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Use of Cuban mint (*Plectranthus amboinicus*) as a botanical insecticide against aphids (*Aphis gossypii*: Aphididae) in open field versus greenhouse cucumber (*Cucumis sativus*) production

by

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A thesis submitted to the Anton de Kom University of Suriname, Faculty of Technology, Suriname, in fulfillment of the requirements for the degree of Master of Science (MSc) in Sustainable Management of Natural Resources

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PREFACE

This thesis is carried out as a completion of the master education in Sustainable Management of Natural Resources at the Technological Faculty of the Anton the Kom University of Suriname. The study is done to test the efficacy of the crude plant extract of Cuban Mint plant as botanical pesticide against an aphid pest in cucumber. The idea to do this came into my mind when I noticed, as a researcher of the Ministry of Agriculture, that the highly fragranced Cuban mint plant was not attacked by any insect pest. After some preliminary testing with the crude extracts of this plant against aphids, I decided to conduct further testing through my thesis project.

I am grateful for completing the Master in Sustainable Management in Natural Resources (SMNR) for giving me the opportunity to integrate my career into the thesis. I am also thankful to the Flemish Inter University Counsel VLIR (Vlaamse Inter-universitaire Raad) for giving support for this MSc. degree program in Suriname.

I am grateful to all people who have contributed either directly or indirectly to this thesis. I thank my supervisor Ms. Lydia Ori, Ph.D. and examiner Mr. Riad Nurmohammed, Ph.D. for their valuable input and support throughout the thesis period. In particular, I owe my deepest gratitude to my co-supervisor Ms. Kathleen Burke, M.Sc. for always supporting and advising me with my research project. Last and definitely the first, thanks to the Almighty God, who gave me everything I needed to complete this successful research.

If farm and economics go wrong, nothing else will go right in agriculture

-M.S. Swaminathan

EXECUTIVE SUMMARY

Aphis gossypii is worldwide an important pest of cucumber (*Cucumis sativus*) by causing direct sucking damage or functioning as a virus vector. This insect is also an important pest of cucumber in Suriname. During this research study, two different concentrations (100 g/l and 200 g/l) of crude extract of Cuban mint *Plectranthus amboinicus* had been tested for their insecticidal activity on *A. gossypii* in cucumber and was compared with the commercial pesticide abamectin. The experiment was executed at the Anton de Kom University (AdeKUS), located at the Leysweg in district Paramaribo. A Randomized Block Design (RBD), with 3 treatments and 3 replications was used in both the greenhouse and in the open field at the AdeKUS location. This whole experiment was executed twice; in a wet season and in an extremely dry season in 2017. The three treatments; 100 g mint/l, 200 g mint/l and abamectin were applied for four weeks on cucumber plants, which were intentionally infested with *A. gossypii*. The results reveal that, the recorded average number of live aphids of 100g/l mint was significantly higher than from 200 g/l and abamectin, Tukey's test ($P \leq 0.05$) while 200 g/l mint and abamectin did not significantly differ. During the rainy season (experiment 1), control with abamectin resp. 200 g mint/l reduced the population of the aphids in week 4 for 95.4 % resp. 79.6 % from the pre-spray. Meanwhile, during the extreme dry season (experiment 2), control with abamectin resp. 200 g/l reduced the population of aphids in week 4 with 85.1 % resp. 26.8 % from pre-spray. Also, mint extract with a dose of 100 g/l reduced the population during the rainy season in week 4 only for 16.0 %, while the population during the dry season increased during week 4 with 24.0 % for this dose. The results obtained in this study revealed that 100 g Cuban mint/l was not effective to control *A. gossypii* at all, while 200 g Cuban mint/l was as effective as abamectin. It is worth noting that mint extract with a dose of 200g /l did not control the aphid pest effectively in extreme dry season when the population was very high. The effect of all the 3 treatments on the number of aphids as well as on the cucumber production in the greenhouse did not significantly differ ($p < 0.05$) from the open field condition.

Keywords: ***Aphis gossypii,*** **Cucumber, Biopesticide, Climate Smart Agriculture, Integrated Pest Management, *Plectranthus amboinicus***

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List of Symbols and Abbreviations

Biopesticides - certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals (Environmental Protection Agency United States, 2016).

Organic agriculture - a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system (Food and Agriculture Organization [FAO], 1999).

Integrated Pest Management (IPM) - an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides (Food and Agriculture Organization [FAO], 2018).

Predator - an organism that preys, destroys, or devours another organism (retrieved from https://www.kerbtier.de/Pages/Content/enGlossar.html, n.d.).

Parasitoid - an organism that lives at the expense of another and finally killing its host (retrieved from https://www.kerbtier.de/Pages/Content/enGlossar.html, n.d.).

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CHAPTER 1. INTRODUCTION

1.1. Background information

The cotton aphid, *Aphis gossypii* which is a small soft bodied insect is distributed worldwide, but is especially abundant in the tropics. This insect is widespread throughout South America and the Caribbean including Suriname (CABI, 2018). It is an important pest in different agricultural crops which is known to cause enormous crop losses worldwide (Martin, 2015) and also in Suriname (Segeren, 1983).

In Suriname *A. gossypii* species occur in different crops such as cucumber, okra, hot pepper and eggplant (Segeren, 1983). However, this aphid pest often occurs on cucumber in the dry seasons (Entomology division, LVV, 2016). Cucumber is mostly consumed uncooked and in general harvested minimum twice a week which implies that the chosen pesticide needs to have a maximum pre harvest Interval of 3 days during the fruiting period. Cucumber are pollinated by bees and the applied pesticide should not be harmful for bees.

Although this insect can be controlled with local available chemical pesticides, there are organic farmers/ backyard growers who prefer bio pesticides. Organic farming is an important tool to promote sustainable farming (Hagemann, 2015) and does not allow use of conventional pesticides. Unfortunately, there are only few commercial bio pesticides available in Suriname (van Dijk, personal communication, July 2017). The available bio pesticides which are successfully used for aphid-control include liquid detergent and commercial bio pesticides such as agricultural soaps, neem oil or soy-oil based pesticides of which most of them are quite expensive. Abamectin is also an effective pesticide against aphid pests, but not recognized by all organizations as bio pesticide anymore (Environmental Protection Agency, personal communication, 2018); possibly because of its toxicity for fish and bees, this pesticide is considered as a conventional pesticide (University of Hertfordshire, 2018). Although the Ministry of Agriculture, Animal Husbandry and Fisheries (LVV) in Suriname recommends use of this pesticide, caution has to be taken for development of resistance and therefore, alternatives have to be researched.

The Cuban mint *P. amboinicus* has a strong smell and is used worldwide as herb, medicinal plant and also as botanical pesticide. Up till now, this plant has only been used as medicinal plant and spice in Suriname. Because of the limited choice of bio pesticides for organic farmers in Suriname and the high prices of ready-made botanicals, farmers should have the option to prepare these natural pesticides by themselves. In Suriname, little research has been done on the effectiveness of extracts of local available plants as botanicals (LVV, personal communication, July 2017).

1.2. Statement of the problem

Because of the limited choice of bio pesticides for organic farmers in Suriname and the high prices of ready-made botanicals, farmers should have the option to prepare these natural pesticides by themselves. In Suriname, research on the efficacy of extracts of local available

plants as botanicals has not been carried out extensively. (LVV, personal communication, July 2017). In this research crude extracts of the plant *Plectranthus amboinicus* were tested against the aphid, *Aphis* gossypii in the crop cucumber (*Cucumis sativus*).

1.3. Purpose and specific objectives

The purpose of this study is to assess the efficacy of the extract of *Plectranthus amboinicus* against aphids (*Aphis gossypii*) in cucumber (*Cucumis sativa*) production.

The main objectives were:

1. To determine if there is a difference in pest population and yield among various treatments
2. To determine if there is a difference in the efficacy of the bio pesticide between Greenhouse and Open Field conditions and between two growing seasons

The sub-objectives were:

1. To identify the various insect-pests present at the time of the investigation on cucumber production
2. To identify the various natural enemies of the aphid-pests present at the location of the experiment.

1.4. Significance of this study

This study highlights the importance of a plant extract made from the Cuban mint plant *Plectranthus amboinicus* and provide insights on how it can be tested for its efficacy against the aphid *Aphis gossypii* on cucumber production. Furthermore, farmers in Suriname will have access to a new bio pesticide which farmers can grow easy in their backyards and farm fields and which is safe to human health and the environment.

1.5. Limitations of the study

The aphids were grown in cages in a greenhouse under natural conditions because of the absence of growing chambers, where temperature and moisture can be controlled.

1.6. Outline of the study

The thesis will consist of five chapters. Chapter 1 contains background information from which the problem statement, the objectives and research questions are formulated. Chapter 2 consists of a literature study conducted on the biology of the aphid and the use of Cuban mint as a bio pesticide to produce safe cucumber production. Chapter 3 describes the experimental research and the statistical analyses that were conducted while the focus in chapter 4 is to present the findings and the discussion of the study. Chapter 5 presents the conclusions and recommendations of this study.

CHAPTER 2. LITERATURE REVIEW

2.1. Taxonomy, distribution and hostplants of aphids

The aphid or plant lice (common names) belongs to the order Hemiptera, suborder Homoptera (aphids, hoppers, whitefly and scales) and form the superfamily Aphidoidea and family Aphididae.

Aphids (Figure 1) are small (mostly about 1-2 mm long), soft-bodied, pear-shaped insects with long legs, antennae and long slender sucking mouthparts. Some secrete a waxy white or gray material that covers their body. Most species have a pair of tube like structures called cornicles or siphuncules, which project backward out of the hind end of the body. The presence of cornicles distinguishes aphids from all other insects (Figure 2). Aphids are green, yellow, brown, red, or black colored depending on the species and the plants they feed on (Barbercheck, 2014).

There are more than 5000 species of aphids worldwide, from which about 100 species are pests in the agriculture (Eastop, 2000). From the 10 species, recorded as pests in Suriname (Dinther, 1960), one species namely *Aphis gossypii* is used in this study.

Figure 1. *Aphis gossypii*

Reprinted from [*Aphis gossypii*] (2011), by National Center for Biotechnology Information. Retrieved from: http://prgdb.crg.eu/wiki/Species:Aphis_gossypii

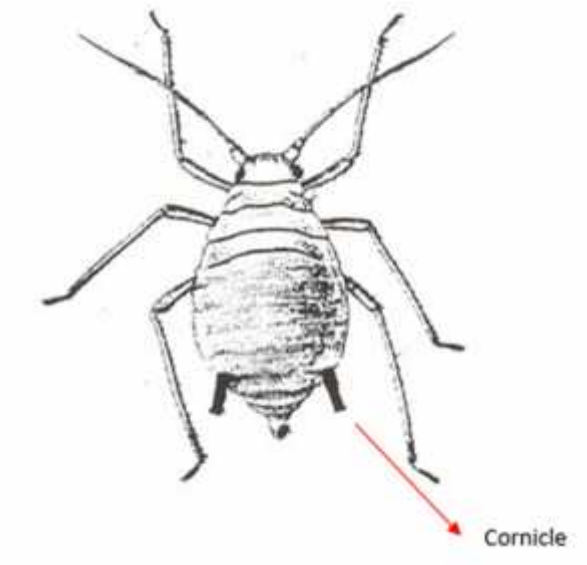

Figure 2. *Aphis gossypii* with cornicle

Reprinted from [*Aphis gossypii*] (n.d), Copyright 2018 by ENTOCARE Retrieved and adapted from: https://www.entocare.nl/pests/aphids/cotton-aphid/?lang=en

The taxonomic classification of *Aphis gossypii* is provided below (CABI, 2018):

Domain:	Eukaryota
Kingdom:	Metazoa
Phylum:	Arthropoda
Subphylum:	Uniramia
Class:	Insecta
Order:	Hemiptera
Suborder:	Sternorrhyncha
Unknown:	Aphidoidea
Family:	Aphididae
Genus:	Aphis
Species:	*Aphis gossypii*

Aphis gossypii has a worldwide distribution, including in Asia, Africa, North-, Central- and South America, Caribbean and Europe. It is commonly present in tropical areas, but in regions with a cold climate it is found on crops grown in greenhouses (CABI, 2018).
The most important hostplants of *A. gossypii* are*: Cucumis sativus* (cucumber) *Abelmoschus esculentus* (okra) *Apium graveolens* (celery) *Averrhoa carambola* (carambola) *Capsicum annuum* (bell pepper) *Capsicum frutescens* (chilli) *Citrullus lanatus* (watermelon) *Cucurbita moschata* (pumpkin) *Gossypium* (cotton) (CABI, 2018).

2.2. Biology and Life cycle of *A. gossypii*

The biology of aphids is quite complex. Adult aphids are usually wingless, but most species can be winged (alate), depending on the circumstances like overcrowding, stress, or when alternating the host plant. They can then move to other plants when the quality of food source decreases. Aphids mostly appear in dense groups on leaves or stems (Biological Services Australia, 2015). Most of the aphids are female, rarely lay eggs and reproduce asexually by giving birth to live immature aphids called nymphs. These nymphs lack wings, are born complete and immediately start feeding on plant sap. Each nymph grows quickly and molt, through a number of immature stages to an adult (female) that can then begin to reproduce. The time taken by aphids to develop depends on many factors, such as: host plant species and its food quality, climatic conditions and population density. Under optimal conditions the development of young aphids to adults are just completed in a few days (Barbercheck, 2014). *A. gossypii* lives for two to three weeks, produces three to ten young aphids daily; it can multiply itself four times (in aubergine) to twelve times (in cucumber) in seven days (Biological Services Australia, 2015). Some aphid species produce males just to winter in cooler climates. In this case, adults mate and females lay eggs that do not hatch until temperatures increase in spring. In warmer climates, and in greenhouses the populations may reproduce asexually for the entire year (Manners, 2017). Because each adult female aphid can produce numerous nymphs in a short period of time, aphid populations and their damage can rapidly increase.

2.3. Damage by aphids

Aphids cause direct- and indirect damage to plants (Barbercheck, 2014). This direct damage is caused by feeding activity, which results in decreased growth rates and reduced vigor; mottling, yellowing, browning or curling of the leaves; wilting, low yields and eventually plant death. Infested flower buds and fruits can be malformed or dropped. While aphids pierce the plant tissue and suck plant sap, they can inject toxic saliva into plants causing curled and distorted leaves. These curled and distorted leaves offer protection for the aphids against natural enemies or applied treatment materials. According to Barbercheck (2014), indirect damage is caused by secreted honeydew from the aphid anus. Honeydew is a sticky, sugary liquid substance produced by aphids as a waste after feeding on plant sap. The accumulated honeydew deposits can function as a growth substrate for a complex of black saprophytic fungi species. The black layer of fungi, named sooty mold, on leaves and other plant parts blocks light and can restrict photosynthesis. Honeydew also attract other insects, such as ants, that feed on the honeydew. Ants can aggressively defend aphids (and their honeydew food source) from predators and parasites, and interfere with the control of aphids by natural enemies. Furthermore, Barbercheck (2014) indicates, that indirect damage is also caused by viruses, transmitted by some aphids especially in cucurbit crops. The direct damage of these aphids are not the major cause of the economic losses in cucurbit crops, but the viruses transmitted by them can cause severe losses. Barbercheck (2014) also indicates that several mosaic diseases are caused by aphid-transmitted viruses such as cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), and papaya ringspot virus (PRSV). And *A. gossypii* is an important carrier of viruses, transmitting at least 44 viruses, such as cucumber mosaic virus. The symptoms of virus infection are mottling, yellowing or curling of leaves, and stunting of plant growth, resulting in reduced growth and yield. The higher the aphid population, the more rapid the virus spread (Barbercheck, 2014).

2.4. Control of aphids

A. Prevention. If possible aphid attacks should be prevented. This can be done by the following actions (Barbercheck, 2014):

- Monitoring the plants regularly: inspection of the plants at least twice a week for presence of aphids to discover infestations early in the growing season. Observation for aphids on the undersides of leaves and new growth. Checking also for the sticky honeydew they produce while feeding and their cast skins, which look like white bits of dust.
- Removing attractive weeds where aphids can survive in absence of the grown crop. Because local data of host weeds of aphids especially *Aphis gossypii* is lacking, weeds grown in the neighborhood can be inspected on crinkled leaves and presence of aphids under the leaves.
- Preventing over-fertilization with nitrogen. Redundant nitrogen causes turgor imbalance in the plant resulting in exudates leakage from the leaf surface. These

exudates contain simple sugars, free nitrogen and simple amino acids, which are attractive food for aphids. Therefor slow-release fertilizers or organic materials such as compost can be used to nourish plants.

B. **Physical control**. This can be done by the following actions (Barbercheck, 2014):

- Spraying plants with water using a spray bottle (for seedlings) or a hose (for established plants) to knock aphids off an infested plant. This can be repeated as necessary until the natural enemies are observed.
- Removing heavily infested plants and stems that may function as infection source can be removed and buried deep in compost pile or soil.
- Controlling ants that feed on the excreted honeydew and protect the aphids from their natural enemies. This can be done by placing a band of sticky material around the trunks of aphid-infested trees or woody plants.

C. **Ethological control measures**. These are based on the behavior of insects. For example, aphids are attracted by yellow color and distracted by reflective light. Based on this information, physical tools and materials are setup to prevent the pest insects from settling on the crops; yellow sticky traps are used to attract aphids and reflective plastic mulch are used for prevention (Bio-Integral Resource Center [BIRC] California, 2015).

D. **Biological Control**. Aphid populations can be suppressed by natural enemies (beneficials) as predators, parasitoids or pathogens (Patterson, 2016). Many predators are generalists; they prey on many different species. Some predators do have a specialized or specific diet. **Ladybeetles** are probably the most well-known of predator beetles that prey on aphids. There are many species from which both the adults and larvae consume aphids. Other predators are adults of the **soldier beetle** (Figure 3). The larvae live in the soil and help to control soil-borne pests. Also many species of **long-legged adult stage flies** (Dolichopodidae) predate on soft-bodied pests. The adults are recognized by their metalic green or blue color (Figure 4). Predators as **Syrphid flies** have about the same size as house flies and hover in flight. The adults, which sometimes resemble bees, are not predaceous. But the larvae, which vary in color from green to brown are aphid predators (Figure 5). The larvae of **predaceous midges** are very small (about 1/10 inch long), but are generalist predators of mites, aphids and other soft-bodied insects. The larvae are yellow to orange in color (Figure 6). The adults are not predatory.

True bugs (Hemiptera); damsel bugs and big-eyed bugs are also generalist predators and both the adults and nymphs eat aphids and other soft-bodied insects, especially on shorter growing plants. Another predator are minute pirate bugs, which are very small (about 1/12 inch long) with black and white color and an X pattern across the back. Both the nymphs and adults are generalist predators feeding on small insects (Figure 7). Also lacewings (Figure 8) are common generalist predators that feed on aphids. Some species of adult lacewings are predaceous while the larvae are very active predators that feed on soft-bodied prey such as mites, aphids, leafhoppers, thrips, whiteflies, and pest eggs.

