CHAPTER 5

SUMMARY

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5. SUMMARY

Mealy bugs infest and attack enormous variety of plant hosts worldwide and they are important insect - pests of citrus, guava and virtually all orchard trees. The mealy bug *Icerya seychellarum seychellarum* (Hemiptera: Monophlebidae) attacks different important economic crops in Egypt. The entomopathogenic fungi are principal pathogens among Hemipteran piercing sucking insects, causing epizootics amongst their host, thus play an important role in regulating their population.

The results of the present study can be summarized in the following points:

1-Pathogenicity of the biocontrol agent *Verticillium lecanii* against *Icerya seychellarum* on citrus in the greenhouse during October 2012

The biological performance of the fungus *Verticillium lecanii* as a biocontrol agent, during 2013 was evaluated for the control of the mealybug *Icerya seychellarum* located on young citrus trees. The results showed that the proportion of mortality percentage increases as the concentration of spore suspension increases. Also, the mortality percentage increase as the time post-application increases. The mortality of *Icerya seychellarum* was 96.59% after 14 days of treatment with the concentration of 1.3×10^5 spores/ml and it was 97.75% at 1.3×10^6spores/ml. The mortality percentage reached 100% after 11 days post-treatment at the concentration of 1.3×10^7 spores/ml and 1.3×10^8 spores/ml. the caculated LT_{50} values of the tested concentrations of the biological agent revealed that LT_{50} values (days) decreases as the concentration increases. The effect of the tested biocontrol agent against insects was similar since the slopes of the LT_{50} lines were more or less the same and they were parallel to each other. The LT_{50} values of the different tested concentrations of *Verticillium lecanii* (1.3×10^5 - 1.3×10^8) tested against *Icerya seychellarum* ranged between 3.70 - 6.40 days. Also, it could be said that the entomopathogenic fungi, *Verticillium lecanii* was effective against the mealy bug *Icerya seychellarum* located on young citrus trees.

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2-Virulence of *Verticillium lecanii* (Zimm.) Viegas passage through rearing on artificial media and an insect host (*Icerya seychellarum*) (Hemiptera: Monophlebidae)

The entomopathogenic fungi are known to lose their desirable features after repeated sub-culturing declining their virulence through successive passages (subcultures). *Verticillium lecanii*, which lose virulence, may sometimes be restored to their former potency by passing them through their natural insect host. The results of the effect on virulence of *V.lecanii* (Zimm.) Viegas passage through an artificial medium and an insect host (*I. seychellarum*) are recorded and from which, it could be concluded that the mortality percentage and LT_{50} values of the adult individuals of the mealy bug *I. seychellarum* exposed to mother culture and its obtained derived subcultures were varied according to differed tested subcultures, time of exposure and the used medium at a single concentration of 1.7×10^{8} spores/ml. Daily recorded mortality percentage proved that the lethal effects of the tested cultures were systematically arranged with increasing the time of exposure in all cases (subcultures) reaching to 94.4, 79.8, 51.7, 44.9 and 36% in treatment with 1^{st}, 2^{nd},3^{rd},4^{th} and 5^{th} sub cultures, respectively when passaged through the MYB artificial medium compared with complete death (100% mortality) of the insect population treated with the mother culture after 13 days. These results proved that the virulence of *V. lecanii* against *I. seychellarum* was decreased with increasing its passage's number through the MYB artificial medium. This was clear when the LT_{50} values of the different passages through the artificial medium (MYB) were compared. The lowest value of LT_{50} for the zero passage (parents) is 4.6 days followed by 5.8, 7.4, 11.3, 13.9 and 17.7 days for the five passages cultures, respectively.

Moreover, these results also confirmed that the passages of *V. lecanii* through a suitable insect-host, (*I. seychellarum*) increased of its virulence,

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showing the lowest value of LT_{50} (4.6 days) for the zero passage followed by 5.3, 6.6, 8.4, 9.0 and 9.47 days for the five derived passages (subcultures), respectively . Comparing the five passages through the natural insect-host, *I. seychellarum* with those through the used artificial medium, it was found that virulence of subcultures obtained form the natural insect host was increased by 1.9 fold relative to that of subcultures obtained from the artificial medium based on their LT_{50} value.

