Efficacy test of micro emulsion formulation of *Lecanicillium lecanii* (= *Verticillium lecanii*) (Zimm.) Zare & W. Gams against four species of mealy bugs by laboratory bioassay

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Abstract

Evaluation of Six oils along with *Lecanicillium lecanii* (= *Verticillium lecanii*) (Zimm.) Zare & W. Gams (LIMO2) conidia to form micro emulsion formulation and Compatibility of oils with *L. lecanii* were also tested in the laboratory and bioassay in the laboratory revealed that, LT₅₀ of Eucalyptus oil +*Lecanicillium lecanii* (E+L), Pungam oils +*Lecanicillium lecanii* (P+L), Neem oil +*Lecanicillium lecanii* (N+L), Mustard oil +*Lecanicillium lecanii* (M+L), Clove oil +*Lecanicillium lecanii* (Cl+L) and Castor oil +*Lecanicillium lecanii* (Ca+L) formulations against *Phenacoccus solenopsis* population were 106.95, 59.15, 54.52, 73.74, 85.28 and 103.90 hours, respectively. The testing of oil formulations against *Paracoccus marginatus* revealed that, LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations were 113.43, 66.37, 54.52, 75.47, 89.78 and 110.12 hours, respectively. Oil formulation revealed that, LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations against *Maconellicoccus hirsutus* population were 103.01, 60.51, 53.19, 69.36, 83.17 and 97.77 hours, respectively. The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against *Ferresia virgata* population were 100.55, 62.94, 52.20, 70.88, 89.54 and 100.22 hours, respectively. Neem oil +*Lecanicillium lecanii* (N+L) combination showed effective against *Phenacoccus solenopsis*, *Paracoccus marginatus* and *Maconellicoccus hirsutus*.

Keywords: oil formulations, *Lecanicillium lecanii*, mealy bugs, LT₅₀

1. Introduction

Of the 700 species of entomopathogenic fungi currently known, only 10 species have been, or are presently being, developed for control (Robert and Hajek, 1992; Hajek & Leger, 1994) [7]. These entomopathogenic hypomycetes fungi have great potential as biological control agents against insects and in an important component within integrated pest management systems. They are being developed world wide for the control of many pests of agricultural importance (Ferron, 1985) [4]. It has emerged as one of the most promising and extensively researched biocontrol agents that can suppress a variety of economically important insect pests (Kaur and Padmaja, 2008) [8]. *Lecanicillium lecanii* (= *Verticillium lecanii*) (Zimm.) Zare & W. Gams is one of the most promising fungal species for the control of whiteflies, aphids and other insect pests.

According to Brown (1971) [3], some species of arthropods of agricultural, veterinary (130 species) and health human importance (102 species) have been found to be resistant to chemical insecticides. In the year 1976, it was also confirmed that many species of insect got resistant to hydrogen cyanide and lead arsenate poisoning. A large number of pesticides being used are poisoning in nature to man and other warm blooded animal. Residues of pesticides are due to inherent physio-chemical properties and depend on several namely (1) crop and their varieties with particularly leaf, stem, fruits etc., (2) climate conditions such as temperature, rainfall (3) pH of soil type, (4) Texture of soil etc. Keeping in view, the ill effects of chemical pesticides on human health and the environment, development of resistance in pests to pesticides and a higher level of pesticide residue in food items. There is a crying need to develop suitable alternatives to chemical pesticides for use in pest control. In the search for new avenues in biological control, the importance of entomopathogens has been highlighted as an environmentally friendly pest control method. Therefore, it is imperative to evolve an effective and ecofriendly method for the management...
of four species of mealybugs (Phenacoccus solenopsis, Paracoccus marginatus, Macoecilococcus hirsutus, Ferrisia virgata) infesting different crops under lab conditions.

2. Materials and Methods

2.1 Isolation & Maintenance of L. lecanii as pure culture
Sabourad’s Dextrose Agar media enriched with yeast extract (SDAY) was used for the production of L. lecanii. The media is composed of Dextrose 40 g, peptone 10 g, Agar 15 g, yeast extract 10 g in 1000 ml distilled water (Bell, 1974) [10]. The inoculated plates were incubated at room temperature (26 ± 1°C) and observed daily for the development of colonies. From such colony, a small quantity of inoculum was taken and transferred to SDAY slants and maintained as a pure culture.