Figure 3. *Soldier* beetle
Reprinted from [Soldier beetle], (n.d.) by Joseph Berger. Retrieved from R. Patterson and R. Ramirez, 2016

Figure 3. Long-legged fly
Reprinted from [Long-legged fly], (n.d.) by Susan Ellis. Retrieved from R. Patterson and R. Ramirez, 2016

Figure 4. *Syrphid* fly
Reprinted from [Syrphid fly], (n.d.) by Susan Ellis. Retrieved from R. Patterson and R. Ramirez, 2016

Figure 5. Predacous midge
Reprinted from [Predacous midge] (n.d.) by Whitney Cranshaw. Retrieved from R. Patterson and R. Ramirez, 2016

Figure 6. Minute bug
Reprinted from [Minute bug] (n.d.) by Jack Dykinga. Retrieved from R. Patterson and R. Ramirez, 2016

Figure 7. Lacewing
Reprinted from [Lacewing] (n.d.) by Whitney Cranshaw. Retrieved from R. Patterson and R. Ramirez, 2016

Some predators on *Aphis gossypii* have been recorded in Suriname; such as *Colleomegilla maculata* (De G.) (Figure 9), *Cycloneda sanguinea* (L.) (Figure 10), *Psyllobora divisa* (F.) (Figure 11), *Hyperaspis festiva* (Figure 12), *Baccha* sp. (Dinther, 1960).

Figure 8. *Colleomegilla maculata*

Reprinted from [*Colleomegilla maculata*], (2018). Copyright 2009 by Thomas Bentley Retrieved from https://bugguide.net/node/view/280162%20https://www.coccinellidae.cl/paginasWebCol/Paginas/Psyllobora_sp_24_Col.php%20http://nathistoc.bio.uci.edu/coleopt/Cycloneda.htm

Figure 9. *Cycloneda sanguinea*

Reprinted from [*Cycloneda sanguinea*] (2018). Copyright 2010 by Mike Quinn Retrieved from https://bugguide.net/node/view/411992

Figure 10. Psyllobora divisa

Reprinted from [*Psyllobora divisa*] (2016). Copyright 2016 by G. González Retrieved from https://www.coccinellidae.cl/paginasWebCol/Paginas/Psyllobora_sp_24_Col.php

Figure 11. Hyperaspis festiva

Reprinted from [*Hyperaspis festiva*], (2006-2013) by R. G González Retrieved from http://coleoptera-neotropical.org/paginas/2_PAISES/Antillas/CUCUJOIDEA/coccinellidae-Antill.html

Figure 12. Parasitic wasp deposits eggs in aphid

Reprinted from [parasitic wasp deposits eggs in aphid], (n. d.). Copyright 1992-2017 by Greenmethods.com Retrieved from https://greenmethods.com/aphidius/

Figure 13. Parasitized aphid

Reprinted [Parasitized aphid] by David Kappaert. Retrieved from R. Patterson and R. Ramirez, 2016

There are also several species of parasitoid wasps that have their own aphid species to parasitize. These parasitoids are very small (about 1/8-inch long) and female wasps have a modified stinger for depositing eggs (Figure 13). The egg is laid into an aphid where the larva develops inside. Parasitized aphids are recognized by their bulbous and light tan to gold colored appearance (Figure 14). The adult wasp makes a circular cut out on the rear-end of the aphid to emerge. By providing a suitable habitat (perennial plantings or border plantings) a source of water and a variety of flowering plants natural enemies can be encouraged. Different flowers provide their nectar as alternative food resources and shelter for natural enemies to complete their life cycle. These beneficial insects can reduce the likelihood of an infestation if they are present in the field early on other hostplants; for example, marigold and sunflower often attract lady beetles.

E. **Botanical pesticide control** Some plants contain components that are toxic to insects. The term botanical is used for the substance obtained from a plant and used typically as pesticide. The use of botanicals in agriculture dates back at least two millennia in Egypt, China, Greece and India. Even in Europe, use of plant extracts started about 150 years ago, after the discovery of many negative environmental impacts of major synthetic chemical pesticides. Often these plants also have other uses than agriculture like household insect repellents or are plants with medicinal applications (El-Wakeil, 2013). A lot of aromatic plants, many from the mint family (Lamiaceae) consist of essential oils, derived by steam distillation, are used as botanicals. The most famous and widely used botanicals are Rotenone (*Derris* sp.), nicotine (tobacco), and pyrethrins (*Chrysanthemum* sp.). These oils have their action as insecticide, repellant, fumigant or as anti-feedant (Mossa, 2016). The use of botanicals is mostly affected by raw material availability, solvent types, plant species and part of the plant, rapid degradation, time of exposure, direct and indirect contact, and weather conditions (El-Wakeil, 2013).

Most botanicals act as contact, respiratory or stomach poisons. Therefore, they are not very selective, but work on a broad range of insects, even on beneficial organisms. Still

botanicals are less toxic and can have less impact on natural enemies if used selectively. Furthermore, the rapid degradation of botanical pesticides reduces the negative impact on beneficial organisms and residues in food. More over resistance to botanicals is not developed as quickly as with synthetic pesticides. Also the preparation and use of plant extracts requires some know-how, but not much material and infrastructures (Foguelman, 2003). Since most of these plant extracts are degraded rapidly by UV light, the duration of their action is short. Therefor frequent spray is recommended, which is labor intensive. Above all, the recommendations followed by farmers are merely not scientifically tested. The sprayed liquid botanicals hardly reach the insect pest hidden in curled leaves; for example, heavily infested plant with aphids. If the infested population is low, the leaves are less curled and spray in that stage is more effective. Developing countries where farmers may not afford synthetic pesticides, can have grateful use of botanicals. These botanicals can also be alternated with the use of microbial bio pesticides, since resistance of the diamondback moth to the bio pesticides *Bacillus thuringiensis* and spinosad because of overuse is proven (El-Wakeil, 2013). Some alternatives of botanicals which can be used in Suriname are given in the table 1 (Stoll, 2000) and (Pluke, 1999).

Table 1: Botanicals for aphid control

Common name botanical	**Scientific name**	**Recommended dose**
African Marigold, "Gena, Afrikaantjes"	*Tagetes erecta*	500g leaves/ 1 liter water
Garlic	*Allium sativum*	1 bulb per 5 liter water
Ginger	*Zingiber officinale*	2 kg rhizomes/ 30 liter water
Lantana	*Lantana camara*	500 g leaves/ 1 liter water
Tobacco, "Tabak'	*Nicotiana tabacum*	1 kg crushed stalks and leaves in 15 liter water
Neem-oil	*Azadirachta indica*	3 cc/liter
"Kwasibita"	*Quassia amara*	500 g chips, 40 cc dish washing liquid in 20 liter water
Turmeric	*Curcuma domestica*	500 g rhizomes /20 liter water

Insecticidal soap and horticultural oils can also provide effective control if applied thoroughly. Oils work by choking soft-bodied insects, and soaps kill these pests by removing their protective surface waxy coating. Also microbials as abamectin, *Beauvaria Bassiana*, *Paecilomyces* sp., *Metarhizium anisopliae* and spinosyns (Spinosad) can also be effective in controlling aphids. (Pesticide research Institute United States, 2018)

F. **Chemical control.** In case of serious infestation chemical pesticide can be applied unless selective and least toxic pesticides are used and pre-harvest interval has been taken into consideration.

LVV recommends mostly abamectin as a foliar application for aphid control in cucumber. Abamectin is available with the trade name Abalotin, Cure and Vertimec and has a pre-harvest interval of 3 days (LVV, personal communication, January 2017). Abamectin is a mixture of avermectins, which are insecticidal or anthelmintic compounds derived from the soil bacterium *Streptomyces avermitilis*. Abamectin is a natural fermentation product of this bacterium, acts on insects by interfering with neural and neuromuscular transmission and has an oral LD50 in rats of 11 mg/kg (Cornell University Cooperative Extension, 1994).

2. 5. Taxonomy of *Plectranthus amboinicus*

The taxonomic classification of the Cuban mint *Plectranthus amboinicus* (Figure 15) is as follows (CABI, 2018):

Kingdom:	Plantae
Division:	Angiosperms
Class:	Eudicots
Order:	Lamiales
Family:	Lamiaceae
Genus:	Plectranthus
Species:	*P. amboinicus* (formerly identified as *Coleus amboinicus*)

2.6. Botany *P. amboinicus*

The lamiaceae family includes some of the most well-known herbs containing essential oils as lavender, sage, basil, mint and oregano. *Plectranthus amboinicus* is a large succulent herb, fleshy and highly aromatic, much branched, 30-90 cm long stems with short soft erect hairs. Leaves have a distinctive smell, are densely covered with soft, short and erect hairs, pubescent. They are also simple, opposite, broadly ovate (2.5-5 cm long), very aromatic and thick. Flowers are shortly pedicelled, 3 mm long, pale purplish in dense whorls at distant intervals in a long slender raceme. Upper calyx lip is ovate, acute, membranous, lower acuminate. Corolla are pale purplish, tube short, throat inflated, lips short.

2.7. Origin and growth conditions *P. amboinicus*

The origin of *P. amboinicus* is unknown, but it may be native to Africa and possibly India (CABI, 2018). This plant grows in full sunny conditions but prefers shade (Don Newcomer's Wild World of Succulents, 2014). It is heat and drought tolerant, can easily propagated from stem cuttings or seed and require little maintenance as pruning at the end of the flowering season (Rice, 2011).

Figure 14. *Plectranthus amboinicus*

Reprinted from [*Plectranthus amboinicus*] (n.d.) by Zuo shou xiang. Retrieved from http://.stuartxchange.org/Oregano.html

2.8. Use of *P. amboinicus*

It has been widely cultivated as a medicinal plant, potherb, ornamental and spice in tropical regions around the world (CABI, 2018). The aromatic leaves are used as a food additive or spice, flavor for meat, soups, fish, and local beer. The leaves are also eaten as a vegetable, as well as for washing clothes, hair and laundry because of its fragrance. The herb is used as a folk remedy for burns and bites, internally as a carminative and anti-asthma, and applied externally as an insect repellant, and is also often grown as an ornamental plant for its attractive leaves and flowers. In Brazil, this species is often grown in agricultural area and also used for its essential oils, as a food additive, vegetable, insect repellant and in religious rituals to prevent spirits (CABI, 2018). The pharmacological activities of the compounds as anti-bacterial, anti-fungal and anti-tumoral, make *Plectranthus* an important genus to search for drug development (Rice, 2011). This expresses the use of *P. amboinicus*: mostly used as medicine (44.02%), followed by food (40.15%) and repellent / insecticide (6%)

(Prashanth, 2017). In Suriname this plant is mostly used as spice for fish and meat. The Indian immigrants in Suriname use this plant to make pickles (pudina chutney).

The essential oil of the whole plant, obtained by steam distillation (0.04-0.05%), contains mostly thymol (41.3%) and carvacrol (13.25%) (Roshan, 2010). The fresh leaves contain 0.055 % volatile oil, dominated by carvacrol. Aqueous leaf extracts consist of tannins, saponins, flavonoids, steroid glycosides, and polyuronides. Crude ethanolic extract of leaves consist of alkaloids, flavonoids, terpenoids, phenols, saponins, carbohydrates and protein (Xiang, 2017).

2.8.1. Use of *P. amboinicus* as botanical pesticide

Essential oils show good potential in the control of insect and mite pests as anti-feedant and repellant and can be affectively applied as spray or fumigant. They may also be selective towards natural enemies. These oils have been used as repellant and anti-feedant in the control of aphids (Digilio, 2008). According to Gunarathna (Gunarathna, 2009) as most of the essential oils are found in common foods and many are approved as food flavorings, no negative effects to humans are expected from use of these plant or constituents as repellents and insecticides. The essential oil of *P. amboinicus* is both tested in the health- and agricultural sector as pesticide. It shows good larvicidal potency for the control of different mosquito larvae, such as African malaria vector, *Anopheles gambiae* (Kweka, 2012), malarial vector *Anopheles stephensi* (Xiang, 2017) and dengue vector *Aedes aegypti* (Mariano, 2017). This oil has also larvicidal activity against larvae of the cacao moth, *Ephestia cautella* Walker (Lepidoptera:Pyralidae) (Sunarti, 2003). Above all the essential oil of *P. amboinicus* has been positively tested against white termites (*Odontotermes obesus* Rhamb) as pesticide, where it has been proved to be more active than synthetic insecticides, Thiodan and Primoban-20 (Xiang, 2017). Finally, it has also been experimented that leaf extracts of *P. amboinicus* triggers plant-defence mechanism against pathogen-attack in rice plants proving its ability to induce systemic resistance in Rice against sheath blight disease (Gurijala, 2016).

2.8.2. Use of *P. amboinicus* in this experiment

In this thesis experiment, crude extract of *P. amboinicus* has been used as a pesticide to control aphids in cucumber. The essential oil of has not been extracted but the plant pulp is used as crude material because this test is done as a potential botanical for farmers, who are supposed to use plant extracts in a simple way. The qualities of this plant as easily grown and chopped, favors it as home-made botanical pesticide.

In this experiment also the LC50 (Lethal Concentration 50) for the crude mint extract has been determined. LC50 is a standard measure of the toxicity of a substance that will kill half of the sample population (50%) of a specific test-animal in a specified period through exposure (Business Dictionary, 2018). This concentration is also used to determine which concentration(s) to use for the efficacy test as “pesticide”. It can be visualized which concentration of the “pesticide” is more effective than the other by plotting the response of

the organisms e.g. insects to various concentrations of a “pesticide”. The best way for the calculation is to fit a regression of the response versus the concentration, or dose and compare between the different concentrations.

Probit (probability unit) analysis is a specialized regression model of binomial response variables. Probit analysis is used to analyze many kinds of dose-response or binomial response experiments in a variety of fields (Vincent, 2008). This analysis is commonly used to determine the relative toxicity of “chemicals” to living organisms. This is done by testing the response of an organism under various concentrations of each of the “chemicals” in question and then comparing the concentrations at which one encounters a response. The response is always binomial (e.g. death/no death) and the relationship between the response and the various concentrations is always sigmoid (S-curve). Probit analysis transforms from sigmoid to linear and then runs a regression on the relationship. This can be done with the use of several statistical software programs. The LC50 is determined by searching the probit list for a probit of 0.5 and then taking the concentration it is associated with (Vincent, 2008).

2.9. Cucumber *Cucumis sativus*

2.9.1. Taxonomy and common names *Cucumis sativus*

The taxonomic classification according to Centre for Agriculture and Bioscience International [CABI] (2018) is as follows:

Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Violales
Family:	Cucurbitaceae
Genus:	Cucumis
Species:	*Cucumis sativus*

According to CABI (2018), the cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family, which consists of 90 genera and 750 species, while the genus *Cucumis* contains nearly 40 species. The common names of *Cucumis sativus* L. are Cucumber, gherkin (English); cohombro, pepino (Spanish); concombre, cornichon (French) Gurke (German); khira (Indian); cetriolo (Italian); augurk and komkommer (Dutch) (CABI, 2018).

2.9.2. Botany *C. sativus*

The cucumber plants are coarse, prostrate annual creeping vines, that use their simple tendrils to climb over trellises or other supporting frames, wrapping around ribbing with thin, spiraling tendrils (CABI, 2018). They have large, prickly, hairy triangular leaves, which are alternate and simple, with 3-7 palmate lobes and serrated margins. The 5-merous

flowers are yellow and bear either female or male organs. The female flowers have the swollen ovary at the base, which will become the edible yellow to green fruit, up to 50cm long (CABI, 2018).

2.9.3. Use and nutritional values *C. sativus*

Cucumber fruits are used raw (sliced) and also as pickles, prepared with vinegar, salt sugar and spices. According to Ware (2018) 1 cup of raw sliced cucumber with peel, weighing around 52 grams (g) contains: 49.52 g of water, 8 calories, 0.34 g of protein, 0.06 g of fat, 1.89 g of carbohydrate, including 0.9 g of fiber and 0.87 g of sugar, 8 milligrams (mg) of calcium, 0.15 mg of iron, 7 mg of magnesium, 12 mg of phosphorus, 76 mg of potassium, 1 mg of sodium, 1.5 mg of vitamin C and 4 micrograms (mcg) of folate. It also contains thiamin, riboflavin, niacin, vitamin B-6, and vitamin A. Cucumbers also contain lignans, which may decrease the risk of cardiovascular disease and several types of cancer.

2.9.4. Origin and growth conditions *C. sativus*

Cucumber plants originate most likely from India, from where they have quickly spread to China and Europe (Haifa, 2014). Cucumber has been cultivated now everywhere for more than 3000 years and grows on moist, well-drained (sandy) soils rich in organic matter and slightly alkaline. It requires an optimum day temperature of 28 °C for growth and prefers full sun exposure in warm and humid climates. *C. sativus* is pollinated by bees that harvest the nectar produced by the flowers (Haifa, 2014). Cucumber can be grown in greenhouse and in open field systems (Yousefi, 2012).

2.9.5. Fertilization *C. sativus*

The recommended fertilizers for cucumbers produced under open field conditions are: 400 g Chicken manure (before planting) and 20 g NPK-Mg (12-12-17-2) per plant (Milton, 2005). The chicken manure of Suriname consists of 0.76 % N, 0.21 % P, 1.1 % K and 0.75 % Mg (Veltkamp, 1975). Chicken granules (Agrogold) consist of 4 % N, 3 % P and 2 % K and can replace Surinamese chicken manure by 1/5 part of the advised weight amount. The recommended pH is 6-7 and nutrient per plant is 5.6 g N, 3.2 g P and 7.8 g K (Milton, 2005).

2.9.6. Pest and diseases *C. sativus*

According to Segeren (1983), the major pest of cucumber in Suriname are: ***Diaphania hyalinata*** (cucumber moth): The moth has white colored wings edged with a thick dark brown to black band and brushy hair at the tip of the abdomen. The larva is bright green, 1-3 cm long, with white dorsal stripes in the final stage. The larva feeds on the young leaves and construct a loose silken structure under the leaves to pupate are.

Diaphania nitidalis (cucumber fruit borer): The moth has yellow wings, which are bordered in dark brown. The wing expanse is about 3 cm. Both sexes have brushy hair at the tip of the

abdomen. The young larvae are nearly white in color with numerous dark gray or black spots. The dark spots are lost at the molt to the fifth instar. Larval color during the last instar is somewhat variable, depending largely on the insect's food source. Before pupation, the larva turns in a red brown color and get a length of 2.5 cm. The presence of the larva in the fruit makes it unmarketable, and fungal or bacterial diseases often develop through the entry hole.

Aphis gossypii (aphid): Yellow to green colored small insects (1-2 mm) are feeding in groups at the underside of the leaves. The sucking damage causes discoloration and malformation of the leaves and may cause fruit-/blossom drop. These insects also transmit viruses, which can lead to enormous yield reduction.

***Tetranychus* sp.** (red spider mite): extremely small, oval shaped, about 0.5 mm and barely visible with the naked eye as reddish spots at the underside of the leaves. They move very slowly and spin webbing between the hairs and nerves of the leaves. Damage can be seen as chlorosis of the leaves where the mites have been feeding. High infestations can cause death of the plants.

