. (2018) and Liu (2008) indicated that there is no phytotoxicity on the taste, crunchy, visual and flavour in broccoli, tomato and green pepper by PH$_3$ fumigation.

5.3. The role of plant volatiles in aphid management

Data presented in Chapter 3 shows the differences between two varieties of *Brassica oleracea* infested with *M. persicae* and uninfested *Brassica* plants by identifying volatile organic compounds in infested and uninfested plant. Many different volatile compounds were reported in previous studies for the infested plants with different species of aphids as a response to aphid infestation (Mathur et al. 2013; Lin et al. 2016).

5.3.1. The differences between infested and uninfested *Brassica* plants with *Myzus persicae* in plant volatile organic compounds (VOCs)

In this Chapter, we are comparing volatile compounds released from uninfested and infested cabbage and broccoli plants with *M. persicae* by using SPME fiber combined with the GC-MS. The results show that the *Brassica* plants infested with *M. persicae* can cause damage and as a result the infested plant released several different VOCs such as alpha- and beta pinene, (E)-3-hexen-1-ol, Myrcene, 1-Hexanone, 5-methyl-1-phenyl-, Limonene, Decane, gamma-Terpinen and Heptane, 2,4,4-trimethyl. This finding consistent with Najar et al. (2015) and Mathur et al. (2013) who reported that alpha- and beta pinene and limonene could increase in *Brassica* plants infested by aphids. The infestation of *Brassica* plants with aphids can release many VOCs as a response to the infestation, these compound including glucosinolate metabolites, phenolics, and terpenoids (de Vos and Jander, 2010; Hien et al., 2014). The interaction between *Brassica* plants infested with aphids can lead to the release of some VOCs that are found in infested plant but some of VOCs disappeared from uninfested plants, such as 3-Hexen-1-ol (E) and beta-pinene. The increase of some volatiles for example (E)-3-hexen-1-ol, beta-pinene and decane can be expected in infested plants because these compounds are well known as the green leaf volatiles and involved in the attraction of parasitoids and predators. This finding is consisted with previous studies (Wei & Kang 2011; Leroy et al. 2012; Li et al. 2017) indicated that green leaf volatiles can be emitted due to pest infestation. Moreover, the experimental results on the VOCs released from broccoli such as Limonene, Undecane, 3,4-dimethyl-, Heptane, alpha-Pinene, Oxalic acid, Citronellol,
Tridecane, n-Decanoic acid, Cyclopentane, pentyl- and n-Hexadecanoic acid has detected from infested broccoli. These results agree with the previous finding (Leroy et al. 2012; Li et al. 2017; Pinto-Zevallos et al. 2018) who reported that volatile compounds were found in the headspace of infested broccoli and has an important role in the attraction of beneficial insects as natural enemies. To conclude this part, the selection of VOCs extracted by using HS-SPME in the uninfested and infested Brassica plants (cabbage and broccoli) with M. persicae depended on the peak areas each compound identified in the treatments.

5.3.2. The attraction of aphids and their parasitoids to Brassica plants

Y-tube olfactometer was used in this experiment to examine the attraction of aphids and parasitoids toward plant samples. The results show that the response of the aphid M. persicae to the host species of Brassica was significantly attracted and influenced by the volatiles released from both infested and uninfested Brassica compared with clean air choice. While, the comparsion between infested and uninfested plants show no differences between the both plant samples with respect to aphid attraction toward infested and uninfested. Aphids can find their host chemically by following the plant VOCs released from both infested and uninfested plants. For this, VOCs can be useful guide for aphids in finding host plants; this result confirms previous studies conducted by (Hatano et al. 2008; Döring 2014; Hori 1999) who showed that aphids can find their host plants based on mainly plant odour and visual cues. Döring (2014) and Verheggen et al. (2013) demonstrated that aphids can be attracted to plant volatiles by using Y-tube olfactometer for host-finding. Therefore, the results in this study show that aphids tend to be attracted to both of damaged and undamaged plants when compared with clean air choice. The explanation of our observation that VOCs can describe the variance in attraction of aphids toward plant and also the VOCs can be increased by some of the compounds induced in response to aphid feeding. This finding consists with Züst and Agrawal (2016) and Hopkins et al. (2017) who indicated that the attraction of aphids can be influenced by plant volatiles.

