24, 48 And 72 Hour Contact Toxicity Test of Peppermint (*Mentha piperita)*, Garlic (*Allium sativum L.)* And Lemon (*Citrus limon L.)* Essential Oil On The Long-Tailed Mealybug (*Pseudococcus longispinus)*

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April 1, 2015

This project is submitted in partial fulfillment of the requirements for Concordia University College of Alberta's EnSc 495 program

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ACKNOWLEDGEMENTS

Many individuals have contributed to make this undergraduate research possible. I begin with thanking one of Concordia's Sessional Instructors, Victor Shegelski for help with statistical data. Next I would like to thank Concordia's Lab Manager Devin Hughes for his help in aquiring all materials needed for this research, as well as maintaining it over the course of the project. Lastly, I express gratitude to my professor and research supervisor, Dr. Sheri Dalton, who provided immense guidance and support through the entire research process.

ABSTRACT

The long-tailed mealybug (*Pseudococcus longispinus)* is a major agricultural pest to over 26 different plant species. They proliferate both sexually and parthenogenetically, taking approximately 4-6 weeks to reach reproductive maturity. Each adult female can produce 75 to 200 live-young over an average 88 day lifespan. Without effective intervention to counteract proliferation, profits may be lost, entire crops depleted and/or other plants in close proximity affected. Essential oils are a promising means of control as they interfere with the mealybug's octopaminergic nervous systems, which act as a neurotransmitter, neuromodulator, and neuro-hormone. However, in mammals and fish octopamine is only a trace amine; therefore, essential oils are designated as a reduced-risk pesticide under Health Canada's Guidelines. There are pest control alternatives such as pesticides, but, these can cause deleterious effects on non-target species and the environment. This research tested the 24, 48 and 72 hour effects of peppermint (*Mentha piperita)*, garlic (*Allium sativum l.)* and lemon (C*itrus limon l.)* essential oil on the long-tailed mealybug. Research shows that there is a significant difference between each oil after 24 (ANOVA: $F(2,6)=2.76$, $p=0.007$), 48 (ANOVA: $F(2,6)=3.45$, $p=0.001$), and 72 (ANOVA: $F(2,6)=5.26$, $p<0.001$) hours. Garlic essential oil (LC50 = 1.65%) had the greatest effect on the long-tailed mealybug in comparison to peppermint (LC50 = 19.0 %) and lemon (LC50 = 48.0%) essential oil.

KEYWORDS: BIOASSAY; PESTICIDES; OCTOPAMINE

INTRODUCTION

The long-tailed mealybug (*Pseudococcus longispinus)*

The long-tailed mealybug (*Pseudococcus longispinus)* is a small (1 to 4 mm long), flattened, oval insect appearing as a miniature sow bug. They are covered with white, powdery wax resembling finely ground meal and adult females produce long (3 to 4 mm) waxy filaments extending from their posterior, thus the origin of their name (Kosztarab, 1996; Schuh and Slater; 1995). Females produce 75 to 200 live young through either parthenogenesis or sexual reproduction, over an average 88 day lifespan (Waterworth, Wright and Millar, 2011). There is a growing demand to find an effective management method for the long-tailed mealy bug (Matthews 1993), because of their wide host range (air plants, coconut and other palms, citrus, hibiscus, orchids, pineapple and potatoes) (El-Minshawy, Karam, El-Sawaf, 1974). In Canada and the United States, the long-tailed mealybug is one of the most common and problematic pest species of exotic plants (eg. palms and orchids) and next to similar scale insects (eg. aphids, beetles), are one of the most difficult to control (Heu, 1990; Howard, 1967). They produce large amounts of sticky liquid waste called honeydew and their needle-like mouthparts cause yellowing of host leaves, distorted growth, premature leaf drop, and with heavy populations, plant death (Flanders 1940). Infestations reduce marketability of products if not remediated prior to export, and commodities may be destroyed or rejected due to their high management difficulty and ease of transfer between plant species (Koul, Walia and Dhaliwal, 2008).

Traditional Management Strategies- Pesticides

In recent years, few environmental issues have aroused as much public concern as the use of and exposure to pesticides (Oliver and Hewitt, 2014; Flaherty et.al, 1982). It is estimated that 2.5 million tons of pesticides are applied to crops worldwide each year and the resulting damage cost reaches $100 billion annually (Pimentel and Burgess, 2014; Brookes and Barfoot, 2014). Mass use is the result of their cost-effectiveness, availability, and wide spectrum of bioactivity. Approximately 0.1% of applied pesticides reach the target pest, leaving the bulk of the pesticides (99.9%) to impact the environment (Horrigan, Lawrence and Walker, 2002). The high toxicity, non-biodegradable properties, and residual effects of pesticides in soil, water and crops, introduce a high probability of harming the environment, ecosystem and/or public health (Health Canada, 2012; CCOHS, 2014). Bassil et.al. (2007) indicates that despite many published studies exposing the relationship between pesticides, human and environmental health, deep controversy still persists (Horrigan, Lawrance and Walker, 2002).

Pesticides comprise a large portion of mealybug control. From the 1940s to the 1990s attempts at eradication included: potassium cyanide, sodium cyanide, sulfur fumigation, chlorinated hydrocarbons (e.g., DDT) and organophosphates (e.g. parathion) (Jeppson, 1953; Oliver and Hewitt, 2014; Frick, 1952). Studies have shown that low application rates (48 g a.i./ha) of ethyl parathion provide adequate control of the grape mealybug (Frick, 1952), however Flaherty et.al (1982) found it became less effective over time (Whalon, 2012). When using less than the minimum concentration to eradicate a pest, the possibility of chemical resistance arises; however, at high concentrations damage may also occur due to plant dehydration (Johnson 2014; Flaherty et al. 1982). Further research is needed to assess highly selective and biodegradable pesticide alternatives, because of the deleterious effects, and resistance which arise from traditional chemical pesticides (Horrigan, Lawrance and Walker, 2002).