Pentatomidae (unidentified species of Stink bugs): Fruits are misshapen because of sucking damage in the young fruits (Entomology Division, LVV, Suriname).

Nematodes (*Meloidogynae* sp.) (Segeren, 1983): These very tiny plant parasitic roundworms cannot be seen with naked eye and live in the soil. After they infest the roots, galls are formed. Galls in the roots are disrupting the nutrient uptake, which results in reduced growth and fast decaying plants in the dry season.

Diseases which are detected by LVV on cucumber in Suriname, but not identified yet, are: **False mildew** (*Pseudoperenospora sp.*): Cucumber plants infected by *P. cubensis* show angular chlorotic lesions bound by leaf veins on the foliage. Infection of cucurbits by this fungus impacts fruit yield and overall plant health (Savory, 2011).

Powdery mildew: Powdery mildew is characterized by obvious patches of whitish mycelium (resembling white powder) on upper and lower leaf surfaces, petioles, and stems. (UConn University of Connecticut, 2016). It is first noticed on the older leaves as pale yellow spots on stems, petioles, and leaves. These spots enlarge as the white, fluffy mycelium grows over plant surfaces and produces spores, which give the lesions a powdery appearance. Infected leaves become dull, chlorotic, and possibly show some degree of wilting in the afternoon heat; eventually they become brown and papery. Because of the infection the photosynthesis in the plant decreases. This leads to growth reduction, premature foliage loss, and consequently a reduction in yield (UConn University of Connecticut, 2016).

Virus: Cucumber mosaic virus (CMV): Four or five days after infection, the leaves become mottled, distorted, and wrinkled, and their edges begin to curl downward (Ferreira, 1992). This results in growth reduction and dwarfed appearance: shorter stem internodes and petioles, and underdeveloped leaves. Infected plants produce few flowers and fruit. Older

leaves get first necrotic areas along the margins which later spread over the entire leaf (Ferreira, 1992).

CHAPTER 3. MATERIALS AND METHOD

3.1. Introduction

The research investigation was carried out at the Anton de Kom University of Suriname (AdeKUS). Experiment 1 of this study was carried out from June 13- September 4, 2017 during a wet season and repeated as experiment 2 from September 4- November 6, 2017 during a dry season. The wet season is characterized by higher rainfalls and lower day temperatures, while the dry season has less rainfall and higher day temperatures. Both experiments had taken place at the Anton de Kom University, located at the Leysweg in Paramaribo. Each of the two experiments were executed simultaneously in a greenhouse and under open field conditions in order to test the efficacy of crude extract of the plant *Plectranthus amboinicus* against aphid (*Aphis gossypii*) pest control. This was done in the crop cucumber (*Cucumis sativa*) crop, grown in plant pots.

The study consisted of two phases:

1. A pre-experiment phase lasted from January to June 2017:
   - A telephone/ field visit Interview with 20 cucumber farmers
   -Identification of the mint plant *Plectranthus amboinicus*
   -Cultivation of *Plectranthus amboinicus* plant
   -Rearing of aphid *Aphis* gossypii in cages
   -Identification of the aphid used in this investigation
   -Probit analyse

2. Experiment phase from June to November 2017 consisted of the following activities:
   -Experimental set up
   -Cucumber cultivation
   -Inoculation aphids on plants
   -Preparation and application of (bio)pesticides
   -Data collection
   -Identification of natural enemies of *A. gossypii* occurring in the thesis field

3.2. Pre-experiment phase lasted from January to June 2017

3.2.1. Interview with 20 cucumber farmers

Twenty cucumber farmers from different farm-locations were interviewed by phone or field visit. These cucumber farmers were asked the following questions:

Do you have aphids as pest in your cucumber crop regularly? If Yes, which control strategies do you apply to control this pest?

3.2.2. Identification of the mint plant as *Plectranthus amboinicus*

Stalks with flowers of the mint plant were brought to the Herbarium of Anton de Kom University where this plant was identified as *Plectranthus amboinicus*.

3.2.3. Cultivation of *Plectranthus amboinicus* plant

Six month before the execution of this experiment, *Plectranthus amboinicus* plants were propagated by 30 cm long cuttings from plants originating from Letitia Vriesdelaan. Ten plants were grown in open field and full soil at Goneshstraat in Livorno area in district Wanica. During the thesis experiment these plants were grown to shrubs on an area of about 10 m². The shrubs were fertilized once in the three months with 100 g. chicken manure per plant and frequently irrigated in dry periods. During the experiment the leaves and shoots of the plants were used to make the botanical pesticide.

3.2.4. Rearing of aphid *Aphis gossypii*

During four weeks, 10-15 seeds were sown weekly to grow in pots in a greenhouse of LVV at Letitia Vriesdelaan 10 for a continuous supply of plants to rear aphids on it. The plants were fertilized with chicken manure and watered daily. Two- to three weeks after the sowing date these plants were transferred into screened cages. Three- to four weeks after the sowing date, the aphids were transferred with aspirators and paintbrushes to the plants in the cages. These actions were continued to get a well settled and high population of aphids for the experiment. These aphids were initially collected from heavily infested and untreated cucumber plants of the Santo Boma and Leidingen area in district Wanica. The cages were finally transferred to the Greenhouse of the Anton de Kom University to have better rearing conditions. Four cages had the size of 60 X 60 X 80 cm and one cage had the size of 100 X 100 X 100 cm. De cages were made of a PVC frame covered by organza screen.

3.2.5. Identification of the aphid used in this thesis

Aphids were collected from the cages and stored in glass vials with 70 % ethanol for slide preparation. These were prepared in the entomological laboratory of the LVV as per Manya Stoetzl method (Dooley, 2002), as follows:

-the aphids were transferred to a 10% KOH solution in a covered watch glass and heated to a temperature of 110 F for 15 minutes in an oven.
-The access body fat was removed with an insect pin and the specimens were transferred to 70 % ethanol again with a small spatula. Then body content was removed by gently pressing down on the abdomen.
-The specimens were pumped in distilled water and left for 5 minutes.
-Excessive water was removed gently with a pipette and some drops of acid fuchsine stain was mixed and specimens were left in this mixture for 5 minutes
-The specimens were transferred to 70 % ethanol and left for 5 minutes.
-The specimens were transferred to 95 % ethanol and left for 5 minutes.
-The specimens were transferred to clove oil for 5 minutes and excess ethanol was gently removed by pumping.
-Each specimen was transferred to a slide, where excess clove oil was removed with a piece of tissue paper.

-A drop of Euparal was placed on the specimen and carefully topped with a cover slip.
-The slide with specimen was observed under the light microscope for identification with the use of an identification key (Blackman, 2017).

An insect key is a tool used to determine the species of a given insect. This key (Blackman, 2017) consists of couplets or choice between 2 options based on a description of a particular feature (e.g. insect size, antennae shape). The option, which best matched the aphid insect being identified was chosen. This choice led to another couplet. The process continued until a final couplet that identifies the aphid insect, had been chosen. The list of materials used in this experiment is attached in appendix 1 on page 57.

3.2.6. Probit analysis

This study was conducted to determine the dose-mortality response of *A. gossypii* to *P. amboinicus* crude extract.

Four groups of 100 aphids were placed separately in petri-dishes with a diameter of 100 mm under room temperature of 28 °C. The edges of the petri-dishes were greased with liquid fluon to prevent aphids from climbing and escaping. About 10 ml of separate concentrations of mint extract were sprayed on each group of aphids. The 5 different concentrations were 200g mint/l, 100g mint/l, 50 g mint/l and 0 g mint/l (water as control treatment). All the exposed aphids were observed after 24 hours, whereas the dead- versus live aphids per petri-dish were counted.

Statistical analysis: Mortality data obtained from this study were subjected to statistical analysis with the Probit method using SPSS Statistics 20 software.

3.2.7. Identification of natural enemies of *A. gossypii* occurring in the experimental plots

Various natural enemies that were noticed in the experimental plots including entomopathogenic fungi, beetles, flies and parasitic wasps, were preserved, photographed and identified. During this research, Denise Sewkaransing, a student in the Bachelor of Science program of the department of Agriculture Production at the Anton de Kom University of Suriname, carried out a short study project in which she assisted in the current research with the identification of the observed parasitic wasps and entomopathogenic fungi. The identification of these natural enemies up to the Family and where possible up to the Genus level, was done under supervision of her Faculty supervisor K. Burke MSc. and through communication with scientists on the Research Gate platform who contributed in confirming the identification. Her draft report was submitted in May 2018 and revisions are still in progress. The entomopathogenic fungi which occurred as natural enemies of the aphids during rearing, were necessarily suppressed by the biological fungicide *Bacillus subtilis*. The parasitized aphids as well as the beetles, syrphid flies and larvae were removed manually to minimize the influence on the data.

3.2.8. Growing of cucumber, Inoculation aphids on plants, Preparation of botanical

First growth medium for the cucumber plants was prepared. The medium consisted of a mixture of humus soil, shell sand and gravel sand in a 7:2:1 ratio. To eliminate soil pest and diseases the medium was steamed at 90 °C in a self-made barrel steamer on a gas stove (Figure 16). This mixture was filled in plant pots with a volume of 12 liters. The average weight of the mixture in each pot was 7.0 kg. Because the chemical analysis of the soil mixture (appendix 5 on page 64) showed a high salt content, the mixture was daily rinsed during one week with water to desalinate. All the pots were placed in a greenhouse, which functioned as a nursery. The greenhouse was made with a metal frame, plastic roof and 2 m height screen wall to protect against big animals (birds, lizards and -insects (mole crickets). Small insects could enter the greenhouse.

Each pot was sown with 2 cucumber seeds of the variety CU 4320 F1Hybrid (Lion seed co. Ltd.), from which one germinated plant was removed. The healthiest plant per pot was allowed to grow under fertilization of 20 g. NPK (12-12-17-2) divided in two gifts and 20 g. Agrogold- Chicken manure granules (with NPK 4-3-2). Thus the total amount of N given to 1 plant was 3.2 g N. According to the analyses (appendix 5 on page 64) the soil already contained 2.7 g N (nitrogen) in experiment 1 and 3.5 g N in experiment 2. Although the recommended amount of N nutrient for each plant is 5.6 g N and the soil already contained 2.7 g resp. 3.5 g, the plant was given 3.2 g N. The plants were consciously over fertilized with N to stimulate the infestation with aphids. There was no need to add calcium to the soil because the pH of soil was 7.1 resp.7.6 and 7.5. The plants in the greenhouse were irrigated daily. Collected rain water from a plastic tank were used for irrigation with an irrigation spray tube. These climbing plants were supported with a trellis system made by rope and wood sticks/ poles. Pest which could influence the growth of the plants before the actual treatment of the study, were controlled with pesticides. Therefore, *Bacillus thuringiensis* was used against caterpillars and lambda cyhalothrin was used outside the green house and open field plots to control fire ants. During the actual treatment of the study the fungicide *Bacillus subtilis* was used against leaf spots.

After the emergence of the fourth leaf of each plant, the third leaf was inoculated with about 25 aphids. These aphids were taken from the rearing cages. The leaves were monitored daily on population growth of the aphids and the occurring natural enemies were removed.

The tested botanical was made by grinding leaves and shoots of *P. amboinicus* plant together with water in a kitchen blender. After leaving the mixture to overnight, in the early morning it was strained and applied thoroughly under the leaves with a knap-sack sprayer.

Figure 15. Self-made soil steamer with barrels during experiment at AdeKUS Leysweg

3.3. Experiment design and (bio) pesticides application

A Randomized Block Design was used in this experiment. Each 10 plants inoculated with insect pests were assigned to one of the 3 treatments (A, B and C) in 3 replications. The dependent variable is the number of aphids reported in each treatment condition. If the treatments (B and/or C, biological control method) is/are effective, the number of insect pests should be significantly more or same as treatment A (common pesticide control option).

The plants with insect pests were assigned to 3 Blocks. Each block represented a replication and consisted of the 3 treatments with 30 plants. One set up of 30 plants with 3 treatments was used in a Greenhouse and one set up under Open Field conditions. The two plots were located on a distance of about 50 m from each other to prevent influence.

The three treatments for aphids, at two factors (1. in Open Field and 2. Greenhouse) were:
treatment A: 100 g leaves of *Plectranthus amboinicus* in 1-liter water
treatment B: 200 g leaves of *Plectranthus amboinicus* in 1- liter water
treatment C: Abamectin (control)

Each plant was grown in a 12- liter plant pot/ bucket and placed in:
3 beds/blocks of 12 m X 2 m in open field, each block consisting of 30 plants.
3 blocks of 12 m X 2m in Greenhouse (8X15 m), each block consisting of 30 plants.
The total amount of plants in Open Field was 90 (3X30) and in the Greenhouse was 90(3X30). The plant distance was 30 cm in the row, 75 cm between the rows, 2 rows per bed/block. Figure 17 gives an overview of the lay-out of the experiment.

The treatments B and C were applied twice a week, while treatment A was applied once a week. This whole experiment was executed twice, in a wet season during 13 June- 4 September 2017 and in an extremely dry season during 7 September-6 November 2017.

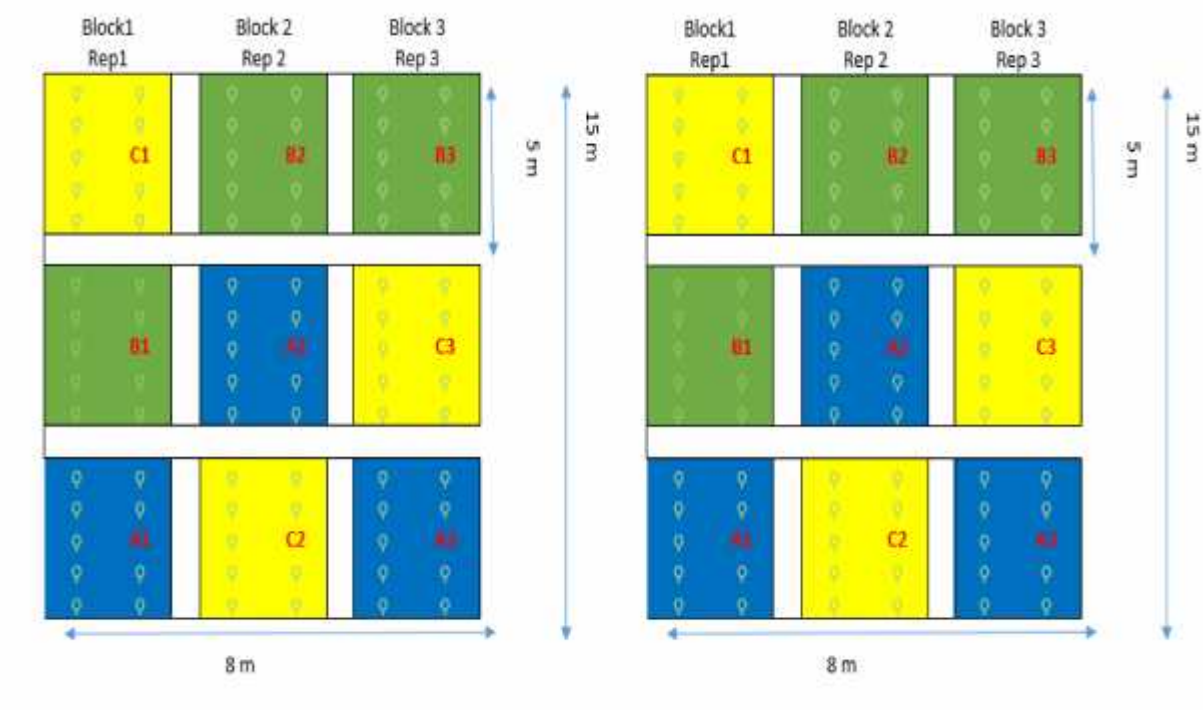

Figure 16. Layout of the experiment (Randomized Block Design) executed in the Greenhouse (left) and in Open Field (right) at AdeKUS Leysweg.

The spray treatments started for experiment 1 in week 9 (after sowing) and for experiment 2 in week 7. For both experiments the plants were sprayed during 4 weeks, twice a week. This was done on every Tuesday and Friday and the weekly data collection took place on every Monday.

For treatment A 100 g plant parts per liter water was used and for treatment B 200 g per liter. The total necessary liquid for each treatment was about 4-6 liters. For the control treatment C, a dose of 0.5 cc/l of the biopesticide abamectin (trade name: Abalotin) was used. During the spray application, the neighbor treatment plants were covered with a plastic sheet to avoid drift and contamination.

3.4. Data collection

The number of aphids was counted as follows; from every treatment the third top leaf of each a-select chosen three plants were removed and the amount of the aphids were recorded in the laboratory using a counter and stereomicroscope. The first data on aphid population was collected one day before the first spray application as a pre-treatment data.

The first flowering date and harvest data, namely the fruit weight per treatment and fruit length were recorded. During the cultivation of the crop all pest and diseases were recorded.

The day temperature in the Greenhouse during the first experiment was measured with a digital thermometer. During the second experiment the temperature was measured with a data-logger. Temperature data of the Open Field was obtained from the Meteorological Service of Suriname (Ministry of Public Works). The Meteorological Service measured the data at 8.30 hrs. a.m., 14.30 hrs. p.m. and 18.30 hrs. p.m.

3.5. Statistical analysis

Treatment data for aphids was analyzed with the software SPSS Statistics 20.0 using the General Linear Model (GLM). In this experiment three treatments (100g mint/l, 200 g mint/l and abamectin) were compared to each other and the Analysis of Variance (ANOVA) was used. The number of aphids (response) functioned as the dependent variable. As stated by the Australian Pesticides and veterinary Medicines Authority (2015), before conducting a parametric analyses of variance, the following assumptions should be met to ensure that the analysis is valid:

-Independency of the 3 different groups (pesticide treatments in this case)
-Homogeneity of variance
-Normality of distribution

It is assumed that the three treatments were independent because the trial was set up in that way. The normality was tested with SPSS. Because the data had no normal distribution, this was normalized by application of a transformation as used in comparable studies (Shinthiya, 2017). In this case a square root plus 0.5 transformation normalized the distribution. The addition of 0.5 was used to avoid zeros in the data. Transformed data were submitted to a Randomized Block analysis of variance (ANOVA) ($P< 0.05$) and differences among the 3 different treatments for every week separately were compared using Tukey's test ($P \leq 0.05$) and a Post Hoc Multiple Comparison.

Data of each week was analyzed separately because the aphid population of each week was also affected by climate factors and weekly observations cannot be considered as replication. To have a general conclusion on the efficacy of the mint botanical also an overall data analysis of week 1-4 together has also been included.

Differences between Greenhouse and Open Field system between treatments were also compared. To observe the effect of the treatments over time, reduction % in aphid occurrence was also calculated for the fourth week: reduction % = 100% - (mean aphid on week 4) / (mean aphid on pre-spray).