There is potential for VOCs to be involved with the pest management by using them in biological control as a natural product for attracting natural enemies to be used in aphid control in many crops.
The results represent in this chapter about Y-tube olfactometer bioassays and attraction of aphid parasitoids show that the infested *Brassica* plants with *M. persicae* can be influenced the attraction of aphid parasitoids *A. colemani* and *A. abdominalis* to plant volatiles. The results from the olfactometer demonstrated that both tested parasitoids *A. colemani* and *A. abdominalis* responded significantly to the volatiles released from infested plants compared with the attraction to either uninfested plants or clean air choices. While, *A. colemani* and *A. abdominalis* showed no response when compared between clean air and uninfested cabbage. Kalule and Wright (2004) reported that parasitoids can be attracted to healthy plants. This was also found by Van Emden et al. (2008) who elucidated that parasitoids can be more attracted to the infested plant. The response of *A. colemani* and *A. abdominalis* to the infested *B. oleracea* preferring aphid-induced volatiles because some compounds may release from infested plant more than uninfested plants or new compounds can release as a result infestation. Therefore, the plant VOCs are significantly attractive natural aphid enemies as a response to the aphid feeding on *Brassica* plants. Da Silva et al. (2016) showed that parasitoid *A. colemani* could attract to volatiles released from *Brassica juncea* and preferred plants damaged by green peach aphids alone. There is potential for volatile plant compounds to be used in several ways: (1) some volatile compounds can be used to attract natural enemies as parasitoids; (2) some volatile compounds such as limonene are toxic to many pests; (3) identification of plant volatiles that could be used as a diagnostic tool for pests. From the above points, the introduction of a new method of pest management is important to reduce the use of synthetic pesticides. Another way to reduce chemical pesticides is using the alternatives such as chemicals replacement with biopesticides for insect pest management.

To sum up this chapter, the HS-SPME combined with GC-MS analysis for the volatiles released from both treatments provided a description of the differences between the infested and uninfested *Brassica* tested crops (cabbage and broccoli) and the role of these volatiles in attracting natural enemies. We found that 29 volatile compounds were released from cabbage and 25 volatile compounds from broccoli. These were identified by using SPME combined with GC-MS. The differences between VOCs released from infested and uninfested plants showed that VOCs released from infested plant were higher than VOCs released from uninfested plant. Y-tube olfactometer data showed no significant differences between infested and uninfested *Brassica*
plants on attractiveness for aphids. There are, however, significant differences in the attractiveness of parasitoids toward plants infested with green peach aphids.

5.4. Aphicidal properties of essential oils

Green peach aphid *M. persicae* is considered a polyphagous species which can be found worldwide. This species has been reported to feed on more than 500 species of plants from 40 plant families. Aphids damage on the plant comes from their feeding on plant sap that causes yellowish and leaf curling to plant. On the other side, *M. persicae* has been involved in plant virus transmission over 180 viruses. *Myzus persicae* has shown resistance to several chemical pesticides, therefore, an alternative way to aphid management and reduce chemical pesticides due to their negative sides is needed. Botanical pesticides are an alternative to the chemicals that are currently widely used against many insect pests.