Essential Oil

Essential oils have received much attention because of their specificity of action, bio-degradable nature, and potential for commercial application (Isman and Machial, 2006; Khater, 2011). An essential oil is defined as: any volatile oil producing strong aromatic components, giving a distinct flavour or odour to a plant (Mondal and Khalequzzaman, 2006). Essential oils are found in glandular hairs or secretory cavities of plant-cell walls and are present as fluid droplets in the leaves, stems, bark, flowers, roots and/or fruits of different plants (Bakr et.al, 2010; Isman, 2000; Isman and Machial, 2006). Essential oils provide various functions for plants including: heat or cold protection, attractant or repellent qualities and direct use as a defence material (Koul, Waliia and Haliwal 2008; Abdelmajeed, Danial and Hasnaa, 2013). A number of essential oils have been implemented for protection of stored commodities; however, the majority of use is in the Mediterranean region and Southern Asia (Koul et.al 2008; Mondal, Khalequzzaman, 2006).

Essential Oil Potential

Many essential oils show a broad spectrum of activity by targeting the octopaminergic nervous system, which acts as an anti-feedant, repellent, ovi-position deterrent, and growth regulator (Oliver and Hewitt, 2014). The octopaminergic nervous system is found in the long-tailed mealybug, however, it is unclear how octopamine (OCT) acts within the species (Kostyukousky et.al, 2002); Koul, Waliia and haliwal (2008) and Khater (2011) assume that it carries out similar functions within most invertebrates. OCT belongs to a group of compounds known as biogenic amines, and is the monohidroxilic analog of norepinephrine. It is present in relatively high concentrations in neuronal and non-neuronal tissues, acting as a neurotransmitter, neuromodulator, and neurohormone (Jeppson, 1953; Isman, 2006). In the peripheral nervous system, OCT controls flight muscles, peripheral organs, and most sense organs. In the central nervous system, OCT regulates motivation, desensitizes sensory inputs and spurs arousal. It also maintains various rhythmic behaviors, hygiene behavior, and social behaviors, including establishment of labor, as well as learning and memory (Franco, Zada and Mendel, 2009). As a neurotransmitter, OCT regulates endocrine gland activity and in the firefly, controls the emission of light (Jeppson, 1953; Khater, 2011). As a neurohormone, OCT is released into hemolymph, transported to target tissues, and induces mobilization of lipids and carbohydrates. This prepares insects for a period of extended activity or may assist in recovery from a period of increased energy demand (Hamlen, 1975; Jeppson, 1953; Kostyukousky et.al, 2002)

The octopaminergic nervous system is not shared with mammal or fish species in toxicological tests, therefore, they meet the criteria for "reduced risk" pesticides under Health Canada Guidelines (2012) (Koul, Waliia and haliwal 2008; Stroh et al., 1998, Tripathi et.al. 2000, Kostyukovsky et al.,2002). Already well received by consumers for use against home and garden pests, essential oils have the potential to provide effective pest management in agricultural situations, particularly for organic food production (Isman, 2000; Isman and Machial, 2006). It is observed that resistance develops slower with essential oil based pesticides because of their complex mixture of constituents (Pimentel and Burgess, 2014; Whalon et.al, 2012). While chemical resistance continues to be an issue for many synthetic pesticides, essential oils may be a promising means of control.

The purpose of this research was to determine if essential oils could be used in the reduction or eradication of the long-tailed mealybug. Three types of essential oil were tested (garlic, peppermint and lemon) to determine the lethal concentration of 50% of the population (LC50) through a 24, 48 and 72 hour contact bioassay. The oil producing the lowest LC50 after 72 hours was deemed most effective as it would be the most comparable to an agricultural setting.

METHODS

Long-tailed Mealybug Habitat

To produce the number of mealybugs needed for each bioassay a growth chamber and contained habitat was set up in a level two bio-safety facility at Concordia University College, Edmonton, Alberta. The habitat was created with a glass fish tank (~ 2 cubic ft.) enclosed with fine muslin cloth (hole size = ~ 0.02mm^2). Butternut squashes and honey were used to provide a sufficient fresh food supply (Meyerdirk et.al, 2002). The habitat was maintained at 25° C and 22$\pm$ 3 % relative humidity for optimal reproduction. Aside from periods of maintenance, the fish tank was kept dark (L/D, 0/24), benefiting the larvae which prefer a dark habitat. In addition, less light limits the rigorous movement of adults (Meyerdirk et,al, 2002).

Long-tailed Mealybug Collection

Thirty (20 females; 10 males) long-tailed mealybugs were collected from pony-tail palm (*Beaucarnea recurvate*) leaves at 25-52009 RR 214, Sherwood Park, Alberta. Males and females were distinguished by physical appearance (adult females have two long filaments extending from their posterior; males produce wings and lack the two rear filaments (El-Minshawy, Karam and El-Sawaf, 1974). After collection each specimen was brought to the previously created habitat and randomly placed on the butternut squashes. The population was allowed to settle and reproduce before the first bioassay was conducted (~5 weeks).

Pony-Tail Palm (Beaucarnea recurvate) Maintenance

Two pony-tail palms were purchased on September 30, 2014 at Salisbury Greenhouse (52337 Range Road 232, Sherwood Park, AB), and kept at Concordia University College's greenhouse. The palms were of medium maturity growing in 10" pots. All plants were monitored and subjected to 25 $\pm$ 3° C, 16:8 LD and 20-40% relative humidity. Each pot was watered once per week.