2.2 Preparation of micro emulsion
Oil-in-water formulation was prepared by mixing the surfactant mixed oil phase with the spore suspension in the aqueous phase. Spores were harvested from 14 days old culture of L. lecanii strain (LIMO2), using 0.01% Tween-80 and spore suspensions were prepared by centrifuging the conidia in 0.2% Tween-80, after decanting the supernatant in the centrifuge tubes and the suspension was thoroughly mixed using a vortex mixer. The procedure of washing the conidia was repeated three times to eliminate Tween-80 and the washed conidia suspended in distilled water, formed the conidial stock 200μl, which was mixed with 9.8ml of distilled water. The required concentration of conidium was prepared using Neubauer haemocytometer. Oil phase of the conidial samples was prepared with sterilized neem oil, clove oil, pungam oil, castor oil, mustard oil and eucalyptus oil at three concentrations (1, 2 and 3%). Triton-X-100 was used as a non-ionic surfactant, Na₂CO₃ (Sodium Carbonate) as stabilizer and paraffin liquid as an antifoaming agent. One per cent oil formulation consists of 1% oil, 1% Triton-X-100, 0.5% paraffin liquid, 1% Na₂CO₃ and 96.5% of the aqueous phase. For 2% and 3% formulations the concentration of oil as well as surfactants was increased to twice and thrice respectively. The mixtures of these two phases were then homogenized using the magnetic stirrer for 60 minutes, to get a stable formulation (Plate 2s).

2.3 Bioassay
Six oil formulations were prepared using eucalyptus oil, neem oil, pungam oil, clove oil, mustard oil and castor oil with the L. lecanii (LIMO2) strain. Different species of mealybug adults were treated as a batch of 10 kept in petriplates by spray application of 1%, 2%, 3% oil formulation at 10⁵ conidia per ml using an atomizer. Fresh cotton leaves were provided as feed everyday and containers were cleaned daily. Petriplates were placed in an environmental chamber set at 25 ± 1°C. The insects were treated for two consecutive days and controls were treated with an equal volume of water with 0.02% Tween-80. Bioassays were setup with three replicates for each treatment. Mortality data were collected at 24h intervals for three days. The dead insects were transferred to Petridishes with a moist filter paper to facilitate mycosis. Before transferring the dead insects into the Petridishes, their surfaces were immediately sterilized with 1% sodium hypochlorite followed by three rinses with sterile distilled water and bioassays were repeated twice. The median lethal time (LT₅₀) was calculated from the cumulative mortality data on each day post treatment, using probit analysis (Finney, 1971) [3].

3. Results and Discussions

3.1 Efficacy test of formulations by laboratory bioassay against four species of mealy bugs

3.1.1 Median lethal Time (LT₅₀) of oil in water formulations of Lecanicillium lecanii (LIMO2) against Phenacoccus solenopsis
The LT₅₀ of eucalyptus oil, pungam oil, neem oil, mustard oil, clove oil and castor oil formulations assessed against P. solenopsis population were 106.95, 59.15, 54.52, 73.74, 85.28 and 103.90 hours, respectively. The LT₅₀ of eucalyptus oil, pungam oil, neem oil, mustard oil, clove oil and castor oil formulations assessed against P. solenopsis population were 277.39, 127.23, 115.24, 165.91, 203.90 and 268.04 hours, respectively (Table 1) (Plate 1). In the present investigation, the lowest LT₅₀ and LT₅₀ was recorded by neem oil formulation as 54.52 and 115.24 hours, respectively followed by pungam oil, mustard oil, clove oil, castor oil and eucalyptus oil formulations assessed against P. solenopsis population. Oils can substantially enhance the efficacy of entomopathogens against insects (Prior et al., 1988) [10].