This chapter described the role of plant essential oils in aphid management. Several essential oils have a broad spectrum of properties against many insect species including aphids. Essential oils are used in several ways for controlling insects and can be applied to crops in fields or on stored products (Isman 2000). The essential oils used in this study were black pepper, eucalyptus, rosemary and tea tree because they are known to have many actions against many pests such as, antifeedant, repellent, ovicidal and insecticidal activities as reported in (Isman et al. 2011). Furthermore, essential oils can be greatly effective on the pesticide resistant insects (Isman et al. 2011; Farajzadeh et al. 2014). In this chapter, the effects of essential oils varied according to the use of essential oils application such as use pure oil and binary combination between two types of essential oils, exposure time and the concentration used on *M. persicae*. The essential oil activity was observed within 1, 2, 3, and 5%. The results show that all tested essential oils applied by contact (direct spray on aphids) or residue (filter paper treated with different concentration of essential oils) demonstrated a toxic effect on *M. persicae*, although they differed in their efficacy. Two binary essential oil combinations achieved higher mortality than the single essential oil. Synergists are low in toxicity, have little or no inherent insecticidal properties, and have very short residual activity (Buss & Park-Brown 2002). There is potential for these essential oils as important alternatives as naturally occurring *M. persicae* control agents.
The results of this study showed the insecticidal effects of black pepper, eucalyptus, rosemary and tea tree essential oils and their binary combinations (BE, BR, BT, ER, ET and TR) in various concentrations on *M. persicae*. However, there were differences in the bioinsecticidal effects of ten essential oil treatments (4 pure and 6 binary combination oils) despite the fact that they all had significant aphidicial activity on *M. persicae* with mortality varying based on the concentration of essential oils, especially with high test doses for 24 hours exposure time. The efficacy of essential oils depends upon their chemical composition of each constituent present in the mixture. Limonene, α-pinene and camphene are major constituents in several essential oils and have demonstrated aphicidal activities against the woolly beech aphid *Phyllaphis fagi* and the palm aphid *Cerataphis brasiliensis* also limonene is able to attract aphid natural enemies so that using essential oil contained a high amount of limonene could act as an attractant of parasitoids and predators (Yazdgerdian et al. 2015). The compound eugenol and 1,8-cineole have been reported against many insects including aphids (Yazdgerdian et al. 2015; Liska et al. 2010). Other major constituents such as terpinene-4-ol, terpinene, 4-carene, α-phellandrene also have insecticidal effect against several insect species including aphids, and these compounds are found in the essential oils used in these experiments (black pepper, eucalyptus, rosemary and tea tree) (Kasmi et al. 2017; Dambolena et al. 2016; Abtew et al. 2015). Our results are similar with the results presented by (Sampson et al. 2005; Digilio et al. 2008) who indicated that an increase in the mortality of aphids reared on diverse of essential oils. The essential oils of black pepper and tea tree in contact treatment resulted in 80% mortality in the green peach aphid. Cloyd et al. (2009) reported results that are comparable with our findings where nearly 100% mortality was caused by essential oils of the black pepper and tea tree against rose-grain aphid. In addition, the residual treatment resulted in 100% mortality by using black pepper and tea tree essential oils, as in our findings, this indicated that the mortality caused by eucalyptus and rosemary oils were high after 24 h exposure time. However, the combination of TR, ET, and BT essential oils can cause over 90% of mortality when using a contact treatment. Görür et al. (2008) showed the parallel effect of essential oils against cabbage aphids and resulted in more than 85% mortality. Our findings of insecticidal effects of the tested combined essential oils in residual treatments resulted in 100% mortality with TR, ET and BT, and more than 96% mortality with BR, BT and ET. The reason for these results comes from essential oils constituents and their volatiles. The result are similar to
those obtained by Digilio et al. (2008) who showed high mortality rate on *M. persicae* and *A. pisum* by using different doses of various essential oils.