Initial Bioassay Assessment

Each bioassay was conducted under laboratory conditions. The initial 24, 48 and 72 hour bioassay used 3 replicates of each dilution (100, 75, 50, 25, 12.5, 6.3, 3(%)). The negative control contained 99.5% isopropyl alcohol and the positive control had no application. All essential oils were mixed with acetone to produce the appropriate dilution. Each dilution was allowed to dry for 1 hour in order for acetone and excess oil to evaporate. Following 1 hour any unevaporated oil was wiped away with paper towel to reduce the possibility of suffocation (Mondal and Khalequzzaman 2006). Three adult long-tailed mealybugs were then added to each glass petri dish (diameter = 6 cm) on palm leaves cut to approximately the same size (1 inch square). Each dish was then covered with a glass lid and labeled with dilution type, concentration and replicate number. To reduce recorder bias each label was covered with tape and numbered. The petri dishes were then randomly placed into a container 25° C, 0:24 LD and 20% relative humidity for the allocated 24, 48 and 72 hours. Conditions were intended to mimic initial growth requirements of the long-tailed mealybug habitat to minimize stress on the species. Following the initial testing it was found that garlic and peppermint produced significant mortality at all concentrations, therefore, dilutions were changed (garlic: 6.3, 3.15, 1.575, 0.788, 0.394, 0.197, 0.098; peppermint: 75,50,25,12.5, 6.3, 3.15, 1.575). Lemon essential oil dilutions were not changed. Five replicates of each dilution were then completed following the procedure outlined on page 10-11.

Data Collection

Following each time increment, mortality was recorded to ensure effects seen were due to the essential oil. Mortality was then calculated in probits to determine an LC50 of the population and significance determined with a 95% confidence value ($p \leq 0.05$). Leaves were also checked for the presence of necrosis, wilting and browning.

RESULTS

Preliminary testing shows that there is a significant difference between garlic, lemon and peppermint essential oil after 24 (ANOVA: $F(2,6)=2.76$, $p=0.007$), 48 (ANOVA: $F(2,6)=3.45$, $p=0.001$) and 72 hours (ANOVA: $F(2,6)=5.26$, $p<0.001$) of exposure over time. The oil producing the lowest LC50 after 72 hours was deemed most effective as it most closely resembles an agricultural application.

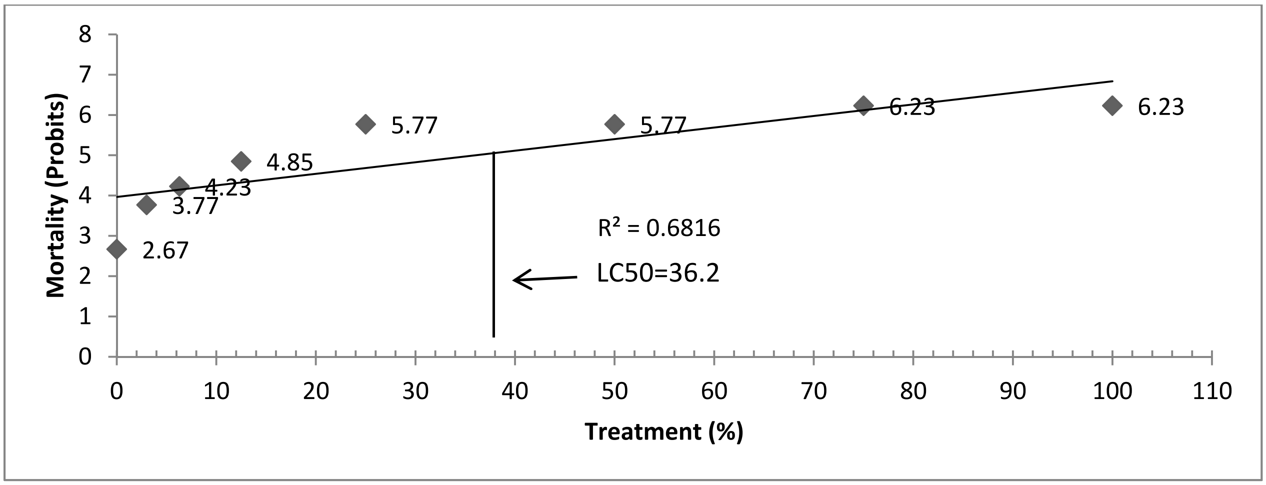

Figure 1 – Preliminary testing; LC50 of the long-tailed mealybug after 72 hours of exposure to lemon essential oil

After 72 hours of exposure, lemon essential oil produced a LC50 of 36.2%, a 30.8% difference from peppermint essential oil (figure 3) and 34.9% difference from garlic (figure 2). There were no 100% mortalities across any dilution. The highest mortality was expressed at 100% with 89% mortality but was constant between both 48 and 72 hours of exposure. 75% dilution increased to 89% mortality and 25% and 50% increased to 78% mortality. There was a significant difference between dilutions (ANOVA: $F(2,21)=30.15$, $p<0.001$) and time (ANOVA: $F(2,21)=3.94$, $p=0.026$) however, no significance between the dilutions and time (ANOVA: $F(2,21)=0.75$, $p=0.711$).

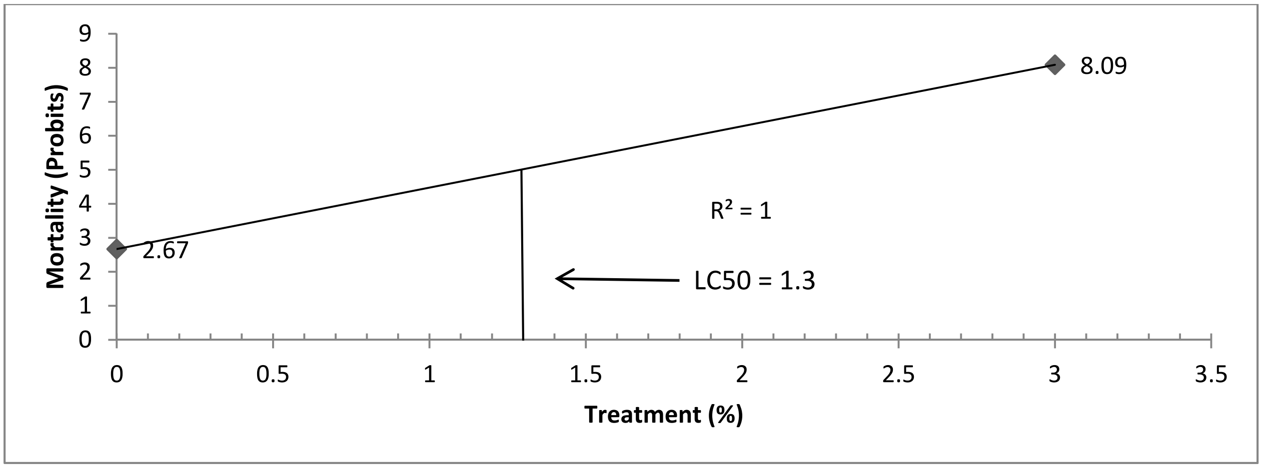

Figure 2 – Preliminary testing; LC50 of the long-tailed mealybug after 72 hours of exposure to garlic essential oil