As the cucumber production data was normally distributed, no square root transformation was applied for this analysis. To test if there were differences in production between the three treatments and also in the production systems, Tukey's test ($P\leq 0.05$) was used in the General Linear Model.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Interview with 20 cucumber farmers

Thirteen of the 20 farmers (65%) interviewed, stated that aphids occur as pests in cucumber plantings. Six of the 20 farmers (30%) reported that aphids do not occur as pests in their cucumber fields. Only one of the 20 farmers did not know if aphids occur as a pest in their cucumber field. The most common pesticides used for the control of aphids are abamectin and lambdacyhalothrin. From the above mentioned 30 % farmers for whom aphid is not a pest, 83 % have indicated that they spray their field preventively with the synthetic pesticide lambdacyhalothrin or cartap hydrochloride. So aphid is an important insect pest in cucumber. Data of these 20 farmers, interviewed during this thesis are presented in appendix 2 on page 58. Although LVV advises only abamectin for aphid pest in cucumber, farmers are using lambdacyhalothrin and cartap hydrochloride, which have a pre harvest interval of one week. If they spray during harvest periods, there is a chance that weekly twice harvested cucumbers have pesticide residues, which poses a risk for human health and environment. This fact emphasizes the need for biocontrol options such as use of botanicals.

4.2. Identification of the aphid used in this thesis

The aphids used in this thesis are identified as *Aphis gossypii.* This is done with the prepared slides of the aphid specimens of this experiment and an identification key (Blackman, 2017) together with a diagrammatic illustration of the aphid with its features. The identification procedure is included in appendix 3 on page 59-61.

4.3. Probit analysis

The probit analysis resulted that the LC 50 of mint leaf extract is 100g/l and about 70% of the studied aphids died at the concentration of 200 g/l. To kill 99% of the aphid population, theoretically 466 g/l will be necessary, which is too much and practically not feasible. Therefor the concentrations of 100g/l and 200 g/l were used in this thesis to test the efficacy of *P. amboinicus* as botanical. The probit analysis is presented in appendix 4 on page 62-64.

4.4. General observations during the cultivation of cucumber

The average rainfall during experiment 1 (13 June-4 September 2017) was 6.4 mm and the average day temperature in open field was 28.5 ˚C. But the average rainfall during experiment 2 (4 September-6 November 2017) as expected in the dry season was 3.7 (lower) and average day temperature was 29.0 ˚C (higher than in the wet season) in open field. The temperature in the Greenhouse during the dry season was 32.5 ˚C, which is 3.5 ˚C higher than Open Field. The temperature in the Greenhouse is higher than the optimum

growth temperature of 28 °C for cucumber. The daily rainfall during 1 June- 30 November 2017 is shown in a graph in Figure 18 and Figure 19 gives the daily temperature in Greenhouse and Open Field during 4 September-6 November 2017.

First blossoms in experiment 1 were observed during week 7, while the plants of experiment 2 bloomed 2 weeks earlier (Table 2). The first harvest in both experiments took place 2 weeks after the first blossoms appeared (Table 2). The harvest period of experiment 1 in the Greenhouse lasted 3 weeks and in experiment 2, this period in Greenhouse as well as in Open Field system lasted 4 weeks. The fruits of experiment 2 were of inferior quality (shape, weight) compared with the fruits of experiment 1.

Table 2. Blossoms and harvest observations of experiment 1 and 2 during the different weeks after sowing

	Experiment 1	Experiment 2
First blossom	Week 7	Week 5
First harvest	Week 9	Week 7
Last harvest	Week 11	Week 10

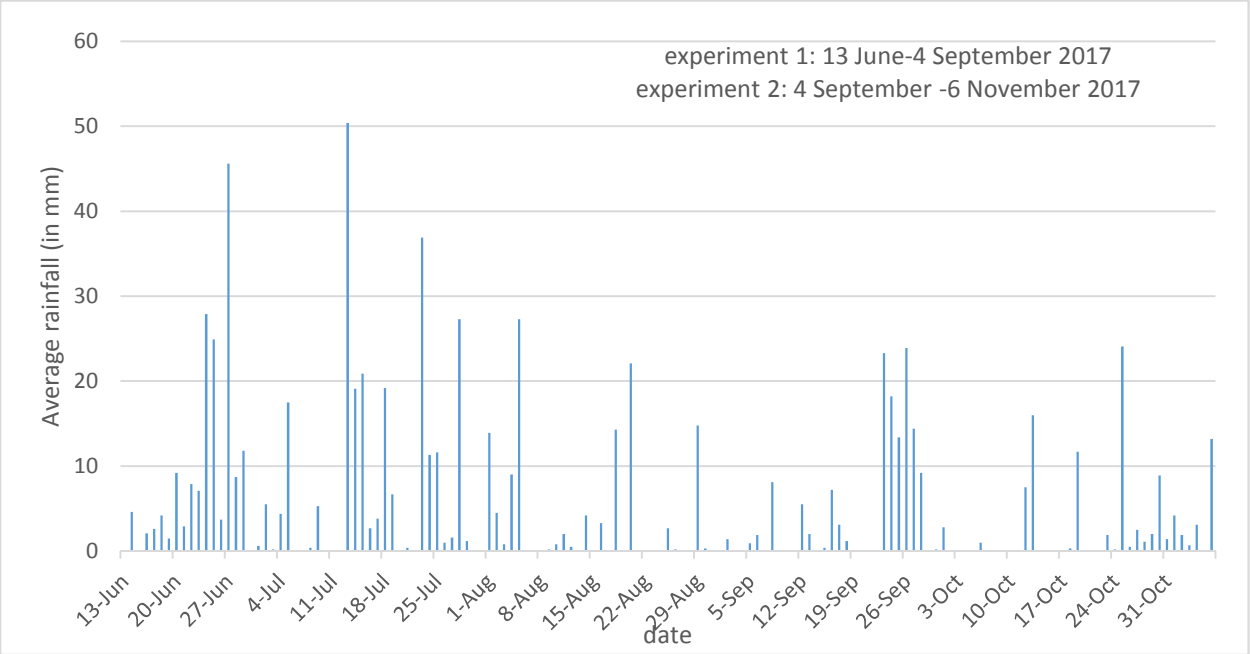

Figure 17. Daily rainfall (mm) during 1 June- 30 November 2017 at location “Zorg and Hoop”

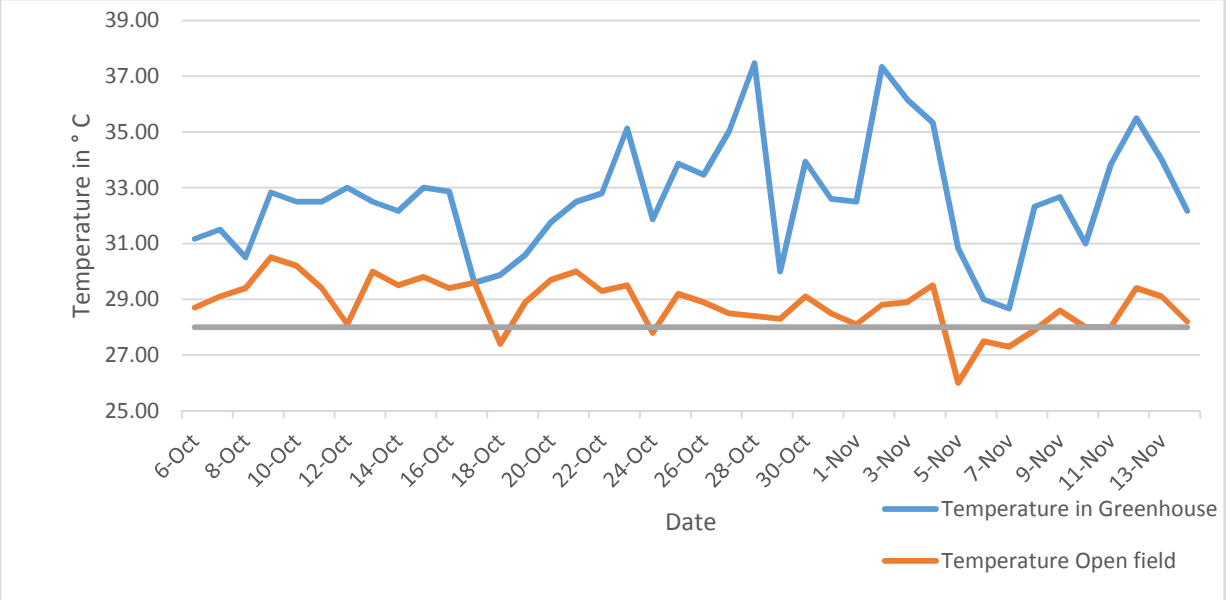

Figure 18. Daily temperature (°C) in Greenhouse and Open Field from 6 October - 13 November 2017 at AdeKUS, Leysweg

4.5. Response of the aphids to the 3 treatments

The main results of the experiment are given in this paragraph. The raw data of the responded aphids are given in appendix 6 and 7 on page 65-71. Average numbers of aphids per sampled leaf in both experiments are given in Table 3 and 4 and shown in Figure 20-23. These tables and figures show that the number of aphids in the Greenhouse is higher than the number of aphids in Open Field condition, which can be caused by the higher temperature (see figure 18) in the Greenhouse as also stated by Manners (2017).

These figures (except for experiment 1 open field system) shows that abamectin has the lowest numbers of aphids, thus had the best performance followed by 200 g/l mint. Figure 20-23 also show population fluctuations and not a continuous decreasing trend during the different weeks. The fluctuation can be caused by weather conditions such as temperature and rainfall fluctuations which can be concluded from Figure 18 and 19. Table 3 and figure 20 reveal that the number of aphids in experiment 1 (rainy season) in the Greenhouse decreased after the treatments started. After week 2 the number of aphids started to increase but were still less than the pre-treatment. Table 4 and Figure 22 show that the number of aphids in experiment 2 (dry season) in the Greenhouse increased during the first week after the start of the treatments, but decreased after week 2 only in the treatments with abamectin and 200g/l mint. It is noticeable that the number of the aphids during the 2 last weeks were stable and did not decrease dramatically; even not with the abamectin treatment. It can be caused by the higher temperature (Figure 18), which stimulates the population increase.

Data of experiment 1 under Open Field systems are not considered in comparison of the means, because only data of week 1 and 2 were obtained.

Table 3. Average number of aphids per sampled leaf with different treatments during 4 weeks in Greenhouse and Open Field of experiment 1

		Average number of aphids (weeks)				
location	treatment	pre-treatment	wk1	wk2	wk3	wk4
Greenhouse	100g mint/l	113	22	13	53	50
	200 g mint/l	153	6	5	34	16
	abamectin	230	1	3	23	6
Openfield	100g mint/l	6	7	14	-	-
	200 g mint/l	4	11	10	-	-
	abamectin	8	12	10	-	-

-no plants left for observation

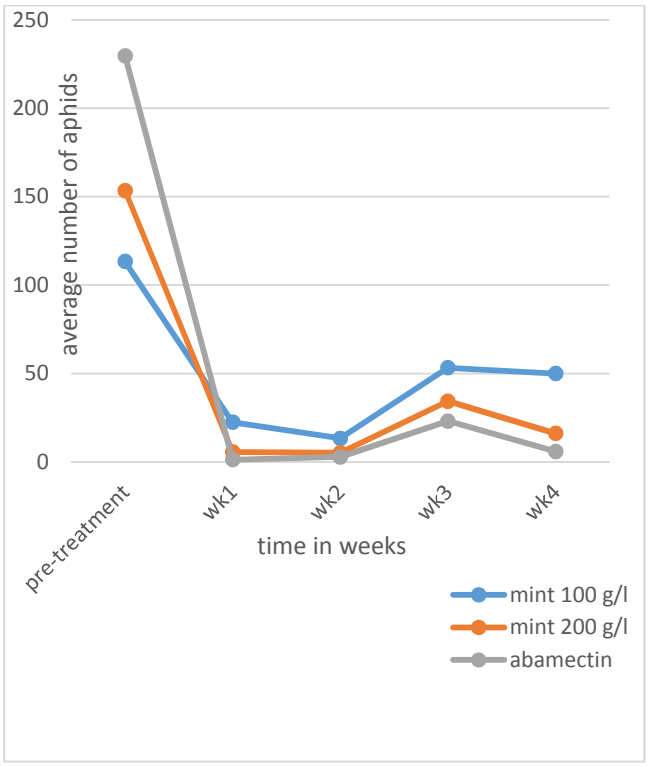

Figure 19. Average number of aphids per sampled leaf with different treatments during 4 weeks in experiment 1 in the greenhouse

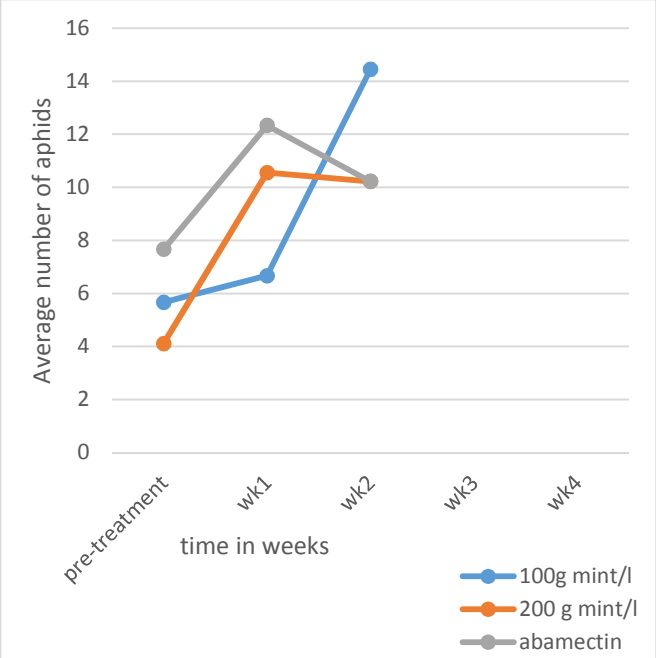

Figure 20. Average number of aphids per sampled leaf with different treatments during 2 weeks in experiment 1 in Open Field

Table 4: Average number of aphids per sampled leaf with different treatments during 4 weeks in Greenhouse versus Open Field in experiment 2

		Average number of aphids (weeks)				
location	treatment	**pre-treatment**	**wk1**	**wk2**	**wk3**	**wk4**
Greenhouse	100g mint/l	114	153	119	126	125
	200 g mint/l	82	215	22	45	36
	abamectin	80	140	14	22	20
Openfield	100g mint/l	53	44	150	130	95
	200 g mint/l	54	53	112	89	63
	abamectin	76	24	81	67	3

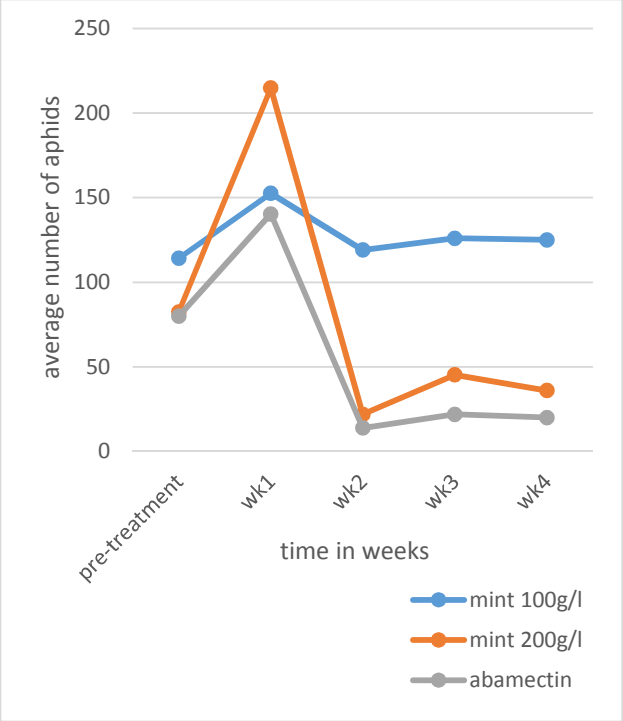

Figure 21. Average number of aphids per sampled leaf with different treatments during 4 weeks in experiment 2 in the Greenhouse

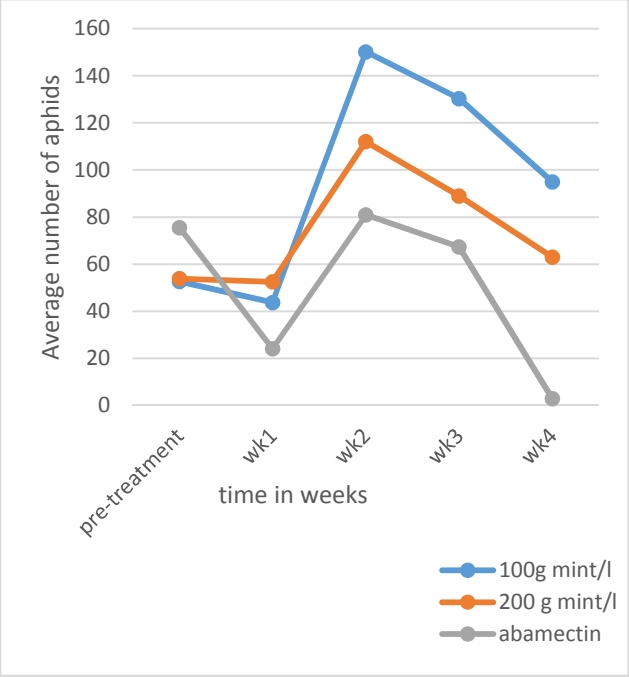

Figure 22. Average number of aphids per sampled leaf with different treatments during experiment 2 in Open Field

Data presented in table 5 shows that in experiment 1, overall after week 1, 2, 3 and 4 there is significance difference (P≤ 0.05) between 100 g mint/l and abamectin treatment and between 100g mint/l and 200 g mint/l. On the other hand, there is no significance difference (P≤ 0.05) between 200 g/l and abamectin. So 200 g mint/l is as effective as

abamectin but much better than 100 g mint/l during rainy season. The statistical analysis with SPSS program regarding Table 5 is attached in appendix 9 on page 73-82. Table 5 shows also that in experiment 2 during week 1, 2, 3 and 4 mostly there is significance difference (P≤ 0.05) between 100 g mint/l and abamectin treatment. But there is no significance difference (P≤ 0.05) between 200 g/l and abamectin. Only one week gives an exemption, where no significance difference (P≤ 0.05) between 200g mint/l and 100 g mint/l. As regards overall comparison of the treatments in experiment 2 there is significance difference (P≤ 0.05) between 100g mint/l, 200 g mint/l and abamectin. This data reveals that 200 g mint/l controls better than 100 g mint/l but is efficient as abamectin in extreme dry season.