3-Mass-production of *Verticillium lecanii* on the different media types

For mass production of *Verticillium lecanii* (Zimmerman) Viegas that originally isolated from Alaska, USA and EMCC Number: 919™ (Egypt microbial culture collection) three methods were evaluated: liquid state, solid state and diphasic system. In the liquid state of production, five media were tested and they were PDB, PSB, MYB, SYB and PCB. Molasses Yeast Broth (MYB) supported maximum spore production (9.380x 10^8 spores/ ml) giving a high biomass production of 0.89 g/100 ml with the liquid nitrogen drying technique, while this media gave 8.70x 10^8 spores ml and a biomass production of 0.69 g/100 ml with the oven (40-45 °C) drying technique. The drying technique by liquid nitrogen supported maximum spores germination percentage (96.99%), while the spores germination percentage of 93.44% was recorded in the oven (40-45 °C) drying technique. In the solid state production, six substrates were tested such as rice, wheat, corn, barley, rice husk and rice straw. Rice grains supported maximum sporulation (1.97×10^9 spores/g) and the highest biomass production (0.96 g/100g). In diphasic state of production, combination of MYB and rice grains produced the greatest amount of spores, (2.5g/100g) and the highest sporulation (2.17×10^9 spores/g).

3. a. Liquid media:

Biomass production count is significantly affected by type of media among five media tested. MYB media produced maximum biomass of 0.69g/100ml and PSB media gave 0.63g/100ml media, followed by PCB, SYB and PDB which produced 0.53, 0.46 and 0.44 g/100ml, respectively

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when these media were oven – dried at 40-45°C. In the case of the liquid nitrogen-dried technique, it was also found that the highest produced biomass was gained in this media(MYB) producing 0.89 g/100ml and (PSB) midea (0.69 g/100ml), followed by PCB, SYB and PDB which produced 0.68, 0.61 and 0.59 g/100ml, respectively. Sporulation is important factor in mass-production and it was found that MYB supported maximum sporulation of 8.7×10^8 spores/ml and 9.38×10^8 spores/ml in the two cases of drying techinques, respectively. The spore's germination percentage indicated that viability of spores had not significant effect on mass-production in all different media although the calculated germination percentage of MYB resulted spores was higher than other tested media (96.44%).

3. b. Solid media:

In solid state production, four cereal grains, rice, wheat, corn and barley and two solid agricultural wastes (rice husk and rice straw) were evaluated. Cereal grain was significantly efficient than, rice husk and rice straw. The maximum spore dust that has been harvested from rice grain was 0.96 g/100g followed by barley, corn, wheat, rice husk and rice straw (0.93, 0.9, 0.87, 0.5 and 0.48 g/100g media, respectively). Sporulation among different media was significant and it was found that rice supporting maximum sporulation of 1.9×10^9 spores/g. The viability of spores in rice media was higher than other media since the viability reached 96.99%.

3. c. Diphasic system:

In diphasic system of production, five liquid media and four solid substrates were tested in different combinations. Combination of MYB + rice, PSB + rice and SYB +rice supported significantly higher biomass production than the other tested treatments yielding 2.5, 2.2 and 2.2

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g/100gmedia. The combination of MYB + rice supporting the maximum spores production (2.17×10^9 spores/g) and the viability percentage of spores was 98.22%.

4-Shelf life and viability of laboratory prepared wettable powder formulation of the entomopathogenic fungi *Verticillium lecanii* (Zimm.) Viegas

Shelf life and viability of laboratory prepared wettable powder formulation of the entomopathogenic fungi *Verticillium lecanii* (Zimm.) Viegas were investigated. The fungal viability was affected by several factors, including moisture content, method of mass production, culture media, as well as the filler types and the storage temperature of the formulation. Three levels of moisture content of 5.2% 9.7 % 13.4% were evaluated. The results showed that the vitality of fungi was higher at the lower moisture level, where the percentages of germination were 77%, 70.66% and 60.66% at the three levels of moisture content, respectively after 180 days of storage on the MYB media and starch as a filler under refrigerator temperature of 4-6 °C, the percentages of geremination at ambient room temperature (30 -35 °C) were 71.33%, 54% and 46.33% , respectively which demonstrates that the low-temperature increases the vitality of fungi and the percentage of germination after long-term storage. Using talc powder as filler, the geremination percentages were 77.33%, 69% and 58.66% under refrigerator conditions (4- 6 °C) and 67%, 47% and 45% at ambient room temperature (30 – 35 °C).

5-Biological performance of certain bio- pesticides, botanical, mineral oils and chemical insecticides aganist the mealy bug *Icerya seychellarum* infecting citrus trees during the summer season of 2013 at Alexandria governorate

In fact, the use of biopesticides with the biological control agents as means or elements of (IPM) program must prove to be effective and selective against the insect-pests. Therefore, the bioinsecticide must meet the

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demand of controlling the pest and increasing the yield via the reduction of the pest population.