The 72 hour exposure to garlic essential oil provided 100% mortality control across all dilutions. After 72 hours of exposure the LC50 was determined to be 1.3%. Treatment had a greater effect then lemon (34.9%) and peppermint (4.1%) There was a significant difference between dilutions (ANOVA: $F(2,21)=4.0$, $p<0.001$), time (ANOVA: $F(2,21)=4$, $p=0.025$) and the interaction of dilution and time (ANOVA: $F(2,21)=4$, $p<0.001$).

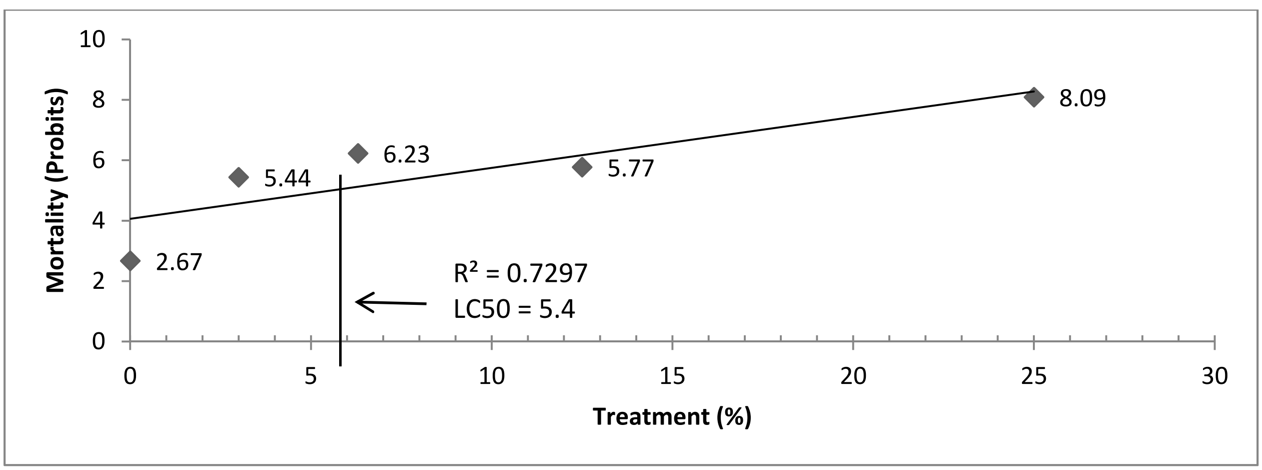

Figure 3 - Preliminary testing; LC50 of the long-tailed mealybug after 72 hours of exposure to peppermint essential oil

After 72 hours of exposure to peppermint essential oil, mortality rates continued to increase across all dilutions. 100% mortality was reached at 25% dilution. There was a drop of

2.5% in the LC50 from the 48 hour exposure. There was a significant difference between the time trials of peppermint essential oil (ANOVA: $F(2,21)=2.24$, $p=0.01$), dilutions (ANOVA: $F(2,21)=46.92$, $p<0.001$) and the interaction between time and dilution (ANOVA: $F(2,21)=2.24$, $p=0.01$).

In preliminary testing garlic and peppermint produced high mortality rates therefore dilutions were altered to produce a more accurate estimate of LC50 (Figure 2 and 3). Lemon was not changed from the initial dilutions (Figure 1). Dilutions were adjusted to start where two consecutive 100% mortalities occurred.

Each of the following figures show mortality in probits after 5 replicates of 7 different dilutions. Only the 72 hours of exposure were shown as there is no significant difference between times for garlic (ANOVA: $F(2,37)=1.489$, $p=0.231$), peppermint (ANOVA: $F(2,37)=0.849$, $p=0.431$) or lemon (ANOVA: $F(2,37)=0.892$, $p=0.114$). Each replicate contained three individual mealybugs within their 3rd instar or adult stage. Negative control (99.5% isopropyl alcohol) samples produced 100% mortality after 24 hours of exposure. The positive control produced 0% mortality after 72 hours of exposure and is included within each graph (2.67 probits).

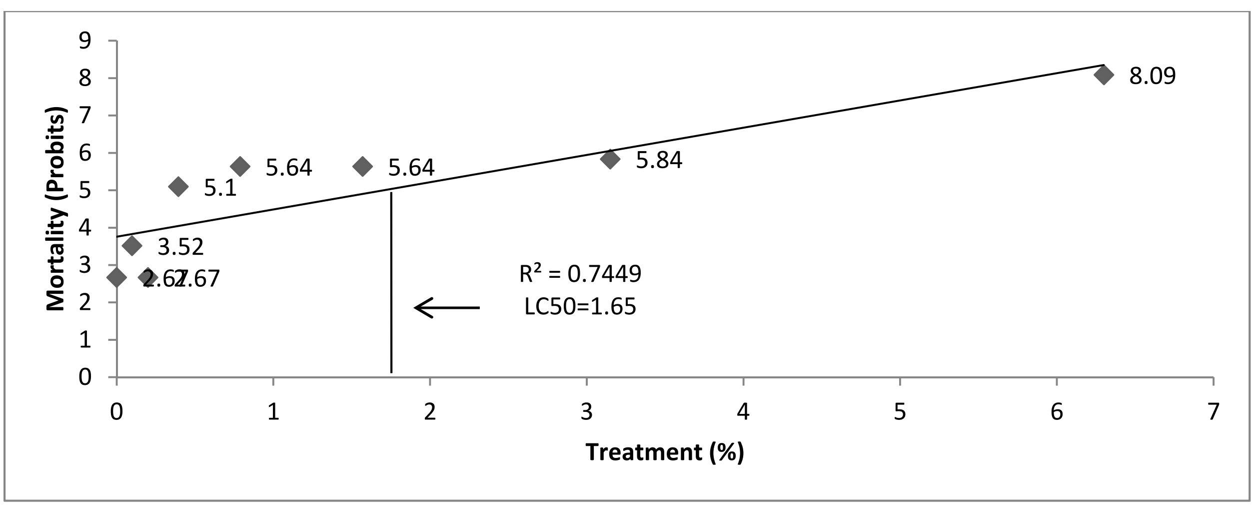

Figure 4 - LC50 of the long-tailed mealybug after 72 hours of exposure to garlic essential oil