Data presented in Table 5 also shows that during the rainy season (experiment 1) control with abamectin resp. 200 g mint/l reduced the population of the aphids in week 4 for 95.04 % resp. 79.55 % from pre-spray. Meanwhile during the extreme dry season (experiment 2) control with abamectin resp. 200 g mint/l reduced the population of aphids in week 4 with 85.06 % resp. 26.84 % from pre-spray. This means that mint extract with a dose of 200g /l gives good control of the aphid pest at low populations (rainy seasons), but is less effective when the population is very high. The above mentioned 79.6 % reduction of the population of *A. gossypii* by 200 g/l mint application is as expected and comparable to 70 % mortality outcome of the executed probit analysis. (See paragraph 4.3). Unfortunately, the population reduction of 26.8 % in the dry season was much lower than the expected 70 %, revealed by the laboratory assay. This can be caused by the hot weather in the field plots with higher temperature than the lab conditions (28 °C), where the probit analysis was executed.
The results of the study reveals that mint extract with a dose of 100 g/l reduced the population during the rainy season only for 15.97 %, while the population during the dry season increased with 23.96 % for this dose. According to the probit analysis of paragraph 4.3, the population should be reduced by 50%. So, in practice this dose does not control the aphid pest sufficiently in all seasons. Finally, it can be concluded that plant extracts are more effective at low infestation levels which is also confirmed by Foguelman (2003).

Raw data of the cucumber production is given in appendix 8 on page 71-72. Table 6 shows that in experiment 1, the number of aphids in open field do not significantly differ from the greenhouse during week 1 but do significantly differ during week 2. In experiment 2 the number of aphids from open field do significantly differ from the Greenhouse during week 1 and 2 but not during week 3 and 4. The overall means of aphids in Open Field do not significantly differ from Greenhouse. This means that the treatments had no difference between Open Field and Greenhouse.

Cucumber production of the 3 different treatments; 100 g mint/l, 200 g mint/l and abamectin do not differ significantly (P≤0.05) from each other (Table 7) in both experiments. Also, the production of the 3 blocks do not significantly differ (P≤0.05) from each other in both experiments. For more detail information, refer to the statistical analysis attached in appendix 10 on page 83-85. This means that the aphid population difference between the different treatments and blocks did not lead to a difference in the fruit production between

the treatments and blocks. This is because of the high virus infection caused by the aphids throughout the most plants and so affected the fruit production evenly in the plots.

Table 5. Means of the response compared between different treatments during experiment 1 and 2

		No. of aphids per leaf*						
	Treatment	**Pre-spray**	**Week1**	**Week2**	**Week3**	**Week4**	**Overall mean**	**Population reduction**
Experiment 1	**100g mint/l**	59.50 (6.57)	14.56 b (3.94)	13.89 b (3.88)	53.22 a** (6.97)	50.00 b** (7.08)	26.69 b (4.95)	15.97%
	200g mint/l	78.78 (7.21)	8.11 ab (2.92)	7.72 ab (2.98)	34.33 a (6.16)	16.11 a (4.25)	13.69 a (3.70)	79.55%
	abamectin	118.67 (8.98)	6.83 a (2.61)	6.56 a (2.73)	23.11 a (4.63)	5.89 a (2.67)	9.30 a (3.00)	95.04%
Experiment 2	**100g mint/l**	83.39 (8.79)	98.17 a (9.40)	134.61 b (11.57)	128.22 b (11.28)	109.67 b (8.86)	117.67 c (10.28)	-23.96%
	200g mint/l	68.11 (8.35)	133.67 a (11.15)	66.94 a (7.84)	67.17 a (7.943)	49.83 ab (6.05)	79.40 b (8.25)	26.84%
	abamectin	77.72 (8.76)	82.22 a (8.26)	47.39 a (6.26)	44.61 a (6.03)	11.61 a (2.72)	46.46 a (5.81)	85.06%

*means of 3 replications and 3 sampled leaves
** data of week 3 and 4 during experiment 1 only regards Greenhouse

Figures in parentheses are $\sqrt{x} + 0.5$ square root transformed values. The test analyses are based on these figures.

Means within a column followed by the same letter(s) do not significantly differ at P≤ 0.05 level, according to Tukey's test. The SPSS-analysis for every week is attached in appendix 9

Table 8 reveals that the cucumber production in greenhouse during experiment 1 was much higher than the production during experiment 2. The reduced production was caused by the high virus infection of the plants during the second experiment. The virus infection also resulted in misshaped fruits. As it is also stated by Barbercheck (2014), this study has

revealed that virus infection can lead to enormous crop losses, more than the aphid as virus-transmitter itself.

The production (table 9) in Greenhouse does not significantly differ (P≤ 0.05) from the production in Open Field condition. Because of the infection by aphids as well as virus in both cultivation systems, the production in the greenhouse do not significantly differ from open field system.

Table 6. Means of the response compared between different cultivation systems during experiments

		No. of aphids per leaf*					
	Treatment	**Pre-spray**	**Week1**	**Week2**	**Week3**	**Week4**	**Overall mean**
Experiment 1	**Greenhouse**	165.48 a	9.81 a	7.15 a	-	-	19.46 a
		(12.79)	(2.99)	(2.80)			(4.09)
	Open field	5.81 b	9.85 a	11.63 b	-	-	10.74 a
		(2.38)	(3.32)	(3.60)			(3.46)
Experiment 2	**Greenhouse**	92.11 a	169.22a	51.63 a	64.44 a	60.52 a	86.45 a
		(9.37)	(12.82)	(6.55)	(7.62)	(7.07)	(8.52)
	Open field	60.70 a	40.15b	114.33 b	95.56 a	53.56 a	75.90 a
		(7.90)	(6.36)	(10.57)	(9.21)	(4.68)	(7.71)

*means of 3 replications and 3 sampled leaves
Figures in parentheses are $\sqrt{x} + 0.5$ square root transformed values. The test analyses are based on these figures.

Means within a column followed by the same letter(s) do not significantly differ at P≤ 0.05 level, according to Tukey's test

Table 7. Means of the production weights (in gram), compared between different treatments, blocks and cultivation systems in experiment 1 and 2

Treatment	**Experiment 1**		**Experiment 2**	
	Mean prod weight Greenhouse (g)	**Mean prod weight Open Field (g)**	**Mean prod weight Greenhouse (g)**	**Mean prod weight Open Field (g)**
100 g mint/l	3075.000 a	-	977.333 a	995.000 a
200 g mint/l	3368.889 a	-	1116.083 a	886.250 a
abamectin	3930.556 a	-	983.333 a	962.917 a
Block				
1	3866.111 a	-	1178.833 a	1035.833 a
2	3342.778 a	-	840.417 a	842.083 a
3	3165.556 a	-	1057.500 a	966.250 a

Means within a column followed by the same letter(s) do not significantly differ at P≤ 0.05 level, according to Tukey's test

Table 8. Production weights (in gram) and amount during experiment 1 and 2

	Experiment 1				Experiment 2			
	GH-total	**GH-per plant**	**OF-total**	**OF-per plant**	**GH-total**	**GH-per plant**	**OF-total**	**OF-per plant**
Prod. weight (kg)	93.37	1.04	-	-	36.92	0.41	34.13	0.38
Prod. amount	334	334/90	-	-	215	2.4	198	2.20

GH= Greenhouse OF=Open field

Table 9. Means of the production weights (in gram) compared between the cultivation systems

Cultivation System	**Experiment1**	**Experiment 2**
Greenhouse	2780.00	1025.58 a
Open field	-	911.67 a

Means within a column followed by the same letter(s) do not significantly differ at P≤ 0.05 level, according to Tukey's test

4.6. Other observed pest and diseases in cucumber during test

The most observed insect pests during this test were already mentioned by Segeren (1983). Besides the intentionally introduced aphid (Figure 24.a) the following insect pests were observed during the experiment (see photo collage of observed pest and diseases on page 43-45):

Spodoptera sp. (Figure 24.c): Larvae (Figure 24b) of the moth feed on leaves of young plants (Figure 22d). About 50-75% of the plants in the Greenhouse were infested, while 25-40 % of the plants in the Open Field were damaged during the first experiment (rainy season). The infestation only occurred before the 3 treatments of the experiment. Weekly spray during 3 weeks with *Bacillus thuringiensis* controlled this pest effectively. Only this insect as pest in cucumber was not mentioned by Segeren (1983)

Diaphania hyalinata (cucumber moth) (Figure 24e): Larvae of the moth feed on young leaves and pupate in folded leaves during the first and second experiment. About 10-20 % of the plants in the Greenhouse and 5 % of the plants in Open Field were infested during the first experiment (rainy season). During the second experiment (dry season) only the plants in the Greenhouse were infested for about 5%. This pest occurred before the treatments started and was only controlled by hand-picking the pupating larvae in the folded leaves.

Diaphania nitidalis (cucumber fruit borer) (Figure 24f): larvae of the moth feed in the fruits and burrow down into the flesh. The exit holes were observed in the damaged fruits (Figure 24g) During the first experiment 6 fruits of Open Field were infested and during the second experiment only 3 fruits of Open Field were infested by this fruit borer. During the second experiment only 1 fruit of the Greenhouse was infested.

Tetranychus sp. (red spider mite) (Figure 24h): the low infested leaves showed discolored patches (Figure 22i). These mites infested the plants in the Open Field as well as of the Greenhouse during both experiments. But the infestation occurred through the whole cultivation period, but was higher in the dry season. This pest was controlled once with 2 cc/l fenbutatinoxide (Torque), which has no effect on insects.

Mosaic virus (unidentified): About one week after the inoculation with the aphids, the plants started to show green mosaic patterns on the leaves (Figure 24k). Only during the first 2 weeks after notice the small plants were replaced by others. During the first experiment only 5 % of the plants and during the second experiment about 30-40% of the plants were infested. The plants of the second experiment looked very unhealthy with the mosaic patterns and stunted growth.

Leaf spots (unidentified fungus) (Figure 24j): The cucumber of the Open Field during the first experiment showed leaf spots in the rainy period, which possibly caused death of the plants.

Dried top leaves (no infection): the top growth dried frequently after extreme hot days (Figure 24l)

Figure 23. Photo collection of observed pest and diseases (photo by M.Jagroep, 2017)

Fig.24a. Leaf infested by aphids

Fig.24b. *Spodoptera* sp. Larva, actual size: 3 cm

Fig.24c. *Spodoptera* sp. Moths, actual size: 2 cm

Fig.24d. Damaged leaves by *Spodoptera* sp.

Fig.24e. *Diaphania hyalinata* moth, size: 2 cm

Fig.24f. *Diaphania nitidalis* moth, actual size: 2cm

Fig.24g. Damaged fruits by *D. nitidalis*

Fig.24h. *Tetranychus* sp., actual size: 0.5 mm

Fig.24i. Damage by *Tetranychus* sp.

Fig.24j. Cucumber leaf with leaf spots

Fig.24k. Cucumber leaves with mosaic patterns

Fig.24l. Dried top because of hot weather

4.5. Observed biological control agents of *A. gossypii* during test

Although Dinther (1960) already mentioned the occurrence of some predators of *A. gossypii* in Suriname, this study resulted in additions of natural enemies of this aphid. It is important to note that not only rainy season but also natural enemies, as stated by Patterson (2016) can suppress the aphid population. From the report from Sewkaransing in 2018, the following natural enemies were found and identified:

1. Parasitic wasp from the order Hymenoptera and family Braconidae (Figure 25)
2. Parasitic wasp from the order Hymenoptera and family Aphelinidae (Figure 26)
3. Parasitic wasp from the order Hymenoptera and family Encyrtidae (Figure 27)

The parasitized aphids were black mummies, where the parasitic wasp left through an exit hole (Figure 26). The above mentioned parasitoids are described in the report of Sewkaransing, 2018)

4. Four different predatory beetle species from the order Coleoptera and family Coccinellidae (Figures 29, 30, 31, 32).
5. Two different species of predatory flies belonging to the order Diptera and family: Syrphidae were found (Figures 33 and 34)

6. Four different species of entopathogenic fungi described in Sewkaransing (2018) were: *Fusarium* sp. (Figure 35), identified by K. Burke in 2017, a green colored unidentified species (Figure 36), a white colored unidentified species (Figure 37) and a pink colored unidentified species (Figure 38). These fungi were also found during the treatments, but on the aphids under the old leaves.

Figure 24. *Order: Hymenoptera*, family: Braconidae, actual size 1.0 mm.

Copied from Evaluatie van entomopathogenen en parasitoïden van bladluizen (Aphididae) op komkommer in Suriname (p. 20) by Denise Sewkaransing, 2018

Figure 25. Order: Braconidae, family Aphelinidae, actual size 1.0 mm.

Copied from Evaluatie van entomopathogenen en parasitoïden van bladluizen (Aphididae) op komkommer in Suriname (p. 20) by Denise Sewkaransing, 2018

Figure 26. *Order Hymenoptera, family: Encyrtida*e, actual size: 0.7 mm.

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Figure 27. Parasitized aphid with exit hole, actual size 2 mm. (photo by M. Jagroep, 2017)

Figure 28. Order: Coleoptera, family: Coccinellidae; actual size 5 mm. (Photo by M.Jagroep, 2017)

Figure 29. Order: Coleoptera, family: Coccinellidae; actual size; 1 mm. (Photo by M.Jagroep, 2017)

Figure 30. Order: Coleoptera, Family; Coccinellidae; actual size 2 mm. (Photo by M.Jagroep, 2017)

Figure 31. Order: Coleoptera, Family: Coccinellidae; actual size 3 mm. (Photo by M.Jagroep, 2017)

Figure 32. Order: Diptera; Family: Syrphidae, actual size: 5-7 cm. (Photo by M. Jagroep, 2017)

Figure 33. Order: Diptera; Family: Syrphidae, actual size: 4-5 mm. (Photo by M. Jagroep, 2017)

Figure 34. *Fusarium* sp. culture (left) and its microscopic slide (right)

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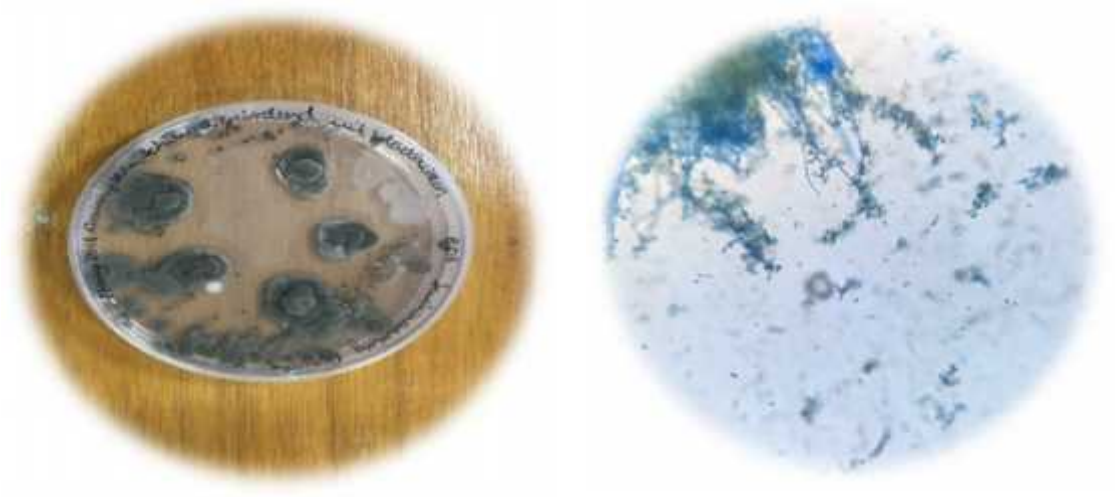

Figure 35. Green fungus culture (left) and its microscopic slide (right)

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Figure 36. White fungus culture (left) and its microscopic slide (right)

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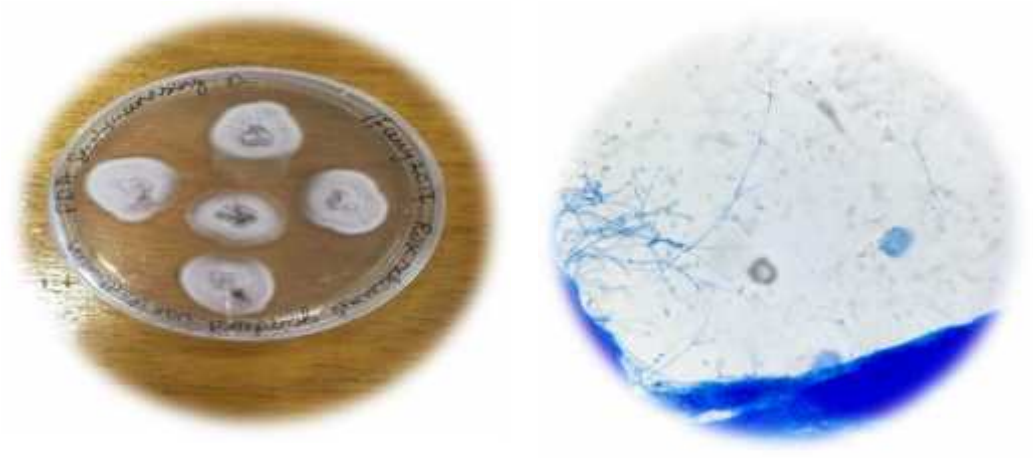

Figure 37. Pink fungus culture (left) and its microscopic slide (right)

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CHAPTER 5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The purpose of the study was to investigate if crude extract of Cuban Mint effectively controls A. *gossypii* in cucumber under Greenhouse and Open field conditions and also during different seasons. Based on the results obtained during the research investigation, the following conclusions can be drawn:

Crude extract of mint *P. amboinicus* with a dose of 100 g/l does not to control the aphid *A. gossypii* in cucumber effectively, both in the rainy- as well as in the dry season for both Greenhouse- as Open Field conditions.

However crude extract of mint *P. amboinicus* with a dose of 200 g/l does control aphid *A. gossypii* in cucumber in the rainy season but less in the dry season for both Greenhouse- as Open Field conditions.

Although there was a significant difference in the number of aphids in the 3 treatments, the production of the cucumber in the 3 treatments did not significantly differ from each other, possibly because of the indirect damage by the aphids.

There was no significant difference in the effectiveness of the aphid-control between Open Field and Greenhouse.

The aphid pest population can also be suppressed by natural enemies. The natural enemies of A. gossypii found during this study are 4 beetle species (Coleoptera; coccinellidae), 2 syrphid fly species (Diptera; Syrphidae), 3 parasitic wasp species (Hymenoptera; Braconidae, Aphelinidae, Encyrtidae) and 4 fungus species (Fusarium sp. and 3 unidentified species).

During this study, the following pests, as also listed by Segeren (1983) were found at the experimental site of the Anton De Kom University: *Diaphania hyalinata, Tetranychus sp., Diaphania nitidalis.* One *Spodoptera* sp., not mentioned as pest in cucumber by Segeren (1983), Mosaic virus (unidentified) and Leaf spots (unidentified fungus) was also present in this experiment.

