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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 (LlMO2) 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, LT_{50} of Eucalyptus oil +*Lecanicillium lecanii* (E+L), Pungam oil+*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, LT_{50} 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, LT_{50} 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 LT_{50} 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, LT_{50}

1. Introduction
Of the 700 species of entomopathgenic fungi currently known, only 10 species have been, or are presently being, developed for control (Robert and Hajek, 1992; Hajek & Leger, 1994) [7, 6]. 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 men and other warm blooded animals and also leave residues. 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 (*Phen*acoccus solenopsis, *Paracoccus marginatus, Maconellicoccus hirsutus, Ferrisia virgata*) infesting different crops under lab conditions

2. Materials and Methods

2.1 Isolation & Maintainance 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) [1]. The inoculated plates were incubated at room temperature (26 ± 1^0C) 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 (LlMO2), using 0.01% Tween-80 and spore suspensions were prepared by centrifuging the conidia in 0.02% 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 conidia 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%). TritonX-100 was used as a non-ionic surfactant, Na_2CO_3 (Sodium Carbonate) as stabilizer and paraffin liquid as an antifoaming agent. One per cent oil formulation consists of 1% oil, 1% TritonX-100, 0.5% paraffin liquid, 1% Na_2CO_3 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* (LlMO2) 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 10^8 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 (LT_{50}) was calculated from the cumulative mortality data on each day post treatment, using probit analysis (Finney, 1971) [5].

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 (LT_{50}) of oil in water formulations of *Lecanicillium lecanii* (LlMO2) against *Phenacoccus solenopsis*

The LT_{50} 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 LT_{95} 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 LT_{50} and LT_{95} 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 (LT_{50}) of oil in water formulations of *Lecanicillium lecanii* (LlMO2) against *Paracoccus marginatus*

The LT_{50} 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 LT_{95} 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, lowest LT_{50} and LT_{95} 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 (LT_{50}) of oil in water formulations of *Lecanicillium lecanii* (LlMO2) against *Maconellicoccus hirsutus*

The LT_{50} 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 LT_{95} of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against *M. hirsutus* population were 255.85, 124.43, 109.12, 150.13, 191.76 and 248.16 hours, respectively (Table 3) (Plate 1). In the present investigation, the lowest LT_{50} and LT_{95} 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 plutus*.

3.1.4 Median lethal Time (LT_{50}) of oil in water formulations of *Lecanicillium lecanii* (LlMO2) against *Ferrisia virgata*

The LT_{50} of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against *F. virgata* population were

100.55, 62.94, 52.20, 70.88, 89.54 and 100.22 hours, respectively. The LT_{95} of (E+L), (P+L), (N+L), (M+L), (Cl+L) and (Ca+L) formulations assessed against *F. virgata* population were 256.55, 130.24, 106.75, 153.96, 209.07 and 247.72 hours, respectively (Table 4) (Plate 2). In the present investigation, the lowest LT_{50} and LT_{95} was recorded by neem oil formulation as 52.20 and 106.75 hours, respectively followed by pungam oil, mustard oil, clove oil, castor oil and eucalyptus oil formulations assessed against *F. virgata* population. Bhanu prakash *et al.* (2015) [2] 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* (LlMO2) against *Phenacoccus solenopsis*

Formulation type	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
Eucalyptus oil	y = 2.24x + 0.41	0.0265	106.95	73.45	155.72	277.39	133.95	574.49
Pungam oil	y = 2.65x + 0.49	0.0241	59.15	50.31	69.54	127.23	89.54	180.78
Neem oil	y = 2.79x + 0.40	0.0021	54.52	47.05	63.17	115.24	84.05	158.01
Mustard oil	y = 2.48x + 0.45	0.0442	73.74	59.33	91.67	165.91	105.31	261.39
Clove oil	y = 2.35x + 0.48	0.0380	85.28	64.82	112.19	203.90	116.57	356.65
Castor oil	y = 2.28x + 0.38	0.0487	103.90	72.22	149.49	268.04	131.66	545.68

All liness are significantly a good fit at 1% (P = 0.05)

Table 2: Time- mortality response of oil in water formulation of *Lecanicillium lecanii* (LlMO2) against *Paracoccus marginatus*

Formulation type	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
Eucalyptus oil	y = 2.14x + 0.47	0.8635	113.43	75.97	163.36	297.43	138.65	638.04
Pungam oil	y = 2.26x + 0.32	0.9997	66.37	55.35	79.58	142.11	97.06	208.07
Neem oil	y = 2.73x + 0.44	0.6607	54.52	48.67	66.17	120.76	86.68	168.25
Mustard oil	y = 2.44x + 0.48	0.3517	75.47	60.30	94.44	170.45	107.03	271.45
Clove oil	y = 2.28x + 0.53	0.3243	89.78	67.02	120.28	216.62	120.55	389.24
Castor oil	y = 2.19x + 0.44	0.2981	110.12	74.70	162.34	287.18	136.28	605.18