After 72 hours of exposure garlic essential oil produced an LC50 of 1.65%, which is 17.35 % lower than peppermint essential oil (Figure 5) and 46.35 % lower than lemon essential oil (Figure 6). Garlic provided 100% mortality at 6.3 %, however drops to 83% mortality at 3.15%. This is similar to the initial bioassay which produced 100% mortality at 6.3, and 3.15%. Both 1.5% and 0.788% exhibited the same mortality of 80%. At 0.394% dilution there was 57% mortality, at 0.2%, 21% mortality and at the lowest dilution 0.098%, 13% mortality. There was a significant difference between garlic dilutions (ANOVA: F(2,37)=83.46, p<0.001); however, there was no significant difference between time (ANOVA:F(2,37)=2.38, p=0.095) and interaction of time and dilution (ANOVA: F(2,37)=1.51, p=0.107). This research demonstrates that garlic essential oil provides the most adequate control in the shortest period of time with the lowest dilution; therefore, is recommended for use against long-tailed mealybug populations.

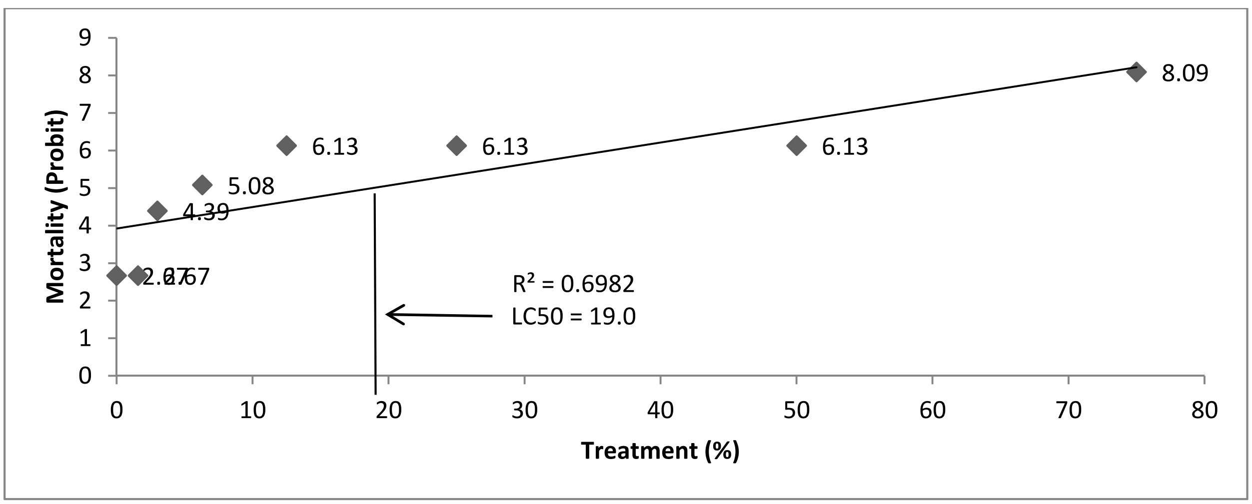

Figure 5 - LC50 of the long-tailed mealybug after 72 hours of exposure to peppermint essential Oil

After 24 hours of exposure to peppermint essential oil the LC50 was determined to be 22.0%, after 48 hours 21.0% and after 72 hours 19.0%. At 75% dilution there was 100% mortality however this drops to 87% when at 50% dilution. At 25% and 12.5% dilution there was also 87% mortality exhibited. At 6.3% there was a 53% mortality a 34% drop from the

12.5% dilution. At 3.15% there was an increase of 1% to 27% mortality. At the lowest dilution of 1.57% there was 0% mortality. There is a significant difference between the dilutions (ANOVA: F(2,37)=75.189, p=<0.001) however, time intervals (ANOVA: F(2,37)=0.849, p=0.431) and the interaction between time and dilution (ANOVA: F(2,37)=0.498, p=0.929) had no significant difference. This indicates that peppermint may hinder the long-tailed mealybug; however, dilutions would have to remain high to provide adequate control.