5.2. Recommendations

The efficacy of 200 g/l mint (with regard to the number of aphids) was lower than abamectin in the dry season, but still 200 g/l mint treatment did not have a lower cucumber production than abamectin. Even a lower efficacy of 200 g/l mint than abamectin, may provide benefits to the grower and will be acceptable if the fruit production is not affected and this concentration of mint has little or no effects on natural enemies. So it is recommended to use crude extract of *P. amboinicus* as alternative for abamectin against *A. gossypii*. This can be done with a dose of 200g/l only during rainy seasons or at low aphid populations if sprayed twice a week and thoroughly at the underside of the leaves. Also the application should not be done less than 2-3 hours before a rainfall. Another benefit of this crude extract is to be used as alternative for abamectin to prevent resistance against abamectin, which is one of least available insecticide with a low pre-harvest interval in Suriname. The effectiveness can be improved by keeping the aphid pest population low

through monitoring the crop and discovering the pest early so that population built up can be prevented.

It is costly to rear biological control agents commercially for the agricultural sector in Suriname. So if these natural enemies are protected and conserved in the agricultural environment by applying sustainable agriculture practices, they can have a great contribution to the biological control of *A. gossypii*. Therefore, farmers can be trained to recognize the natural enemies. Also comprehensive studies in the future with the observed microbial fungi in this experiment as bio pesticides can give promising results to international manufacturers of microbial pesticides.

As the effect of Cuban mint extract has not been tested on natural enemies in this thesis, future research on this matter is recommended. A combination of 200 g/l mint treatment and presence of natural enemies can give excellent control if the mint extract has no adverse effect on natural enemies.

It is remarkable that *D. nitidalis* -fruitborer, which is also one of the major cucumber pests in Suriname, occurred at a low level during this experiment. This low infestation can be contributed to the treatment with *P. amboinicus*. Thus, it can be investigated in future studies if *P. amboinicus* also controls *D. nitidalis*.

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APPENDICES

Appendix 1. Materials used for experiment

Interview with 20 cucumber farmers: phone
Identification of the mint plant as *Plectranthus amboinicus*: plant material
Cultivation of *Plectranthus amboinicus* plants: chicken manure granules, cuttings of *Plectranthus amboinicus*, pruner
Rearing of aphid *Aphis gossypii* : organza and PVC-tubes for cages, potting soil, humus soil, chicken manure granules, calcium, cucumber seeds, watering can, plant pots (0.5 liter, 1liter and 5 liter) with trays, aspirator, paint brushes, woodsticks for plants
Probit analyse: petri dishes with diameter of 10 cm, fluon liquid, spray bottles, counter, *Plectranthus amboinicus* extract, aphids
Identification of the aphids and natural enemies: 70% - and 90% ethanol, 10% KOH, Euparal, acid fuchsine, clove oil, object glass, cover glass, stereo microscope, microscope, forceps, pins, pipettes, identification key for aphids, oven, camera

Growing of cucumber: Cucumber seeds, 10 liter plastic buckets (as plantpots), soil steamer, gas cylinder, gas stove, humus, shell sand, fine gravel sand, chicken manure granules, NPK (12-12-17-2), irrigation spray tube, EC-meter, rope, wood sticks
Preparation and application of (bio)pesticides: *Plectranthus amboinicus* plant parts, blender, buckets, water, weigh scale, sieve, knapsack sprayer, pesticides (Abamectin, Lambda Cyhalothrin, *Bacillus thuringiensis*, *Bacillus subtilis*)
Inoculation aphids on plants: paint brush, aspirators, plastic bags
Data collection: Data logger for Temperature and Relative Humidity, camera, weigh scale, permanent marker, stereo microscope, aphids
Identification of the natural enemies: 70% ethanol, 10% KOH, object glass, cover glass, stereo microscope, microscope, forceps, stain, identification keys for hymenoptera and fungus

Appendix 2. List of interviewed cucumber farmers and experience with aphids as pest

nr	Name	Address	Aphid as pest	Control method
1	Naipal, S	Oryza Uitkijk and Welgedacht A 312, Wanica	Yes	No control
2	Bineshri,		No	Preventive control with lambdacyhalothrin
3	Jagroep, J	Okrodam 44, Wanica	Do not know, but leaves are yellowing in dry season	
4	Mattaw, B	Magentaweg, Wanica	Yes	Plant extracts
5	Silos, M	Mariënburg, Commewijne	Yes	Abamectin, Neemal
6	Del Prado, G	Ministry Agriculture Letitia Vriesdelaan, Paramaribo	Yes	Abamectin
7	Bhansing, M	Weg naar Zee, Wanica	Yes	No control
8	Saeri, M	Okrodam 42, Wanica	Yes	Lambdacyhalothrin
9	Dassasing, R	Bolletrihé 9, Wanica	Yes	Malathion
10	Soekhan, H	Bolletrihé 29, Wanica	Yes	Abamectin, Lambdacyhalothrin, Cartap hydrochloride (Padan)
11	Mahadew, A	Bolletrihé 92, Wanica	No, only ants	Preventive control with lambdacyhalothrin
12	Ajodhia	Paloeloeweg 191, Saramacca	Yes	Abamectin, Lambdacyhalothrin only on ant nests
13	Rodjan, K	Commisaris Roblesweg 26, Commewijne	Yes	Abamectin
14	Ramautar, M	Josikreek 264	Yes	Abamectin
15	Moenna	Damboenton, Saramacca	No	Preventive control with Cartap hydrochloride (Padan)
16	Doerdjan	Tout lui Fout kanaal, Wanica	Yes	Neemal
17	Dihal, S	Jagtlust, Commewijne	No	No control
18	Sewkaransing, S	Helena Christinaweg	No	Preventive control with mixture lambdacyhalothrin and malathion
19	Lachman, P	Verl.Houttuinweg 173	Yes	Abamectin
20	Gauri	Verl. Bergenshoopweg 37	No	Preventive control with lambdacyhalothrin

Appendix 3. Identification of the aphid used in this thesis

The aphids used in this thesis are identified as *Aphis gossypii* . This is done with the prepared slides of the aphid specimens of this experiment and an identification key (Blackman, 2017) together with a diagrammatic illustration of the aphid with its features (Figure 37).

Some of the pictures of the prepared slides are included and mentioned in the identification procedure (Figures 39-42).

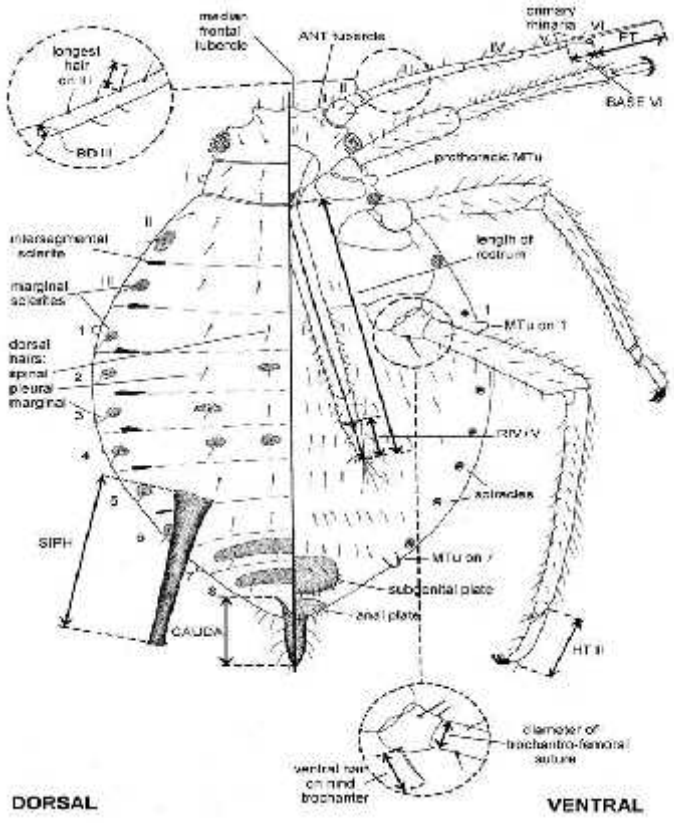


Figure 38. Diagrammatic illustration of an aphid

Diagrammatic illustration of an aphid showing dorsal (L) and ventral (R) morphological features used in the keys in this book, the abbreviations used, and ways to measure certain morphometric parameters.

Antennal (ANT) and thoracic segments are numbered I-VI and I-III respectively, ANT III onwards being the ANT flagellum, and ANT VI comprising BASE and processus terminalis (PT). The ratio of ANT VI BASE to PT ("ANT PT/BASE") is a frequently used discriminant. Abdominal segments are numbered 1-8. Insets show measurements of ANT and trochantral hairs, basal diameter of ANT III (BD III) and diameter of trochantro-femoral suture. The last two segments of the rostrum usually form a combined structure (R IV+V), the length of which is often compared with that of the 2nd segment of the hind tarsus (HT II). Members of the tribe Aphidini typically have marginal tubercles (MTu) on the prothorax and abdominal tergites (ABD TERG) 1 and 7, but some have them also on other segments. (copy right unknown, retrieved from http://www.aphidsonworldsplants.info/Fig_1.htm)

The protocol with different steps/options including the features to identify the aphid as *Aphis gossypii* is as follows:

1. SIPH present, conical or tubular. ANT PT/BASE more than 1.25. Eyes multifaceted .....4 (Figure 40)

4. Head without spicules, or with a few minute ones ventrally. ANT tubercles undeveloped or variably developed, if well-developed then with smooth divergent inner faces 15

15. Cauda tongue-shaped, finger-shaped, tapering or triangular, usually much longer than basal width, if not then it has 10 or more hairs. SIPH pale or dark, imbricated, without any marked subapical annular incision. ANT tubercles variably developed, if weakly developed then ABD TERG 1 and 7 have MTu. Spiracular apertures reniform. Dorsum with or without dark markings 16
(Figure 22)

16. ANT tubercles little developed, or if somewhat developed then with middle part of front of head also projecting forward somewhat, so that the outline is sinuous in dorsal view. SIPH dark or pale with dark apices, never with polygonal reticulation. ABD TERG 1 and 7 constantly with MTu (although these may be very small). Body oval with length of SIPH usually 0.5 or less than the distance between their bases 22

22. SIPH tapering gradually over most of length, without any subapical constriction and usually with a small or moderate flange. Tergum smooth, wrinkled, or reticulated, but without bead-like spicules arranged in polygons .....24 (Figure 21)

24. Dorsal abdomen with or without dark markings, but without an extensive solid black sclerite. Cauda pale or dark, but if black then usually with more than 7 hairs 25

25. Stridulatory apparatus not present 28

28. SIPH 0.10-0.26 × BL. ANT III usually without rhinaria (except in alatiform specimens). ANT PT/BASE 1.4-4.7. ANT PT 1.7-3.2 × R IV+V. Cauda usually tongue- or finger-shaped. Dorsal abdomen with or without dark markings 29

29. MTu only sporadically occurring on ABD TERG 2-5 and then small and placed on marginal sclerites (if these are present). Cauda tongue- or finger-shaped, distinctly longer than its basal width, with 4-24 hairs 30

30. SIPH uniformly dark 31

31. Hind tibiae pale for more than half of length. ANT PT/BASE 1.4-3.5. R IV+V 0.85-1.3 (-1.5) × HT II. SIPH 0.7-2.5 × cauda 32

32 Cauda pale, dusky or dark, bearing 4-7(-8) hairs. Longest hind femoral hairs only 0.4-0.7 × diameter of trochantro-femoral suture. (Also check: ANT PT/BASE 2.0-3.5, R IV+V 1.1-1.5 × HT II, SIPH 1.3-2.5 × cauda, alata with secondary rhinaria distributed ANT III 3-15, IV almost always 0) …..*Aphis gossypii* (Plate 7c, Figure 38)

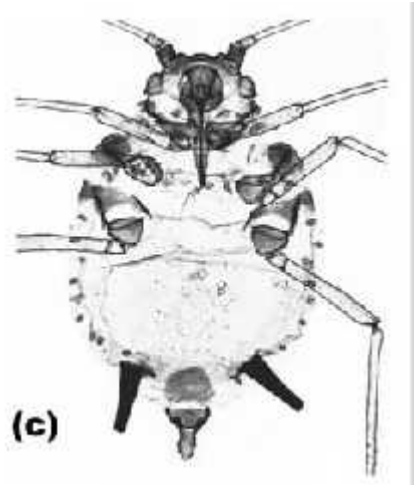
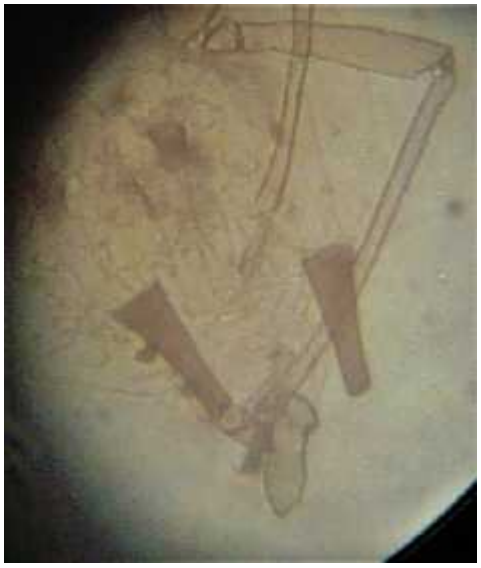


Figure 39. (left) illustration of *Aphis gossypii*

Reprinted from [Plate 7c] Copy right holder unknown. Retrieved from http://www.aphidsonworldsplants.info/Polyphagous_II.htm

Figure 40. (right) Microscopic photo of A. gossypii on prepared slide. (photo by M. Jagroep, 2017)



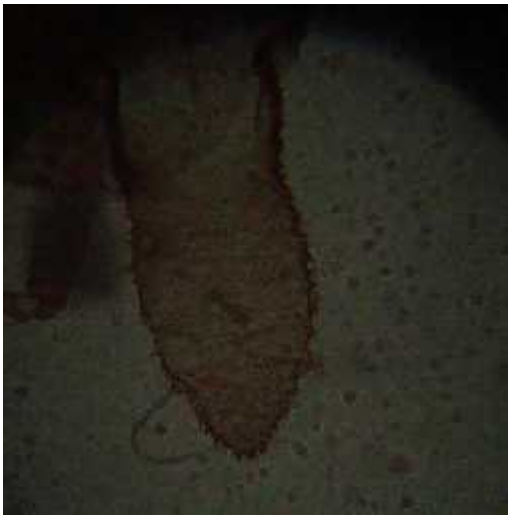


Figure 41. (left) Microscopic photo of A. gossypii showing Siphuncule and tongue shaped cauda (photo by M.Jagroep, 2017)

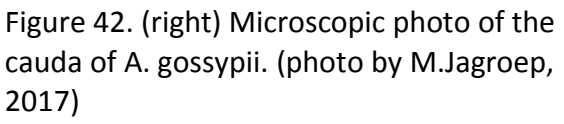


Figure 42. (right) Microscopic photo of the cauda of A. gossypii. (photo by M.Jagroep, 2017)

Appendix 4. Probit analysis

Table 10 shows the amount of dead aphids in different concentrations of the mint extract. These figures were log-transformed and plotted in a curve (Figure 23) with SPSS software. Because the exact concentration at 50 % mortality (0.5) could not read from the curve, this was done from the cell counts and residuals (table 11) and from the confidence limits at a probability of 0.5 (table 12). The concentration with 50 % mortality is regressed at 104.450, so the LC50 is about 100g/l.

The LC 50 of mint leaf extract is 100g/l and about 70% of the studied aphids died at the concentration of 200 g/l. To kill 99% of the aphid population, theoretically 466 g/l will be necessary, which is too much and practically not feasible. Therefor the concentrations of 100g/l and 200 g/l were used in this thesis to test the efficacy of *P. amboinicus* as botanical.

Table 10: Response of the aphids to different concentration of mint leaf extract

Concentration leafextract in g/l	Total aphids	Dead aphids
0	100	11
50	100	39
100	100	60
200	100	70

Table 11: Cell counts and residuals

	Number	concentration	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability	
	1	.000	100	11	20.945	-9.945	.209	
	2	50.000	100	39	33.350	5.650	.333	
LOGIT	3	100.000	100	60	48.586	11.414	.486	
	4	200.000	100	70			77.120	-7.120

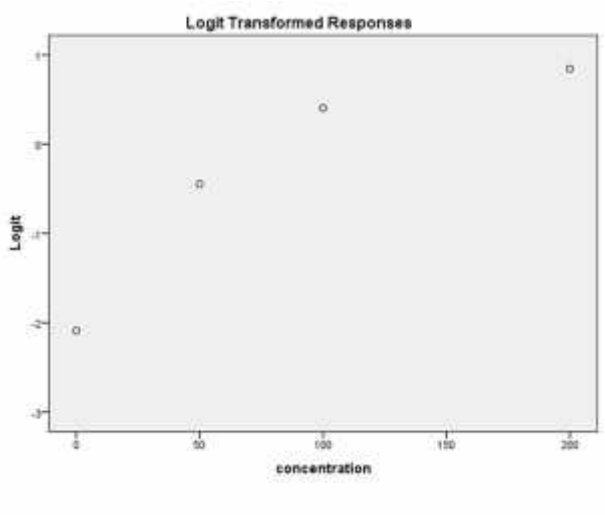


Figure 43. Logit transformed responses[i]

Table 12. Confidence limits

Probability	95% Confidence Limits for concentration		
	Estimate	Lower Bound	Upper Bound
.010	-256.897	.	.
.020	-201.592	.	.
.030	-168.900	.	.
.040	-145.463	.	.
.050	-127.092	.	.
.060	-111.923	.	.
.070	-98.960	.	.
.080	-87.609	.	.
.090	-77.488	.	.
.100	-68.334	.	.
.150	-31.954	.	.
.200	-4.564	.	.
.250	18.058	.	.
.300	37.821	.	.
.350	55.770	.	.