3.1.2 Median lethal Time (LT₅₀) of oil in water formulations of Lecanicillium lecanii (LIMO2) against Paracoccus marginatus
The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against P. marginatus population were 113.43, 66.37, 54.52, 75.47, 89.78 and 110.12 hours, respectively. The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against P. marginatus population were 297.43, 142.11, 120.76, 170.45, 216.62 and 287.18 hours, respectively (Table 2). In the present investigation, the lowest LT₅₀ and LT₅₀ were recorded by neem oil formulation as 54.52 and 120.76 hours, respectively followed by pungam oil, mustard oil, clove oil, castor oil and eucalyptus oil formulations assessed against P. marginatus population. Oil carriers can also distribute the inoculum over the intersegmental membranes, which are more readily penetrated by entomopathogenic fungi (Lisansky, 1989) [9].

3.1.3 Median lethal Time (LT₅₀) of oil in water formulations of Lecanicillium lecanii (LIMO2) against Macoecilococcus hirsutus
The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against M. hirsutus population were 103.01, 60.51, 53.19, 69.36, 83.17 and 97.77 hours, respectively. The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against M. hirsutus population were 255.85, 127.23, 115.24, 165.91, 203.90 and 268.04 hours, respectively (Table 3) (Plate 1). In the present investigation, the lowest LT₅₀ and LT₅₀ were recorded by neem oil formulation as 53.19 and 109.12 hours, respectively followed by pungam oil, mustard oil, clove oil, castor oil and eucalyptus oil formulations assessed against M. hirsutus population. Prior et al. (1992) [11] found that a conidial suspension of B. bassiana in coconut oil, water and 0.01% Tween-80 was infective against the cocoa weevil pest, Pantorhytes platus.

3.1.4 Median lethal Time (LT₅₀) of oil in water formulations of Lecanicillium lecanii (LIMO2) against Ferrisia virgata
The LT₅₀ of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against F. virgata population were

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population. Bhanu prakash et al. (2015) reported that the enhanced efficacy of formulation is generally attributed to the fact that oils are excellent stickers, promoting contact between the formulated active ingredient and the lipophilic insect cuticle and increasing rain-fastness on the waxy leaf cuticle of treated host plants. The good pest control achieved in the field trial is a positive indication for the inclusion of this fungus in the integrated pest management programmes.

### Table 1: Time- mortality response of oil in water formulation of Lecanicillium lecanii (LMO2) against Phenacoccus solenopsis

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Regression equation</th>
<th>Calculated $\chi^2$</th>
<th>Fiducial limits $L_{T50}$ (Hours)</th>
<th>Fiducial limits $L_{T95}$ (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>(y = 2.24x + 0.41)</td>
<td>0.0265</td>
<td>106.95</td>
<td>73.45</td>
</tr>
<tr>
<td>Pungam oil</td>
<td>(y = 2.65x + 0.49)</td>
<td>0.0241</td>
<td>59.15</td>
<td>50.31</td>
</tr>
<tr>
<td>Neem oil</td>
<td>(y = 2.79x + 0.40)</td>
<td>0.0021</td>
<td>54.52</td>
<td>47.05</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>(y = 2.48x + 0.45)</td>
<td>0.0442</td>
<td>73.74</td>
<td>59.33</td>
</tr>
<tr>
<td>Clove oil</td>
<td>(y = 2.35x + 0.48)</td>
<td>0.0380</td>
<td>85.28</td>
<td>64.82</td>
</tr>
<tr>
<td>Castor oil</td>
<td>(y = 2.28x + 0.38)</td>
<td>0.0487</td>
<td>103.90</td>
<td>72.22</td>
</tr>
</tbody>
</table>

All lines are significantly a good fit at 1% ($P = 0.05$).