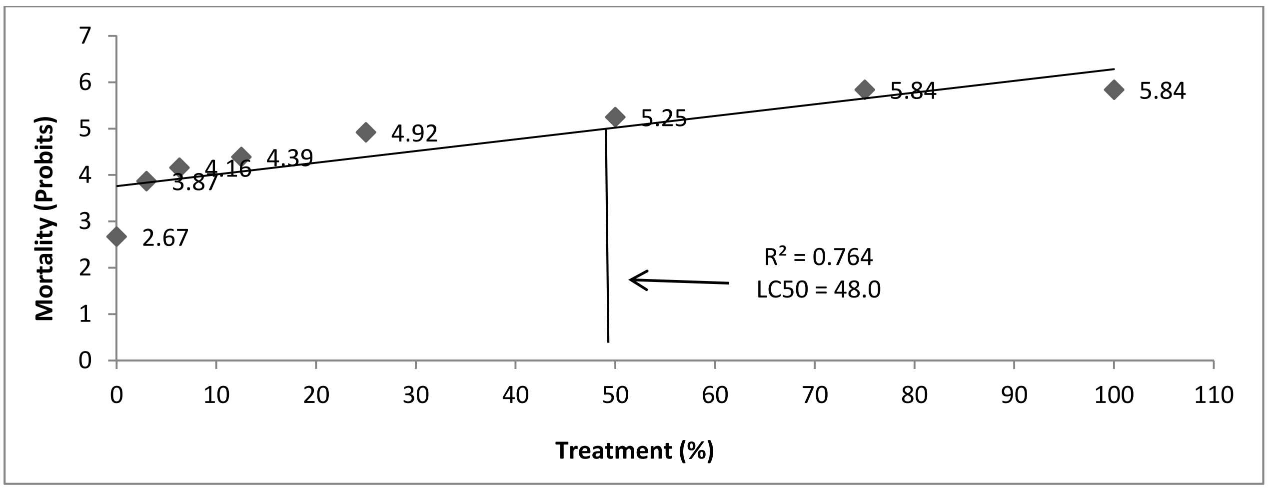

Figure 6 – LC50 of the long-tailed mealybug after 72 hours of exposure to lemon essential oil

After 24 hours of exposure to lemon essential oil, the LC50 was determined to be 51.0%, after 48 hours 50.5% and after 72 hours 50.2%. The 100% and 80% dilutions produced the same effect with 80 % mortality. At 50%, the mortality dropped to 60% a 20% difference. 25% dilution provided 47% mortality, 12.5%, 20% mortality and 3%, 13% mortality. There was a significant difference between the essential oil dilutions (ANOVA: F(2,37)=45.340, p<0.001) ,however, no significant difference between time (ANOVA: F(2,37)=0.892, p=0.115) or interaction of time and dilution (ANOVA: F(2, 37)=0.049, p=1). This suggests that lemon essential oil provided the least effective control of the long-tailed mealybug and does not provide appropriate eradication of the species.

DISCUSSION

Garlic, lemon and peppermint essential oil were tested to determine an alternative to pesticides. This research has determined that for the greatest mortality at the smallest dilution, garlic is the most promising control (Figure 4). On a cost and efficiency basis, garlic essential oil should be considered for eradicating the long-tailed mealybug in an agricultural setting.

Garlic essential oil provided the greatest mortality against the long-tailed mealybug, at the lowest dilution (LC50 = 1.65%, 72 hrs), and shortest time (LC50 = 1.7%, 24hrs). These results are similar to those found by Mousa et al (2013) who reported a 68.9% reduction of leafhoppers and planthoppers with garlic in comparison to a common pesticide (pest ban) with 68.9%. Within the same study garlic essential oil (90.96%, at 3%) were also determined to be the most effective in the reduction of aphids in comparison to pest ban (89.44%) (Mousa *et al*., 2013). Park and Shin (2005) observed garlic essential oil against the Japanese termite, (*Reticulitermes speratus)* which produced 100% mortality (2.0 µL/L and 0.5 µL/L) within 48 hours but decreased to 67% after 72 hours. Similarly in a study by Huang, Chen and Ho (2000), two of the major constituents of garlic essential oil (methyl allyl disulfide and diallyl trisulfide), were tested against the maize weevil (*Sitophilus zeamais)* and red flour beetle (*Tribolium castaneum*) for contact toxicity, fumigant toxicity, and antifeedant activity. Both compounds were determined to reduce hatching rates, where Diallyl trisulfide totally suppressed hatching at 0.32 mg/cm^2 . Methyl allyl disulfide significantly decreased the growth rate, food consumption, and food utilization of adults of both insect species, with optimal feeding deterrence at 44% (6.08 mg/g food for *S. zeamais* and 1.52 mg/g food for *Tribolium castaneum).* Denloye (2010) tested against the cowpea seed beetle (*Callosobruchus maculatus F.),* and found extracts of garlic essential oil (0.11 g/L) more toxic to the cowpea seed beetle than the corresponding ethanol extracts. Results collected for garlic essential oil show that it may be used against a variety of pest species and are a promising alternative to pesticide use.

Lemon essential oil was the least effective at controlling the long-tailed mealybug only producing an LC50 of 48.0% after 72 hours (Figure 6). Karamaouna et.al. (2013) found similar results as indicated in figure 6 when conducting a lemon essential oil bioassay through spray applications on grape leaves bearing clusters of the grape mealybug (*Pseudococcus ficus*). The study found the LC50 value ranged from 2.7 to 8.1 mg/mL. Lack of control may be due to the waxy coating that adult mealybugs produce or feeding habits where they habituate on citrus plant species (Hoffmann and Frodsham, 1993; El- Minshawy, Karam and El-Sawaf, 1974). Research on other similar scale insects has been conducted suggesting that lemon essential oil does provide growth inhibition and effective antifeedant action; however, it is species dependent. Michaelakis et.al (2009) exposed mosquito larvae (*Culex pipiens*) to lemon essential oil and found a LC50 of 30.1 mg/L (~ 3% dilution). The cabinet beetle (*Trogoderma granarium)* was also exposed to lemon essential oil. At a concentration of 8%, lemon essential oil was effective at control, providing anti-feedant activities with minimal loss in treated grains (Sagheer et.al 2013). In a contact toxicity bioassay, another pest species, the black soybean weevil (*Rhyssomatus subtilis)* was significantly affected by *Citrus limon L* with 100% mortality at 5 uL/cm2 (Zunino et.al. 2012). In a similar study Moravvej and Abbar (2008) observed that the cowpea seed beetle (*Callosobruchus maculatus)* was also susceptible to the fumigant properties of lemon essential oil *(*LC50; 235 &Amp; plus mn;1 L^{-1} at 24 h exposure). Although this is seen to inhibit other pest species it is not recommended for control of the long-tailed mealybug.