All liness are significantly a good fit at 1% (P = 0.05)

Table 3: Time- mortality response of oil in water formulation of *Lecanicillium lecanii* (LlMO2) against *Maconellicoccus hirsutus*

Formulation type	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
Eucalyptus oil	y = 2.36x + 0.19	0.5651	103.01	72.86	145.63	255.85	131.50	497.78
Pungam oil	y = 2.90x + 0.01	0.0031	60.51	51.78	70.71	124.43	89.93	172.15
Neem oil	y = 2.98x + 0.11	0.0635	53.19	46.30	61.12	109.12	81.72	145.61
Mustard oil	y = 2.67x + 0.20	0.0010	69.36	57.16	84.16	150.13	100.33	224.63
Clove oil	y = 2.48x + 0.28	0.1537	83.17	64.39	107.40	191.76	114.56	321.13
Castor oil	y = 2.08x + 0.80	0.5580	97.77	69.79	136.96	248.16	126.97	484.99

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* (LlMO2) against *Ferrisia virgate*

Formulation type	Regression equation	Calculated χ^2	LT_{50} (Hours)	Fiducial limits		LT_{95} (Hours)	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
Eucalyptus oil	y = 2.04x + 0.83	1.2775	100.55	70.97	142,45	256.55	129.17	509.56
Pungam oil	y = 2.84x + 0.05	0.6763	62.94	53.44	74.14	130.24	92.70	182.98
Neem oil	y = 3.01x + 0.09	0.0172	52.20	45.53	59.84	106.75	80.56	141.47
Mustard oil	y = 2.63x + 0.02	0.1727	70.88	58.09	86.49	153.96	101.93	232.55
Clove oil	y = 2.35x + 0.36	2.3774	89.54	67.62	118.58	209.07	120.34	363.22
Castor oil	y = 2.41x + 0.16	0.1358	100.22	71.66	140.17	247.72	129.28	474.67

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

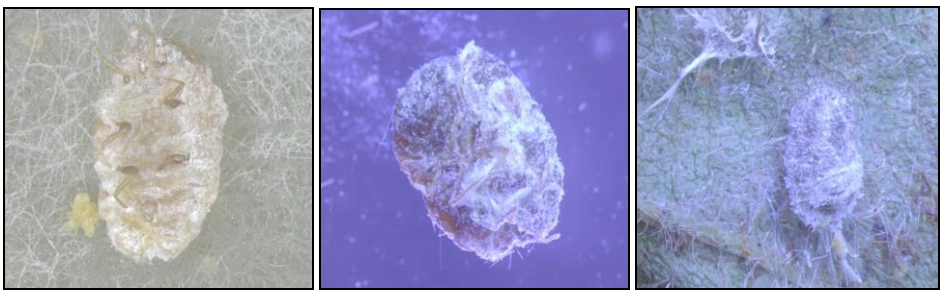
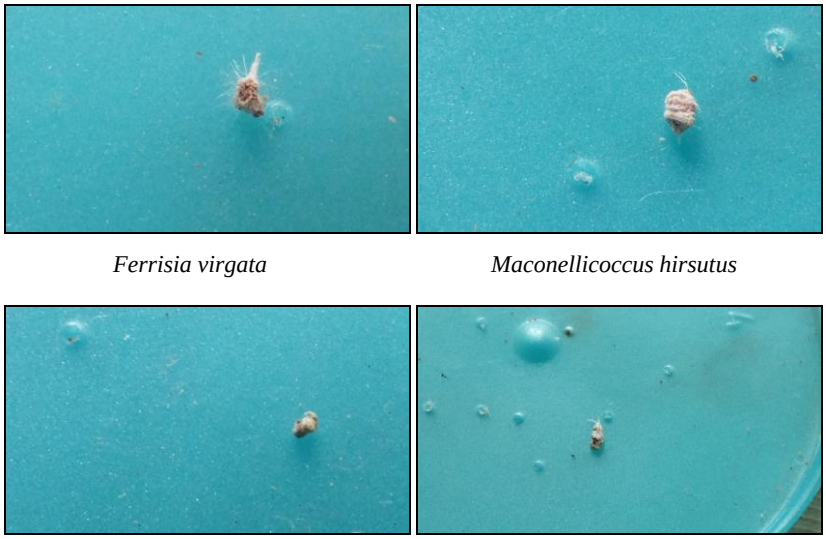


Plate 1: Mealybug affected due to oil in water formulation of oils



Ferrisia virgata *Maconellicoccus hirsutus*

Phenacoccus solenopsis *Paracoccus marginatus*

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

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