LOGIT[a]	.400	72.565	.	.
	.450	88.669	.	.
	.500	104.450	.	.
	.550	120.230	.	.
	.600	136.334	.	.
	.650	153.129	.	.
	.700	171.078	.	.
	.750	190.841	.	.
	.800	213.464	.	.
	.850	240.853	.	.
	.900	277.233	.	.
	.910	286.387	.	.
	.920	296.508	.	.
	.930	307.859	.	.
	.940	320.822	.	.
	.950	335.991	.	.
	.960	354.362	.	.
	.970	377.799	.	.
	.980	410.491	.	.
	.990	465.796	.	.

a. A heterogeneity factor is used

Appendix 5. Soil analysis results (Adek University Suriname)

experiment	Lab MNr	pH H2O (1:2.5)	EC (mS)	Tot. N (%)	Tot. N (g)	Tot. P (ppm P)	Tot. K (ppm K)
exp.1 *	2017-238	7.1	6	0.39	2.8	185	116
exp.2 GH	2017-458	7.6	1427	0.45	3.5	472	301
exp.2 OF	2017-457	7.5	1863	0.50	3.5	567	431

GH=Greenhouse OF=Open field

*The same result was valuable for Open field as well as for greenhouse soil

Appendix 6 Raw data of the responded aphids to the treatments during experiment1

location	week	sample	block	treatment	aphids	location	week	sample	block	treatment	aphids
GH	0	1	1	A	97	OF	0	1	1	A	1
GH	0	2	1	A	184	OF	0	2	1	A	3
GH	0	3	1	A	225	OF	0	3	1	A	1
GH	0	1	1	B	73	OF	0	1	1	B	0
GH	0	2	1	B	133	OF	0	2	1	B	0
GH	0	3	1	B	225	OF	0	3	1	B	1
GH	0	1	1	C	115	OF	0	1	1	C	3
GH	0	2	1	C	216	OF	0	2	1	C	8
GH	0	3	1	C	490	OF	0	3	1	C	15
GH	0	1	2	A	71	OF	0	1	2	A	10
GH	0	2	2	A	151	OF	0	2	2	A	0
GH	0	3	2	A	96	OF	0	3	2	A	28
GH	0	1	2	B	258	OF	0	1	2	B	2
GH	0	2	2	B	345	OF	0	2	2	B	1
GH	0	3	2	B	87	OF	0	3	2	B	11
GH	0	1	2	C	131	OF	0	1	2	C	3
GH	0	2	2	C	149	OF	0	2	2	C	5
GH	0	3	2	C	374	OF	0	3	2	C	0
GH	0	1	3	A	56	OF	0	1	3	A	3
GH	0	2	3	A	72	OF	0	2	3	A	5
GH	0	3	3	A	68	OF	0	3	3	A	0
GH	0	1	3	B	101	OF	0	1	3	B	1
GH	0	2	3	B	105	OF	0	2	3	B	13
GH	0	3	3	B	54	OF	0	3	3	B	8
GH	0	1	3	C	249	OF	0	1	3	C	25
GH	0	2	3	C	107	OF	0	2	3	C	0
GH	0	3	3	C	236	OF	0	3	3	C	10
GH	1	1	1	A	25	OF	1	1	1	A	3
GH	1	2	1	A	18	OF	1	2	1	A	6
GH	1	3	1	A	13	OF	1	3	1	A	4
GH	1	1	1	B	3	OF	1	1	1	B	4
GH	1	2	1	B	0	OF	1	2	1	B	2
GH	1	3	1	B	2	OF	1	3	1	B	0
GH	1	1	1	C	0	OF	1	1	1	C	11
GH	1	2	1	C	2	OF	1	2	1	C	9
GH	1	3	1	C	0	OF	1	3	1	C	30
GH	1	1	2	A	12	OF	1	1	2	A	12
GH	1	2	2	A	56	OF	1	2	2	A	9
GH	1	3	2	A	31	OF	1	3	2	A	15
GH	1	1	2	B	15	OF	1	1	2	B	38
GH	1	2	2	B	8	OF	1	2	2	B	5

GH	1	3	2	B	14	OF	1	3	2	B	10
GH	1	1	2	C	2	OF	1	1	2	C	8
GH	1	2	2	C	0	OF	1	2	2	C	10
GH	1	3	2	C	1	OF	1	3	2	C	16
GH	1	1	3	A	15	OF	1	1	3	A	10
GH	1	2	3	A	19	OF	1	2	3	A	0
GH	1	3	3	A	13	OF	1	3	3	A	1
GH	1	1	3	B	1	OF	1	1	3	B	10
GH	1	2	3	B	2	OF	1	2	3	B	20
GH	1	3	3	B	6	OF	1	3	3	B	6
GH	1	1	3	C	0	OF	1	1	3	C	4
GH	1	2	3	C	2	OF	1	2	3	C	15
GH	1	3	3	C	5	OF	1	3	3	C	8
GH	2	1	1	A	31	OF	2	1	1	A	12
GH	2	2	1	A	22	OF	2	2	1	A	12
GH	2	3	1	A	20	OF	2	3	1	A	18
GH	2	1	1	B	14	OF	2	1	1	B	4
GH	2	2	1	B	6	OF	2	2	1	B	5
GH	2	3	1	B	10	OF	2	3	1	B	5
GH	2	1	1	C	2	OF	2	1	1	C	8
GH	2	2	1	C	3	OF	2	2	1	C	13
GH	2	3	1	C	8	OF	2	3	1	C	22
GH	2	1	2	A	4	OF	2	1	2	A	15
GH	2	2	2	A	1	OF	2	2	2	A	10
GH	2	3	2	A	11	OF	2	3	2	A	5
GH	2	1	2	B	2	OF	2	1	2	B	32
GH	2	2	2	B	1	OF	2	2	2	B	1
GH	2	3	2	B	1	OF	2	3	2	B	22
GH	2	1	2	C	0	OF	2	1	2	C	8
GH	2	2	2	C	6	OF	2	2	2	C	2
GH	2	3	2	C	5	OF	2	3	2	C	7
GH	2	1	3	A	8	OF	2	1	3	A	0
GH	2	2	3	A	12	OF	2	2	3	A	7
GH	2	3	3	A	11	OF	2	3	3	A	51
GH	2	1	3	B	7	OF	2	1	3	B	8
GH	2	2	3	B	4	OF	2	2	3	B	3
GH	2	3	3	B	2	OF	2	3	3	B	12
GH	2	1	3	C	0	OF	2	1	3	C	5
GH	2	2	3	C	1	OF	2	2	3	C	11
GH	2	3	3	C	1	OF	2	3	3	C	16
GH	3	1	1	A	98	OF	3	1	1	A	
GH	3	2	1	A	24	OF	3	2	1	A	
GH	3	3	1	A	213	OF	3	3	1	A	
GH	3	1	1	B	73	OF	3	1	1	B	
GH	3	2	1	B	38	OF	3	2	1	B	
GH	3	3	1	B	25	OF	3	3	1	B	

GH	3	1	1	C	49	OF	3	1	1	C
GH	3	2	1	C	0	OF	3	2	1	C
GH	3	3	1	C	59	OF	3	3	1	C
GH	3	1	2	A	17	OF	3	1	2	A
GH	3	2	2	A	26	OF	3	2	2	A
GH	3	3	2	A	27	OF	3	3	2	A
GH	3	1	2	B	43	OF	3	1	2	B
GH	3	2	2	B	46	OF	3	2	2	B
GH	3	3	2	B	18	OF	3	3	2	B
GH	3	1	2	C	42	OF	3	1	2	C
GH	3	2	2	C	21	OF	3	2	2	C
GH	3	3	2	C	9	OF	3	3	2	C
GH	3	1	3	A	23	OF	3	1	3	A
GH	3	2	3	A	11	OF	3	2	3	A
GH	3	3	3	A	40	OF	3	3	3	A
GH	3	1	3	B	38	OF	3	1	3	B
GH	3	2	3	B	12	OF	3	2	3	B
GH	3	3	3	B	16	OF	3	3	3	B
GH	3	1	3	C	19	OF	3	1	3	C
GH	3	2	3	C	7	OF	3	2	3	C
GH	3	3	3	C	2	OF	3	3	3	C
GH	4	1	1	A	118	OF	4	1	1	A
GH	4	2	1	A	50	OF	4	2	1	A
GH	4	3	1	A	69	OF	4	3	1	A
GH	4	1	1	B	25	OF	4	1	1	B
GH	4	2	1	B	23	OF	4	2	1	B
GH	4	3	1	B	39	OF	4	3	1	B
GH	4	1	1	C	7	OF	4	1	1	C
GH	4	2	1	C	2	OF	4	2	1	C
GH	4	3	1	C	7	OF	4	3	1	C
GH	4	1	2	A	71	OF	4	1	2	A
GH	4	2	2	A	56	OF	4	2	2	A
GH	4	3	2	A	49	OF	4	3	2	A
GH	4	1	2	B	20	OF	4	1	2	B
GH	4	2	2	B	6	OF	4	2	2	B
GH	4	3	2	B	16	OF	4	3	2	B
GH	4	1	2	C	1	OF	4	1	2	C
GH	4	2	2	C	1	OF	4	2	2	C
GH	4	3	2	C	3	OF	4	3	2	C
GH	4	1	3	A	10	OF	4	1	3	A
GH	4	2	3	A	22	OF	4	2	3	A
GH	4	3	3	A	5	OF	4	3	3	A
GH	4	1	3	B	3	OF	4	1	3	B
GH	4	2	3	B	7	OF	4	2	3	B
GH	4	3	3	B	6	OF	4	3	3	B
GH	4	1	3	C	7	OF	4	1	3	C

GH	4	2	3	C	3	OF	4	2	3	C
GH	4	3	3	C	22	OF	4	3	3	C

Appendix 7 Raw data of the responded aphids to the treatments during experiment2

location	week	sample	block	treatment	aphids	location	week	sample	block	treatment	aphids
GH	0	1	1	A	69	OF	0	1	1	A	11
GH	0	2	1	A	11	OF	0	2	1	A	40
GH	0	3	1	A	370	OF	0	3	1	A	3
GH	0	1	1	B	111	OF	0	1	1	B	16
GH	0	2	1	B	190	OF	0	2	1	B	26
GH	0	3	1	B	96	OF	0	3	1	B	36
GH	0	1	1	C	34	OF	0	1	1	C	18
GH	0	2	1	C	318	OF	0	2	1	C	70
GH	0	3	1	C	68	OF	0	3	1	C	95
GH	0	1	2	A	180	OF	0	1	2	A	35
GH	0	2	2	A	95	OF	0	2	2	A	115
GH	0	3	2	A	86	OF	0	3	2	A	78
GH	0	1	2	B	72	OF	0	1	2	B	86
GH	0	2	2	B	33	OF	0	2	2	B	32
GH	0	3	2	B	105	OF	0	3	2	B	52
GH	0	1	2	C	32	OF	0	1	2	C	44
GH	0	2	2	C	81	OF	0	2	2	C	34
GH	0	3	2	C	32	OF	0	3	2	C	88
GH	0	1	3	A	50	OF	0	1	3	A	68
GH	0	2	3	A	75	OF	0	2	3	A	79
GH	0	3	3	A	91	OF	0	3	3	A	45
GH	0	1	3	B	25	OF	0	1	3	B	108
GH	0	2	3	B	29	OF	0	2	3	B	87
GH	0	3	3	B	80	OF	0	3	3	B	42
GH	0	1	3	C	35	OF	0	1	3	C	110
GH	0	2	3	C	31	OF	0	2	3	C	135
GH	0	3	3	C	88	OF	0	3	3	C	86
GH	1	1	1	A	248	OF	1	1	1	A	21
GH	1	2	1	A	69	OF	1	2	1	A	38
GH	1	3	1	A	490	OF	1	3	1	A	57
GH	1	1	1	B	202	OF	1	1	1	B	40
GH	1	2	1	B	326	OF	1	2	1	B	75
GH	1	3	1	B	154	OF	1	3	1	B	98
GH	1	1	1	C	55	OF	1	1	1	C	34
GH	1	2	1	C	180	OF	1	2	1	C	2
GH	1	3	1	C	84	OF	1	3	1	C	49
GH	1	1	2	A	64	OF	1	1	2	A	43
GH	1	2	2	A	166	OF	1	2	2	A	23

GH	1	3	2	A	178	OF	1	3	2	A	46
GH	1	1	2	B	359	OF	1	1	2	B	43
GH	1	2	2	B	244	OF	1	2	2	B	37
GH	1	3	2	B	324	OF	1	3	2	B	38
GH	1	1	2	C	184	OF	1	1	2	C	0
GH	1	2	2	C	57	OF	1	2	2	C	7
GH	1	3	2	C	286	OF	1	3	2	C	8
GH	1	1	3	A	46	OF	1	1	3	A	42
GH	1	2	3	A	55	OF	1	2	3	A	30
GH	1	3	3	A	57	OF	1	3	3	A	94
GH	1	1	3	B	111	OF	1	1	3	B	76
GH	1	2	3	B	73	OF	1	2	3	B	50
GH	1	3	3	B	140	OF	1	3	3	B	16
GH	1	1	3	C	69	OF	1	1	3	C	14
GH	1	2	3	C	223	OF	1	2	3	C	23
GH	1	3	3	C	125	OF	1	3	3	C	80
GH	2	1	1	A	151	OF	2	1	1	A	176
GH	2	2	1	A	60	OF	2	2	1	A	62
GH	2	3	1	A	150	OF	2	3	1	A	108
GH	2	1	1	B	4	OF	2	1	1	B	73
GH	2	2	1	B	12	OF	2	2	1	B	50
GH	2	3	1	B	29	OF	2	3	1	B	142
GH	2	1	1	C	14	OF	2	1	1	C	31
GH	2	2	1	C	6	OF	2	2	1	C	56
GH	2	3	1	C	33	OF	2	3	1	C	22
GH	2	1	2	A	20	OF	2	1	2	A	320
GH	2	2	2	A	73	OF	2	2	2	A	106
GH	2	3	2	A	112	OF	2	3	2	A	306
GH	2	1	2	B	10	OF	2	1	2	B	216
GH	2	2	2	B	10	OF	2	2	2	B	79
GH	2	3	2	B	51	OF	2	3	2	B	106
GH	2	1	2	C	2	OF	2	1	2	C	4
GH	2	2	2	C	5	OF	2	2	2	C	158
GH	2	3	2	C	17	OF	2	3	2	C	94
GH	2	1	3	A	182	OF	2	1	3	A	79
GH	2	2	3	A	63	OF	2	2	3	A	92
GH	2	3	3	A	261	OF	2	3	3	A	102
GH	2	1	3	B	16	OF	2	1	3	B	91
GH	2	2	3	B	10	OF	2	2	3	B	103
GH	2	3	3	B	55	OF	2	3	3	B	148
GH	2	1	3	C	3	OF	2	1	3	C	186
GH	2	2	3	C	19	OF	2	2	3	C	21
GH	2	3	3	C	26	OF	2	3	3	C	156
GH	3	1	1	A	218	OF	3	1	1	A	69
GH	3	2	1	A	80	OF	3	2	1	A	124
GH	3	3	1	A	144	OF	3	3	1	A	138

GH	3	1	1	B	10	OF	3	1	1	B	69
GH	3	2	1	B	187	OF	3	2	1	B	62
GH	3	3	1	B	12	OF	3	3	1	B	161
GH	3	1	1	C	2	OF	3	1	1	C	53
GH	3	2	1	C	38	OF	3	2	1	C	59
GH	3	3	1	C	41	OF	3	3	1	C	358
GH	3	1	2	A	127	OF	3	1	2	A	149
GH	3	2	2	A	95	OF	3	2	2	A	61
GH	3	3	2	A	228	OF	3	3	2	A	450
GH	3	1	2	B	24	OF	3	1	2	B	20
GH	3	2	2	B	51	OF	3	2	2	B	132
GH	3	3	2	B	25	OF	3	3	2	B	38
GH	3	1	2	C	12	OF	3	1	2	C	4
GH	3	2	2	C	9	OF	3	2	2	C	32
GH	3	3	2	C	21	OF	3	3	2	C	5
GH	3	1	3	A	88	OF	3	1	3	A	28
GH	3	2	3	A	66	OF	3	2	3	A	89
GH	3	3	3	A	89	OF	3	3	3	A	65
GH	3	1	3	B	17	OF	3	1	3	B	168
GH	3	2	3	B	22	OF	3	2	3	B	23
GH	3	3	3	B	60	OF	3	3	3	B	128
GH	3	1	3	C	39	OF	3	1	3	C	35
GH	3	2	3	C	17	OF	3	2	3	C	51
GH	3	3	3	C	18	OF	3	3	3	C	9
GH	4	1	1	A	77	OF	4	1	1	A	0
GH	4	2	1	A	36	OF	4	2	1	A	0
GH	4	3	1	A	170	OF	4	3	1	A	0
GH	4	1	1	B	12	OF	4	1	1	B	227
GH	4	2	1	B	59	OF	4	2	1	B	59
GH	4	3	1	B	96	OF	4	3	1	B	74
GH	4	1	1	C	56	OF	4	1	1	C	0
GH	4	2	1	C	8	OF	4	2	1	C	0
GH	4	3	1	C	43	OF	4	3	1	C	0
GH	4	1	2	A	93	OF	4	1	2	A	286
GH	4	2	2	A	118	OF	4	2	2	A	358
GH	4	3	2	A	191	OF	4	3	2	A	199
GH	4	1	2	B	2	OF	4	1	2	B	102
GH	4	2	2	B	0	OF	4	2	2	B	102
GH	4	3	2	B	83	OF	4	3	2	B	5
GH	4	1	2	C	13	OF	4	1	2	C	25
GH	4	2	2	C	0	OF	4	2	2	C	0
GH	4	3	2	C	38	OF	4	3	2	C	0
GH	4	1	3	A	130	OF	4	1	3	A	5
GH	4	2	3	A	41	OF	4	2	3	A	4
GH	4	3	3	A	266	OF	4	3	3	A	0
GH	4	1	3	B	35	OF	4	1	3	B	0

GH	4	2	3	B	11	OF	4	2	3	B	0
GH	4	3	3	B	30	OF	4	3	3	B	0
GH	4	1	3	C	18	OF	4	1	3	C	0
GH	4	2	3	C	4	OF	4	2	3	C	0
GH	4	3	3	C	4	OF	4	3	3	C	0

Appendix 8 Raw data of production during experiment 1 and 2

			EXPERIMENT 1					EXPERIMENT 2	
location	**week**	**block**	**treatment**	**weight(g)**	**location**	**week**	**block**	**treatment**	**weight(g)**
GH	9	1	100g/l mint	2110	GH	**7**	**1**	**100g/l mint**	430
GH	9	1	200g/l mint	1055	GH	7	1	200g/l mint	1105
GH	9	1	Abamectin	0	GH	7	1	Abamectin	0
GH	9	2	100g/l mint	1745	GH	7	2	100g/l mint	685
GH	9	2	200g/l mint	0	GH	7	2	200g/l mint	835
GH	9	2	Abamectin	140	GH	7	2	Abamectin	280
GH	9	3	100g/l mint	1740	GH	7	3	100g/l mint	625
GH	9	3	200g/l mint	810	GH	7	3	200g/l mint	965
GH	9	3	Abamectin	1675	GH	7	3	Abamectin	325
GH	10	1	100g/l mint	2720	GH	8	1	100g/l mint	965
GH	10	1	200g/l mint	5325	GH	8	1	200g/l mint	2085
GH	10	1	Abamectin	7000	GH	8	1	Abamectin	1315
GH	10	2	100g/l mint	4375	GH	8	2	100g/l mint	1145
GH	10	2	200g/l mint	3905	GH	8	2	200g/l mint	1755
GH	10	2	Abamectin	4875	GH	8	2	Abamectin	1825
GH	10	3	100g/l mint	4070	GH	8	3	100g/l mint	2395
GH	10	3	200g/l mint	4535	GH	8	3	200g/l mint	820
GH	10	3	Abamectin	2950	GH	8	3	Abamectin	1220
GH	11	1	100g/l mint	3240	GH	9	1	100g/l mint	1848
GH	11	1	200g/l mint	4405	GH	9	1	200g/l mint	2073
GH	11	1	Abamectin	8940	GH	9	1	Abamectin	1995
GH	11	2	100g/l mint	2820	GH	9	2	100g/l mint	630
GH	11	2	200g/l mint	6410	GH	9	2	200g/l mint	1100
GH	11	2	Abamectin	5815	GH	9	2	Abamectin	730
GH	11	3	100g/l mint	4855	GH	9	3	100g/l mint	2140
GH	11	3	200g/l mint	3875	GH	9	3	200g/l mint	1570
GH	11	3	Abamectin	3980	GH	9	3	Abamectin	1640
GH	12	1	100g/l mint	1470	GH	10	1	100g/l mint	160
GH	12	1	200g/l mint	3320	GH	10	1	200g/l mint	550
GH	12	1	Abamectin	5995	GH	10	1	Abamectin	1620
GH	12	2	100g/l mint	2690	GH	10	2	100g/l mint	375
GH	12	2	200g/l mint	1680	GH	10	2	200g/l mint	180
GH	12	2	Abamectin	3565	GH	10	2	Abamectin	545