Peppermint essential oil was also seen as a promising control method of the long-tailed mealybug, producing an LC50 of 19.0 % after 72 hours (Figure 5). Tripathi *et al*., 2000 researched the deterrent ability of peppermint essential oil concluding that it was effective against ants, flies, lice and moths. Sinthusiri and Soonwera (2013) studied the effects of peppermint essential oil on the housefly (*Musca domestica L*). The results were similar to the mortality of the long-tailed mealybug producing a LC50 of 2.62 mg/cm^2 at 10% dilution. The carmine spider mite (*Tetranychus cinnabarinus Boisd*) also was observed to have similar inhibition (1.83 µg ml^{-1} air) (Sertkaya, Kaya and Soylu, 2010). The greenhouse whitefly (*Trialeurodes vaporariorum),* was reported to have an 83% mortality after 24 hrs of treatment (9.3 X 10^4 l/ml air) (Choi et.al, 2003). These studies indicate that peppermint is an effective means to reduce and repel a variety of insect pests and further research should be conducted to identify the full potential of peppermint essential oil.

Further research is needed to observe the effect of essential oils on non-target species (honey bee) because of a shared octopaminergic nervous system. Imdorf et.al, (1999) tested essential oil of thyme, salvia and hyssop on the Varroa mite (*Varroa destructor Oud) (*a honey bee mite) and found that 20% mortality occurred for bees at concentrations which lead to adequate mite control. However, similar research was also conducted with essential oil of the mint family finding them to deter and reduce mites within the hive without bee mortality (Ebert et.al, 2007; Calderone 2001 et.al). Further research should be conducted to determine the effects of essential oils on pollinators and non-target species.

Other possible complications for this study stem from the application procedure. As there was only one leaf within each petri-dish, its resemblance to a natural habitat is limited. Further research should consider application to plants in a larger, more realistic environment. Residual effects should also be examined because of the volatile nature of essential oil. The re-application rate should be evaluated to determine success rates with complete eradication of all individuals over a longer period of time (Abdelmajeed, Danial and Hasnaa, 2013).

CONCLUSION

This research has brought attention to the effects essential oils have on the long-tailed mealybug. Depending on the intended target, essential oils have a high probability of success towards pest species and limited deleterious effects when compared to pesticides (Health Canada, 2012). Ultimately, it may be in developing countries which are rich in endemic plant biodiversity that essential oils have their greatest impact (Koul, Walia and Dhaliwal, 2008). However, both personal and large scale pest management programs should consider essential oils as an alternative to pesticides.

REFERENCES

Abdelmajeed, N.A. Danial, E.N. Hasnaa, A.S.2013. The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. (66):4.

Bakr.R , Fattah H.M, Salim N.M, Atiya N.H. 2010. "Insecticidal activity of Four Volatile Oils on Two Museum Insects Pests". biolog. Sci., 2 (2): 57- 66.

Bassil. K.L, Vakil. C, Sanborn. M, Cole. D.C. 2007. "Cancer health effects of pesticides: Systematic review". Canadian Family Physician. (53)10: 1704-1711

Bell.V.A, Bonfi glioli R.E, Walker J.S, Lo P.L, Mackay J.F, McGregor S.E. 2009. "Grapevine leafroll associated virus 3 persistence in *Vitis vinifera* remnant roots". J Plant Pathol. 91:527–533.

Brookes, G. Barfoot, P. 2014. Key global economic and environmental impacts of genetically modified (GM) crop use 1996-2012. *Agro FOOD Industry Hi Tech*, *25*, 4.

Calderone N. W.,Bruce. W.A , Allen-Wardell. G, and Shimanuki. H. 1991. Evaluation of botanical compounds for the control of the honey-bee tracheal mite, *Acarapis woodi*. Am. Bee. J 131:589–591.

Canadian Center For Occupational Health and Safety (CCOHS). 2014. Pesticides. http://www.ccohs.ca/oshanswers/chemicals/pesticides/general.html. Accessed December 12, 2014.

Choi. W, Lee. E, Choi. B , Park. H, Ahn. Y. 2003. "Toxicity of Plant Essential Oils to *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)" J. Econ. Entomol. 96(5): 1479-1484.

Ebert, T.A, Kevan, P.G, Bishop.B,L, Kevan., S.D. Downer. R.A. 2007. Oral Toxicity of essential oils and organic acids fed to honey bees (*Apis mellifera). Agricul research and bee world. 46(4): 220-224.*

El-Minshawy, A. H.; Karam, H. H.; El-Sawaf, S. K. 1974. Biological studies on the long tailed mealy bug, Pseudococcus longispinus. Bulletin de la Societe Entomologique d'Egypte. Vol. 58 pp. 385-391.

Flaherty.D.L, Peacock. W.L, Bettiga L, Leavitt. G.M . 1982. “Chemicals losing effect against grape Mealybug”. Calif Agric 36:15–16

Flanders, S.E. 1940. Biological Control of the Long-tailed Mealybug, *Pseudococcus longispinus*. Entemol. (33)5:754-759.

Frick. K.E.1952. “The value of some organic phosphate insecticides in control of grape mealybug”. J Econ Entomol 45:340–341

Franco, J.C, Zada. A, and Mendel. Z. 2009. Novel approaches for the management of mealybug pests. Biorational Control of Arthropod Pests. 1: 233-278.

Grohmann, L, Blenau, W, Erber, J, Ebert, P.R, Strunker, T, Baumann, A. 2003. Molecular and functional characterization of an octopamine receptor from honeybee (*Apis mellifera*) brain. Neurochem. 86(3): 725-735.

Hamlen, R. A. 1975. Insect growth regulator control of longtailed mealybug, hemispherical scale, and *Phenacoccus solani* on ornamental foliage plants. J. Econ. Ent. 68 (2): 223-226.

Health Canada. (2012). “Regulatory Directive DIR2012-01, Guidelines for the Registration of Non-Conventional Pest Control Products”. Canada.

Heu, R. A. 1990. Distribution & Host Records of Agricultural Pests and Other Organisms in Hawaii. State of Hawaii Department of Agriculture: Honolulu. 34 pp.

Hoffmann, M.P. Frodsham, A.C. 1993. Natural Enemies of Vegetable Insect Pests. Cooperative Extension, Cornell University, Ithaca, NY. 63 pp.