Different treatments were evaluated to show the possibility of involving them within an IPM program for controlling the horticultural main insect pest (*Icerya seychellarum*) (Hemiptera) that attack orange trees. The evaluated biopesticides were Bio-Catch® 1.15 % (*Verticillium lecanii*) (a commercial marketed formulation) and a laboratory prepared wettable powder formulation containing *V. lecanii*. Also, a natural product (Nimbecidine® 0.03%) (azadirachtin), two chemical insecticides (Malathion® 57%E.C and Actara® 25 W.G (thiamethoxam) and a mineral oil (Kz oil® 95% E.C) were evaluated.

5. a. Application at sunset

It is obvious that the mineral oil Kz oil® 95% E.C was the most effective compound tested against the mealybug *Icerya seychellarum* (Westwood) followed by Malathion® 57%E.C and Actara® 25 W.G (Thiamethoxam), laboratory prepared wettable powder formulation, Bio-Catch® 1.15 % (*Verticillium lecanii*) and Nimbecidine® giving initial reduction of 90.71%, 89.80%, 87.74%, 54.69%, 53% and 41.60%, respectively, after one week post-application.

Regarding the biological performance on infestation reduction percentages after two weeks post - treatment, the same trend was observed. Kz oil® 95% E.C exhibited high reduction percentage estimated by 89.54% followed by Malathion® (88.66%), Actara® (83.5%), laboratory prepared wettable powder formulation (61.19%), Bio-Catch® (57.61%) and Nimbecidine® (46.51%).

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moreover, after three weeks post-treatments, the reduction percentage showed that the superior treatment was Kz oil® 95% E.C recording a reduction of infestation of 88.37%, followed by Malathion® (87.47%), Actara® (81.39%), laboratory prepared wettable powder formulation (65.41%), Bio-Catch® (63.55%) and Nimbecidine® (53.99%).

It is found that Kz oil® 95% E.C was always in the 1 *st* rank in reducing the insect population all over the period of inspection that lasted for 4 weeks post-application. The data showed that Kz oil® and Malathion® were significantly more effective than the other tested insecticide since they caused reduction percentages of 88.70 and 87.11%, respectively followed by Actara® wich caused 82.02% reduction. There were no significant differences between Bio-catch® and laboratory prepared wettable powder formulation and both gave the same value of reduction (70.13 and 71.68%, respectively) four weeks after application, but the lowest reduction of 61.49% was gained by Nimpecidine®. The results show that the microbial insecticides had high residual effects that lasted for four weeks after application.

5. b. Application in the morning:

It is obvious that the mineral oil Kz oil® was the most effective insecticide tested against the mealybug *Icerya seychellarum* (Westwood) followed by Malathion® and Actara® (thiamethoxam), laboratory prepared wettable powder formulation, Nimbecidine® and Bio-Catch® (*Verticillium lecanii*) giving initial reductions of 91.58%, 91.41%, 85.05%, 47.25%, 40.73% and 39.43% , respectively, after one week post-application.

Regarding the biological performance on infestation reduction percentages after two weeks post treatment, the same trend was observed. Kz oil® exhibited high reduction percentage estimated by 90.41% followed by

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Malathion® (90.18%), Actara® (83.39%), Nimbecidine® (51.13%), laboratory prepared wettable powder formulation (44.67%) and Bio-Catch® (43.98%) whichwas the least efficient tested compound.

Also, after three weeks post-treatments, the reduction percentage showed that the superior treatment was Kz oil® recording a reduction percentage of infestation of 89.14%, followed by Malathion® (88.51%), Actara® (82.31%), laboratory prepared wettable powder formulation (54.67%), Bio-Catch® (52.97%) and Nimbecidine® (51.76%).

It is found that Kz oil® was in the 1 *st* rank in reducing the insect population all over the period of inspection that lasted for 4 weeks post-application.The data showed that Kz oil® and Malathion® are significantly more effective than Actara® they caused reduction percentages of 88.61 and 88.25%, respectively followed by Actara® caused 80.78% reduction. There were significantly differences between Bio-catch® and laboratory prepared wettable powder formulation causing reduction percentages of 60.88 and 66.86%, respectively after four weeks of application. The lowest reduction percentage of 57.06% was recorded for Nimpecidine®. The results showed that the microbial insecticides were effective and have high residual effects and lasted for four weeks after application.

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