GH	12	3	100g/l mint	1750	GH	10	3	100g/l mint	330
GH	12	3	200g/l mint	2970	GH	10	3	200g/l mint	355
GH	12	3	Abamectin	3330	GH	10	3	Abamectin	305
GH	13	1	100g/l mint	125	OF	7	1	100g/l mint	385
GH	13	1	200g/l mint	520	OF	7	1	200g/l mint	190
GH	13	1	Abamectin	595	OF	7	1	Abamectin	0
GH	13	2	100g/l mint	565	OF	7	2	100g/l mint	190
GH	13	2	200g/l mint	210	OF	7	2	200g/l mint	260
GH	13	2	Abamectin	1435	OF	7	2	Abamectin	0
GH	13	3	100g/l mint	345	OF	7	3	100g/l mint	285
GH	13	3	200g/l mint	0	OF	7	3	200g/l mint	510
GH	13	3	Abamectin	1165	OF	7	3	Abamectin	255
					OF	8	1	100g/l mint	2110
					OF	8	1	200g/l mint	2200
					OF	8	1	Abamectin	715
					OF	8	2	100g/l mint	1455
					OF	8	2	200g/l mint	1365
					OF	8	2	Abamectin	2330
					OF	8	3	100g/l mint	1795
					OF	8	3	200g/l mint	1020
					OF	8	3	Abamectin	1610
					OF	9	1	100g/l mint	2425
					OF	9	1	200g/l mint	1830
					OF	9	1	Abamectin	220
					OF	9	2	100g/l mint	1120
					OF	9	2	200g/l mint	940
					OF	9	2	Abamectin	2095
					OF	9	3	100g/l mint	895
					OF	9	3	200g/l mint	1700
					OF	9	3	Abamectin	1710
					OF	10	1	100g/l mint	930
					OF	10	1	200g/l mint	465
					OF	10	1	Abamectin	0
					OF	10	2	100g/l mint	0
					OF	10	2	200g/l mint	0
					OF	10	2	Abamectin	960
					OF	10	3	100g/l mint	350
					OF	10	3	200g/l mint	155
					OF	10	3	Abamectin	350

Appendix 9 Data analysis in SPSS (regarding table 5)

Experiment 1 week1

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphidsplus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17.411[a]	2	8.706	3.338	.043
Intercept	537.456	1	537.456	206.103	.000
treatment	17.411	2	8.706	3.338	.043
Error	132.993	51	2.608		
Total	687.860	54			
Corrected Total	150.404	53			

a. R Squared = .116 (Adjusted R Squared = .081)

Multiple Comparisons

Dependent Variable: SQRT_aphidsplus

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	1.0204	.53828	.150	-.2790	2.3198
	Abamectin	1.3287*	.53828	.044	.0293	2.6281
200g/l mint	100g/l mint	-1.0204	.53828	.150	-2.3198	.2790
	Abamectin	.3083	.53828	.835	-.9911	1.6077
Abamectin	100g/l mint	-1.3287*	.53828	.044	-2.6281	-.0293
	200g/l mint	-.3083	.53828	.835	-1.6077	.9911

Based on observed means. The error term is Mean Square(Error) = 2.608.

*. The mean difference is significant at the 0.05 level.

SQRT_aphidsplus

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	18	2.6091	
200g/l mint	18	2.9174	2.9174
100g/l mint	18		3.9379
Sig.		.835	.150

Experiment 1 week2:

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphidsplus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.138[a]	2	6.569	3.340	.043
Intercept	552.850	1	552.850	281.125	.000
treatment	13.138	2	6.569	3.340	.043
Error	100.295	51	1.967		
Total	666.283	54			
Corrected Total	113.433	53			

a. R Squared = .116 (Adjusted R Squared = .081)

Multiple Comparisons

Dependent Variable: SQRT_aphidsplus

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	.9008	.46745	.141	-.2276	2.0292
	Abamectin	1.1478*	.46745	.045	.0194	2.2762
200g/l mint	100g/l mint	-.9008	.46745	.141	-2.0292	.2276
	Abamectin	.2470	.46745	.858	-.8814	1.3754
Abamectin	100g/l mint	-1.1478*	.46745	.045	-2.2762	-.0194
	200g/l mint	-.2470	.46745	.858	-1.3754	.8814

Based on observed means. The error term is Mean Square(Error) = 1.967.

*. The mean difference is significant at the 0.05 level.

SQRT_aphidsplus

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	18	2.7348	
200g/l mint	18	2.9818	2.9818
100g/l mint	18		3.8825
Sig.		.858	.141

Experiment 1 week3:

Tests of Between-Subjects Effects

Dependent Variable: SRT_aphidsplus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	25.464[a]	2	12.732	1.722	.200
Intercept	946.146	1	946.146	127.950	.000
treatment	25.464	2	12.732	1.722	.200
Error	177.472	24	7.395		
Total	1149.081	27			
Corrected Total	202.935	26			

a. R Squared = .125 (Adjusted R Squared = .053)

Multiple Comparisons

Dependent Variable: SRT_aphidsplus

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	.8143	1.28190	.802	-2.3869	4.0156
	Abamectin	2.3428	1.28190	.182	-.8585	5.5440
200g/l mint	100g/l mint	-.8143	1.28190	.802	-4.0156	2.3869
	Abamectin	1.5284	1.28190	.469	-1.6728	4.7297
Abamectin	100g/l mint	-2.3428	1.28190	.182	-5.5440	.8585
	200g/l mint	-1.5284	1.28190	.469	-4.7297	1.6728

Based on observed means.

The error term is Mean Square(Error) = 7.395.

SRT_aphidsplus

Tukey HSD

treatment	N	Subset
		1
Abamectin	9	4.6293
200g/l mint	9	6.1577
100g/l mint	9	6.9720
Sig.		.182

Experiment 1 week 4

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphids

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	90.013[a]	2	45.007	12.144	.000
Intercept	469.037	1	469.037	126.554	.000
treatment	90.013	2	45.007	12.144	.000
Error	88.950	24	3.706		
Total	648.000	27			
Corrected Total	178.963	26			

a. R Squared = .503 (Adjusted R Squared = .462)

Multiple Comparisons

Dependent Variable: SQRT_aphids

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	2.8277*	.90753	.013	.5613	5.0940
	Abamectin	4.4147*	.90753	.000	2.1484	6.6811
200g/l mint	100g/l mint	-2.8277*	.90753	.013	-5.0940	-.5613
	Abamectin	1.5871	.90753	.208	-.6793	3.8534
Abamectin	100g/l mint	-4.4147*	.90753	.000	-6.6811	-2.1484
	200g/l mint	-1.5871	.90753	.208	-3.8534	.6793

Based on observed means. The error term is Mean Square(Error) = 3.706.

*. The mean difference is significant at the .05 level.

SQRT_aphids

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	9	2.1673	
200g/l mint	9	3.7544	
100g/l mint	9		6.5821
Sig.		.208	1.000

Experiment 2 week1: **Univariate Analysis of Variance**

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphid plus 0.5

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	76.159[a]	2	38.079	1.761	.182
Intercept	4979.150	1	4979.150	230.282	.000
treatment	76.159	2	38.079	1.761	.182
Error	1102.722	51	21.622		
Total	6158.031	54			
Corrected Total	1178.881	53			

a. R Squared = .065 (Adjusted R Squared = .028)

Post Hoc test -Treatment

Multiple Comparisons

Dependent Variable: SQRT_aphidplus 0.5

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mint 100g	Mint 200g	-1.7436	1.54998	.503	-5.4852	1.9980
	Abamectin	1.1447	1.54998	.742	-2.5969	4.8864
Mint 200g	Mint 100g	1.7436	1.54998	.503	-1.9980	5.4852
	Abamectin	2.8883	1.54998	.160	-.8533	6.6300
Abamectin	Mint 100g	-1.1447	1.54998	.742	-4.8864	2.5969
	Mint 200g	-2.8883	1.54998	.160	-6.6300	.8533

Based on observed means. The error term is Mean Square(Error) = 21.622.

SQRT_aphidplus 0.5

Tukey HSD

treatment	N	Subset
		1
Abamectin	18	8.2581
Mint 100g	18	9.4028
Mint 200g	18	11.1464
Sig.		.160

Means for groups in homogenous subsets are displayed.
Based on the observed means. The error term is Mean Square (Error)= 21.622. Alpha=0.05

Experiment2 week 2: Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphidsplus 0.5

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	267.828[a]	2	133.914	9.639	.000
Intercept	3953.175	1	3953.175	284.551	.000
treatment	267.828	2	133.914	9.639	.000
Error	708.527	51	13.893		
Total	4929.530	54			
Corrected Total	976.355	53			

a. R Squared = .274 (Adjusted R Squared = .246)

Post Hoc test -Treatment

Multiple Comparisons

Dependent Variable: SQRT_aphidsplus 0.5

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mint 100g	Mint 200g	3.7274*	1.24243	.011	.7282	6.7266
	Abamectin	5.3132*	1.24243	.000	2.3140	8.3124
Mint 200g	Mint 100g	-3.7274*	1.24243	.011	-6.7266	-.7282
	Abamectin	1.5857	1.24243	.415	-1.4135	4.5849
Abamectin	Mint 100g	-5.3132*	1.24243	.000	-8.3124	-2.3140
	Mint 200g	-1.5857	1.24243	.415	-4.5849	1.4135

Based on observed means. The error term is Mean Square(Error) = 13.893.

*. The mean difference is significant at the 0.05 level.

SQRT_aphidsplus 0.5

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	18	6.2565	
Mint 200g	18	7.8422	
Mint 100g	18		11.5696
Sig.		.415	1.000

Experiment2 week 3: Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphidplus 0.5

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	254.084[a]	2	127.042	9.487	.000
Intercept	3823.857	1	3823.857	285.543	.000
treatment	254.084	2	127.042	9.487	.000
Error	682.969	51	13.392		
Total	4760.910	54			
Corrected Total	937.053	53			

a. R Squared = .271 (Adjusted R Squared = .243)

Post Hoc test -Treatment

Multiple Comparisons

Dependent Variable: SQRT_aphidplus 0.5

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	3.3336*	1.21982	.023	.3890	6.2782
	Abamectin	5.2500*	1.21982	.000	2.3054	8.1946
200g/l mint	100g/l mint	-3.3336*	1.21982	.023	-6.2782	-.3890
	Abamectin	1.9164	1.21982	.267	-1.0282	4.8610
Abamectin	100g/l mint	-5.2500*	1.21982	.000	-8.1946	-2.3054
	200g/l mint	-1.9164	1.21982	.267	-4.8610	1.0282

Based on observed means. The error term is Mean Square(Error) = 13.392.

*. The mean difference is significant at the 0.05 level.

SQRT_aphidplus 0.5

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	18	6.0262	
200g/l mint	18	7.9426	
100g/l mint	18		11.2762
Sig.		.267	1.000

Experiment 2 week 4: Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: SQRT_aphidsplus 0.5

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	340.324[a]	2	170.162	7.359	.002
Intercept	1864.130	1	1864.130	80.615	.000
treatment	340.324	2	170.162	7.359	.002
Error	1179.321	51	23.124		
Total	3383.774	54			
Corrected Total	1519.645	53			

a. R Squared = .224 (Adjusted R Squared = .194)

Multiple Comparisons

Dependent Variable: SQRT_aphidsplus 0.5

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	2.8037	1.60291	.197	-1.0657	6.6731
	Abamectin	6.1416*	1.60291	.001	2.2722	10.0109
200g/l mint	100g/l mint	-2.8037	1.60291	.197	-6.6731	1.0657
	Abamectin	3.3378	1.60291	.104	-.5316	7.2072
Abamectin	100g/l mint	-6.1416*	1.60291	.001	-10.0109	-2.2722
	200g/l mint	-3.3378	1.60291	.104	-7.2072	.5316

Based on observed means. The error term is Mean Square(Error) = 23.124.

*. The mean difference is significant at the 0.05 level.

SQRT_aphidsplus 0.5

Tukey HSD

treatment	N	Subset	
		1	2
Abamectin	18	2.7157	
200g/l mint	18	6.0535	6.0535
100g/l mint	18		8.8572
Sig.		.104	.197

Experiment 1 without pre-spray overall weeks

Tests of Between-Subjects Effects

Dependent Variable: SQRT_plus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	105.506[a]	2	52.753	11.606	.000
Intercept	2442.294	1	2442.294	537.319	.000
treatment	105.506	2	52.753	11.606	.000
Error	722.709	159	4.545		
Total	3270.508	162			
Corrected Total	828.214	161			

a. R Squared = .127 (Adjusted R Squared = .116)

Multiple Comparisons

Dependent Variable: SQRT_plus

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l	200g/l	1.2474*	.41030	.008	.2767	2.2181
	abamectin	1.9517*	.41030	.000	.9810	2.9225
200g/l	100g/l	-1.2474*	.41030	.008	-2.2181	-.2767
	abamectin	.7043	.41030	.202	-.2664	1.6751
abamectin	100g/l	-1.9517*	.41030	.000	-2.9225	-.9810
	200g/l	-.7043	.41030	.202	-1.6751	.2664

Based on observed means. The error term is Mean Square(Error) = 4.545.

*. The mean difference is significant at the 0.05 level.

SQRT_plus

Tukey HSD

treatment	N	Subset	
		1	2
abamectin	54	2.9974	
200g/l	54	3.7018	
100g/l	54		4.9492
Sig.		.202	1.000

Experiment 2 without pre-spray overall weeks

Tests of Between-Subjects Effects

Dependent Variable: SQRT_plus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	718.792[a]	2	359.396	17.808	.000
Intercept	14214.634	1	14214.634	704.314	.000
treatment	718.792	2	359.396	17.808	.000
Error	4298.819	213	20.182		
Total	19232.245	216			
Corrected Total	5017.611	215			

a. R Squared = .143 (Adjusted R Squared = .135)

Multiple Comparisons

Dependent Variable: SQRT_plus

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l	200g/l	2.0303*	.74874	.020	.2631	3.7975
	abamectin	4.4624*	.74874	.000	2.6952	6.2296
200g/l	100g/l	-2.0303*	.74874	.020	-3.7975	-.2631
	abamectin	2.4321*	.74874	.004	.6649	4.1993
abamectin	100g/l	-4.4624*	.74874	.000	-6.2296	-2.6952
	200g/l	-2.4321*	.74874	.004	-4.1993	-.6649

Based on observed means. The error term is Mean Square(Error) = 20.182.

*. The mean difference is significant at the 0.05 level.

SQRT_plus

Tukey HSD

treatment	N	Subset		
		1	2	3
abamectin	72	5.8141		
200g/l	72		8.2462	
100g/l	72			10.2765
Sig.		1.000	1.000	1.000

Appendix 10 Data analysis in SPSS (regarding table 6)

Experiment2-Effect Cultivation Systems (location) on production

Tests of Between-Subjects Effects

Dependent Variable: yield_weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	233586.125[a]	1	233586.125	.434	.512
Intercept	67552876.125	1	67552876.125	125.470	.000
location	233586.125	1	233586.125	.434	.512
Error	37688020.750	70	538400.296		
Total	105474483.000	72			
Corrected Total	37921606.875	71			

a. R Squared = .006 (Adjusted R Squared = -.008)

Multiple Comparisons

Dependent Variable: yield_weight

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	-15.00	213.745	.997	-526.98	496.98
	abamectin	67.63	213.745	.946	-444.36	579.61
200g/l mint	100g/l mint	15.00	213.745	.997	-496.98	526.98
	abamectin	82.62	213.745	.921	-429.36	594.61
abamectin	100g/l mint	-67.63	213.745	.946	-579.61	444.36
	200g/l mint	-82.62	213.745	.921	-594.61	429.36

Based on observed means. The error term is Mean Square(Error) = 548240.676.

yield_weight

Tukey HSD

treatment	N	Subset
		1
abamectin	24	918.54
100g/l mint	24	986.17
200g/l mint	24	1001.17
Sig.		.921

Experiment 2; Effect Blocks on production

Tests of Between-Subjects Effects

Dependent Variable: yield_weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	483585.583[a]	2	241792.792	.446	.642
Intercept	67552876.125	1	67552876.125	124.503	.000
block	483585.583	2	241792.792	.446	.642
Error	37438021.292	69	542580.019		
Total	105474483.000	72			
Corrected Total	37921606.875	71			

a. R Squared = .013 (Adjusted R Squared = -.016)

Multiple Comparisons

Dependent Variable: yield_weight

Tukey HSD

(I) block	(J) block	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	200.67	212.638	.615	-308.67	710.00
	3	95.46	212.638	.895	-413.88	604.79
2	1	-200.67	212.638	.615	-710.00	308.67
	3	-105.21	212.638	.874	-614.54	404.13
3	1	-95.46	212.638	.895	-604.79	413.88
	2	105.21	212.638	.874	-404.13	614.54

Based on observed means. The error term is Mean Square(Error) = 542580.019.

yield_weight

Tukey HSD

block	N	Subset
		1
2	24	866.67
3	24	971.88
1	24	1067.33
Sig.		.615

Alpha = 0.05.

Experiment 1: Effect of treatments on production

Tests of Between-Subjects Effects

Dependent Variable: yield_weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10171093.333[a]	2	5085546.667	1.110	.339
Intercept	347778000.000	1	347778000.000	75.910	.000
treatment	10171093.333	2	5085546.667	1.110	.339
Error	192422106.667	42	4581478.730		
Total	550371200.000	45			
Corrected Total	202593200.000	44			

a. R Squared = .050 (Adjusted R Squared = .005)

Multiple Comparisons

Dependent Variable: yield_weight

Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100g/l mint	200g/l mint	-293.33	781.578	.925	-2192.17	1605.51
	abamectin	-1122.67	781.578	.332	-3021.51	776.17
200g/l mint	100g/l mint	293.33	781.578	.925	-1605.51	2192.17
	abamectin	-829.33	781.578	.543	-2728.17	1069.51
abamectin	100g/l mint	1122.67	781.578	.332	-776.17	3021.51
	200g/l mint	829.33	781.578	.543	-1069.51	2728.17

Based on observed means. The error term is Mean Square(Error) = 4581478.730.

yield_weight

Tukey HSD

treatment	N	Subset
		1
100g/l mint	15	2308.00
200g/l mint	15	2601.33
abamectin	15	3430.67
Sig.		.332

b. Alpha = 0.05.