Horrigan, L. Lawrence, R. S., Walker, P. 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives*, 110(5).

Howard, M. L. 1967. Mealybugs of California. University of California Press: Berkeley& Los Angeles.

Imdorf, A., S. Bogdanov, R. I. Ochoa, and N. Calderone.1999. Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie 30:209–228.

Isman, M.B. 2000. Plant essential oils for pest and disease management. *Crop Prot.,* 19, 603–608.

Isman, M.B. and Machial, C.M. 2006. Pesticides based on plant essential oils: from traditional practice to commercialization. *In* M. Rai and M.C. Carpinella (eds.), *Naturally Occurring Bioactive Compounds*, Elsevier, BV, pp 29–44.

Johnson, P. J. 2014. “Mealybugs on Orchids” Insect Research Collection. South Dakota State University.

Jeppson, L.R. 1953. Systemic insecticides: entomological aspects of systemic insecticides. Journal of Agricultural and Food Chemistry 1: 830-832.

Karamaouna. F,Kimbaris. A, Michaelakis. A, Papachristos. D, Polissiou. M, Papatsakona. P,Tsora. E. 2013. “Insecticidal Activity of Plant Essential Oils Against the Vine Mealybug, *Planococcus ficus”*. Journal of Insect Science 13(142): 1-13.

Khater, H.F. 2011. Biorational Insecticides for integrated pest management. *Benha Veterinary Medical Journal*, In press.

Kosztarab, M. 1996. General section, pp. 16-24. In: Scales Insects of Northeastern North America, Identification, Biology, and Distribution. Virginia Museum of Natural History. Martinsville, VA. 650 pgs.

Koul, O. Walia, S. Dhaliwal, G.S. 2008. Essential Oils as Green Pesticides: Potential and Constraints. *Biopestic. Int. 4*(1): 63–84.

Matthews, G.A., 1993. Insecticide Application in Stores. In: Application Technology for Crop Protection. CAB International, Wallingford, UK., pp: 305-315.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N. and Shaaya, E. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. *Pest Manag. Sci.*, 58,1101–1106.

Meyerdirk, D.E., R. Warkentin, B. Attavian, E. Gersabeck, A. Francis, J. Adams, and G. Francis. 2002. Biological control of pink hibiscus mealybug project manual. United States Dept. of Agric., Animal and Plant health Inspection Service, Plant Protection and Quarantine. 194pp.

Metcalf, C. L. Flint.W.P. 1939. Destructive and Useful Insects, 2nd Ed. McGrawHill Book Company: New York. 981 pp.

Michaelakis.A, Papachristos.D, Kimbaris.A, Koliopoulos.G, Giatropoulos. A, Polissiou. M. 2009. "Citrus essential oils and four enantiomeric pinenes against Culex pipiens (Diptera: Culicidae)". Parasitol. (105)3:769-773.

Mondal, M. Khalequzzaman, M. 2006. Toxicity of essential oils against red flour beetle, *Tribolium castaneum. Bio-sci: (14)43-8.*

Moravvej. G, Abbar.S. 2008. "Fumigant Toxicity of Citrus Oils Against Cowpea Seed Beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)". *Pakistan Journal of Biological Sciences, 11: 48-54.*

Oliver, R. Hewitt, H. G. 2014. Legislation and regulation. *Fungicides in crop protection*, (Ed. 2), 162-176.

Pimentel. D, Burgess. M. 2014. Environmental and Economic Costs of the Application of Pesticides Primarily in the United States: *Integrated Pest Management.* Springer, NY. Press.

Sagheer, M., Mansoor-ul-Hasan, Z. Ali, M. Yasir, Q. Ali, K. Ali, A. Majid and F.Z.A. Khan, 2013. Evaluation of essential oils of different citrus species against *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) collected from Vehari and Faisalabaddistricts of Punjab, Pakistan. Pak. Entomol., 35(1): 37-41.

Schuh, R.T, Slater, J.A. 1995. True bugs of the World (Hemiptera Heteroptera). *Entomol. 2.1(3): 336-340.*

Sertkays. E, Kaya. K, Soylu. S. 2010. "Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetranychus cinnabarinus* Boisd.) (Acarina: Tetranychidae)". Entomol. (31)1:107-112.

Sinthusiri J. Soonwera M. 2013. "Efficacy Of Herbal Essential Oils As Insecticides Against The Housefly, *Musca Domestica* L." Entomol. 44(2): 188-196.

Stroh, J., Wan, M.T., Isman, M.B. and Moul, D.J. 1998. Evaluation of the acute toxicity to juvenile Pacific coho salmon and rainbow trout of some plant essential oils, a formulated product, and the carrier. *Bull. Environ. Contam. Toxicol.*, 60, 923– 930.

Tripathi, A.K., Prajapati, V., Aggarwal, K.K., Sushil Kumar, Prajapti, V., Kumar, S. Kukreja, A.K. Dwivedi, S. and Singh, A.K. 2000. Effects of volatile oil constituents of *Mentha species* against stored grain pests, *Callosobrunchus maculatus* and *Tribolium castanum. J. Med. Arom. Plant Sci.*, 22, 549–556.

Walton VM, Pringle KL. 2004. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A review. S Afr J Enol Vitic 25:54–62

Waterworth, R.A. Wright, I.M. illar, J.G. 2011. Reproductive Biology of Three Cosmopolitan Mealybug (Hemiptera: Pseudococcidae) Species, *Pseudococcus longispinus*, *Pseudococcus viburni*, and *Planococcus ficus. Entomol. 104(2): 249-260.*

Whalon, M., D. Mota-Sanchez, R. Hollingworth, and L. Duynslager. 2012. Arthropod pesticide resistance database. Michigan State University. http://www.pesticideresistance.org/.

Zunino. M.P, Areco. V.A, Zygadlo.J.A. 2012. "Insecticidal activity of three essential oils against two new important soybean pests: Sternechus pinguis (Fabricius) and Rhyssomatus subtilis Fiedler". Bol.latinoam.Caribe plantas med. Aromat. (11)3:269-277.