### 5.4.1. GC-MS analysis of essential oils

The results of GC-MS analysis of all types of essential oils represented in this chapter indicate that essential oils have considerable differences in their chemical composition and their percentage between pure and mixture essential oils. The chemical composition of black pepper has been shown to have several compounds that are important and involved for control of various pests. These constituents were Caryophyllene, D-Limonene, α-pinene, β-pinene, and Sabinene. This finding is consistent with past studies (Bernard et al. 1995; Scott et al. 2008; Tak et al. 2016). While, the results indicated that eucalyptus essential oil has eucalyptol and represented the major chemical composition that made up of 82.25% compared with other chemical compositions. Ben-Issa et al. (2017) showed that eucalyptol has insecticidal activities against many insects including aphids. Additionally, the highest constituents in rosemary essential oil were Eucalyptol, α-pinene, β-pinene and (-)-camphor. These components have pesticide properties that have been used in insect and spider mite control as presented in many past experiments as reported in (Miresmailli et al. 2006; Tak et al. 2016). Likewise, the GC-MS result in this chapter showed that tea tree essential oil contains the main compounds which are (-)-terpinen-4-ol and γ-Terpinene, and these compositions also have insecticidal properties and can affect insect enzymes such as AChE, GST and CarE (Mann et al. 2012; Coronado-Puchau et al. 2013; Liao et al. 2017). Interestingly, the quantity of the highest occurring compounds in the tested essential oils, changed after binary mixing, some constituents appeared in the essential oil combination as origin each oil, for example, chemical compositions in the combination of BE were Caryophyllene, D-Limonene, α-pinene, β-pinene, and Sabinene. These results indicated that disparity of chemical compound amount between two pure essential oils before mixing in same ratio and this may be due to the essential oil constituents being remade after mixing. In BE mixture, the main constitutions were reduced to nearly half compared with black pepper and eucalyptus alone. The BE combination showed most of the new compounds found in the mixture that were not found in the pure oils prior to mixing. This might result in a broad range insecticidal activity. While, in the blend of BR, the main compounds were α-pinene, β-pinene, D-Limonene, Eucalyptol, and Caryophyllene. The combination of BT resulted in (-)-terpinen-4-ol and Caryophyllene being the highest constituents.
in this mixture, this finding is consistent with (Liao et al. 2016) who indicated that the high quantity of terpinen-4-ol and caryophyllene in tea tree *M. alternifolia* oil. Moreover, ER, ET and TR has participated in the same components α-pinene, eucalyptol, γ-Terpinene and (-)camphor, all these compounds have properties against various types of insects and other pests as shown in previous studies.

### 5.4.2. The toxicity of essential oils binary combination

The results indicate that individual oils varied in their toxicity to tested aphids. The binary mixture of essential oils showed additive, synergistic and antagonistic effect; we found that their toxicity was as high as expected especially with short time and the use of low concentration. This indicates that some combinations have a synergistic effect. Synergistic activities have been observed in the BT combination and some other combinations of essential oils, there were also additive interactions and ER showed antagonistic interaction between two essential oil combinations because of the important interaction between essential oils and their components (Faraone et al. 2015). The authors suggested several assumptions that could illuminate the synergistic effect of black pepper and tea tree against *M. persicae* (1) the insecticidal mechanism of black pepper and tea tree might be different by acting on different targets of *M. persicae*, (2) the synergistic effect could be due to a parallel interaction of their mechanism, and (3) the synergistic effect occurs only once they have combined insecticidal effect on *M. persicae*. This finding follows that of Ngamo et al. (2007) and Pavela (2010) who reported that synergistic insecticidal activities could be observed not only in the combination of essential oils but also in their constituents as well as between synthetic insecticides with essential oils. The data showed that spraying a formulation of essential oils on capsicum and cabbage seedlings were active against green peach aphids. Essential oil formulations have insecticidal activities and great potential for aphid control, but there was phytotoxicity on the plant seedlings especially cabbage plants with the spray application.

### 5.4.3. The stability of essential oils during the storage time

The findings of our results indicate that the three temperature degrees (15, 25 and 35°C) had no affect on essential oil storage based on FTIR analysis and there was no change in functional groups
during all periods of storage tested. Our results are consistent with Turek and Stintzing (2012) who reported essential oils demonstrated oil stability at various temperature degrees (5-38°C) and showed no negative effects from high temperatures ever three months storage. The purpose of studies into storage at different temperatures was to determine the best temperature that essential oils can be stored without affect on their consistency.

5.5. Future studies could include:

1- To refined methods for reduce ethyl formate phytotoxicity on store commodities and try another effective combination of fumigants to target fast results and reduction of fumigation time.

2- Using the SPME technology in the field as early diagnostic tool for detection of pests on crops.

3- To use selective compounds from plant volatiles or from essential oils and test on aphids or use as a lure to attract natural enemies in the field for use in IPM system.

4- To conduct chemical analysis of botanical pesticide residues in the plant after treatment.
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