### Table 2: Time- mortality response of oil in water formulation of Lecanicillium lecanii (LMO2) against Paracoccus marginatus

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Regression equation</th>
<th>Calculated $\chi^2$</th>
<th>Fiducial limits $L_{T50}$ (Hours)</th>
<th>Fiducial limits $L_{T95}$ (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>(y = 2.14x + 0.47)</td>
<td>0.8635</td>
<td>113.43</td>
<td>75.97</td>
</tr>
<tr>
<td>Pungam oil</td>
<td>(y = 2.26x + 0.32)</td>
<td>0.9997</td>
<td>66.37</td>
<td>55.35</td>
</tr>
<tr>
<td>Neem oil</td>
<td>(y = 2.73x + 0.44)</td>
<td>0.6607</td>
<td>54.52</td>
<td>48.67</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>(y = 2.44x + 0.48)</td>
<td>0.3517</td>
<td>75.47</td>
<td>60.30</td>
</tr>
<tr>
<td>Clove oil</td>
<td>(y = 2.28x + 0.53)</td>
<td>0.3243</td>
<td>89.78</td>
<td>67.02</td>
</tr>
<tr>
<td>Castor oil</td>
<td>(y = 2.19x + 0.44)</td>
<td>0.2981</td>
<td>110.12</td>
<td>74.70</td>
</tr>
</tbody>
</table>

All lines are significantly a good fit at 1% ($P = 0.05$).

### Table 3: Time- mortality response of oil in water formulation of Lecanicillium lecanii (LMO2) against Maconellicoccus hirsutus

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Regression equation</th>
<th>Calculated $\chi^2$</th>
<th>Fiducial limits $L_{T50}$ (Hours)</th>
<th>Fiducial limits $L_{T95}$ (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>(y = 3.36x + 0.19)</td>
<td>0.5651</td>
<td>103.01</td>
<td>72.86</td>
</tr>
<tr>
<td>Pungam oil</td>
<td>(y = 2.90x + 0.01)</td>
<td>0.0031</td>
<td>60.51</td>
<td>51.78</td>
</tr>
<tr>
<td>Neem oil</td>
<td>(y = 2.98x + 0.11)</td>
<td>0.0635</td>
<td>53.19</td>
<td>46.30</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>(y = 2.67x + 0.20)</td>
<td>0.0010</td>
<td>69.36</td>
<td>57.16</td>
</tr>
<tr>
<td>Clove oil</td>
<td>(y = 2.48x + 0.28)</td>
<td>0.1537</td>
<td>83.17</td>
<td>64.39</td>
</tr>
<tr>
<td>Castor oil</td>
<td>(y = 2.08x + 0.80)</td>
<td>0.5580</td>
<td>97.77</td>
<td>69.79</td>
</tr>
</tbody>
</table>

All limits are significantly a good fit at 1% ($P = 0.05$).

### Table 4: Time- mortality response of oil in water formulation of Lecanicillium lecanii (LMO2) against Ferrisia virgata

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Regression equation</th>
<th>Calculated $\chi^2$</th>
<th>(L_{T50}) (Hours)</th>
<th>Fiducial limits $L_{T50}$ (Hours)</th>
<th>Fiducial limits $L_{T95}$ (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus oil</td>
<td>(y = 2.04x + 0.83)</td>
<td>1.2775</td>
<td>100.55</td>
<td>70.97</td>
<td>142.45</td>
</tr>
<tr>
<td>Pungam oil</td>
<td>(y = 2.84x + 0.05)</td>
<td>0.6763</td>
<td>62.94</td>
<td>53.44</td>
<td>74.14</td>
</tr>
<tr>
<td>Neem oil</td>
<td>(y = 3.01x + 0.09)</td>
<td>0.0172</td>
<td>52.20</td>
<td>45.53</td>
<td>59.84</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>(y = 2.63x + 0.02)</td>
<td>0.1727</td>
<td>70.88</td>
<td>58.09</td>
<td>86.49</td>
</tr>
<tr>
<td>Clove oil</td>
<td>(y = 2.35x + 0.36)</td>
<td>2.3774</td>
<td>89.54</td>
<td>67.62</td>
<td>118.58</td>
</tr>
<tr>
<td>Castor oil</td>
<td>(y = 2.41x + 0.16)</td>
<td>0.1358</td>
<td>100.22</td>
<td>71.66</td>
<td>140.17</td>
</tr>
</tbody>
</table>

All lines are significantly a good fit at 1% ($P = 0.05$).
References

Plate 2: Different species of mealybug infected by L. lecanii

Ferrisia virgata  Maconellicoccus hirsutus

Phenacoccus solenopsis  Paracoccus marginatus