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Development of formulation and optimization of delivery system of most virulent strain (*Lecanicillium lecanii* (l1mo2)) obtained by lab studies

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Abstract
Six oils with three different concentrations (1%, 2% and 3%) were used along with *L. lecanii* (LlMO2) conidia to form micro emulsion formulation. Compatibility of oils with *L. lecanii* were also tested in the laboratory. In case of neem oil, T value calculated for 1% concentration was 91.51 (C) which indicates compatibility. T value calculated for 2% concentration was 84.83 (C) which indicates compatibility. Among all concentrations, 1% and 2% concentrations are compatible with the fungus based on calculated T value. Among the treatments, eucalyptus oil at 1% concentration did not cause any phytotoxicity symptoms.

Keywords: Development, formulation, optimization, (*Lecanicillium lecanii* (l1mo2))

Introduction
Microbial control is a powerful pest management tactic, which involves the purposeful manipulation of pathogenic microorganisms to ensure a reduction in pestilence of a pest. This approach is a part of applied biological control in which the role of human agency is quite imperative. Like human beings insects too are attacked by a wide range of microorganisms like bacteria, fungi, nematode, protozoan and rickettsiae resulting in a reduction of their number. There is a tremendous potential in the micro-organisms and their product in the modern IPM programme because of their high degree of multiplicity of a faster rate, high degree of selectivity and specificity restoring beneficial natural fauna and conserving ecological balance. Coupled with these, their harmless nature to other forms of life, failure of insects to develop resistance against those microbes and more over the compatibility of their products with conventional insecticides have on added impetus in successfully exploitation of such microorganisms in insect control.

Many fungi, which are exclusively used in insect control belong to four distinct classes' viz., Hyphomycetes, Deuteromycetes, Ascomycetes, and Basidiomycetes. The class Phycomycetes and Deuteromycotina include most of the important fungal pathogens and the genera *Entomophthora*, *Metarhizium*, *Beauveria*, *Nomuraea* and *Verticillium* are noteworthy (Bell, 1974: Ferron, 1978) [2, 5]. Fungal diseases are commonly seen in insect orders such as Homoptera, Lepidoptera, Hymenoptera and Diptera (Keller, 1992; Kerwin, 1992) [6, 7].

Of the 700 species of entomopathgenic fungi currently known, only 10 species have been, or are presently being, developed for control (McCoy *et al.*, 1988) [8]. Biopesticides formulation based on *Beauveria bassiana* (Balsam.) Vuillemin, a wide host range insect pathogenic fungus are being marketed and used in insect pest management (Reddy *et al.*, 2008) [9]. *Lecanicillium lecanii* (= *Verticillium lecanii*) (Zimm.) Zare & W. Gams is one of the most promising fungal species for control of whiteflies, aphids and other insect pests.

According to Brown (1971) [3] some 130 species of arthropods of agricultural and veterinary importance and 102 species of importance to human health have been found to be resistant to chemical insecticides A large number of pesticides being used are poisoning in nature to men and other warm blooded animals and also leave residues. Keeping in view, the ill effects of a chemical pesticides on human health and the environment, development of resistance in pests to pesticides and higher level of pesticides 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 environmental friendly pest control method.

Preparation of micro emulsion

Oil-in-water formulation was prepared by mixing the surfactant mixed oil phase with the spore suspension in 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. Required concentration of conidia was prepared using Neubauer haemocytometer. Oil phase of the conidial samples were 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 non-ionic surfactant, Na_2CO_3 (Sodium Carbonate) as stabilizer and paraffin liquid as 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).

Germination assessment

Fifty micro liters of the oil formulation at 1×10^8 conidia per ml was used for inoculating SDAY plates by spread plate method, and four sterile cover slips were randomly placed on each plate. Plates were sealed with parafilm and incubated at 25 ± 1°C. After 24h post incubation, 1ml of formaldehyde (0.5%) was transferred on to each plate to arrest germination as per the method of David *et al*. (2008) [4]. Each cover slip was removed and placed on glass slide for making germinated / un-geminated spore count (500 per each cover slip). For each sample three replicates were observed.

Vegetative growth and conidiation

By using the cork borer, a 5mm hole was made in the middle of SDAY plate and inoculated with 50µl of the formulation at 1 × 108 conidia per ml and the plates were sealed with para film before incubating at 25 ± 1°C. Colony diameter was recorded on 14th day for assessment of conidiogenesis, the spores were flushed out from the plates using 10ml of 0.02% Tween-80. Spore count was done using Neubauer haemocytometer and three replicates were maintained for each sample. Data was submitted to ANOVA and means were computed by the Tukey test ($p \leq 0.05$). Compatibility assessment of the different oils was made using the formula Alves *et al.* (1998) [1].

$$T = \frac{20(VG) + 80(SP)}{100}$$

Where, vegetative growth (VG) and sporulation (SP) were given in relation to the control (100%). T value of 0 to 30 = very toxic; 31 to 45 = toxic; 46 to 60 = moderately toxic; > 60 =compatible.

Plate 1: View of Micro emulsion of different oils

Table 1: "T" values and compatibility classification of six oils on *Lecanicillium lecanii* (LlMO2)

Formulation type	T value		
	1% concentration	2% concentration	3% concentration
Eucalyptus oil	59.20 (MT)	55.19 (MT)	50.38 (MT)
Pungam oil	82.69 (C)	77.62 (C)	73.62 (C)
Neem oil	91.51 (C)	84.83 (C)	80.83 (C)
Mustard oil	73.88 (C)	66.41 (C)	59.67 (MT)
Clove oil	65.61 (C)	62.14 (C)	55.20 (MT)
Castor oil	56.27 (MT)	53.86 (MT)	49.33 (MT)
Control	96.89 (C)	98.77 (C)	97.70 (C)

C = compatible, MT = moderately toxic, T = toxic, Control = without oils and additives

T value of 0 to 30 = very toxic; 31 to 45 = toxic; 46 to 60 = moderately toxic; > 60 =compatible

Table 2: Effect of 1%, 2%, 3% Oil in water emulsion formulation on conidia of *Lecanicillium lecanii* (LlMO2)

Treatment	Concen-tration	Colony diameter		Conidia number per plate		Concen-tration	Colony diameter		Conidia number per plate		Concen-tration	Colony diameter		Conidia number per plate	
		Mean (cm)	Reduction percentage	Mean	Reduction percentage		Mean (cm)	Reduction percentage	Mean	Reduction percentage		Mean (cm)	Reduction percentage	Mean	Reduction percentage
Eucalyptus oil	1%	4.01	10.69	7.93 x 10^8	52.42	2%	3.99	12.11	7.96 x 10^8	53.53	3%	3.94	12.83	7.21 x 10^8	54.53
Pungam oil	1%	4.16	7.35	12.99 x 10^8	22.07	2%	4.13	9.03	12.89 x 10^8	24.75	3%	4.12	8.84	10.83x 10^8	31.71
Neem oil	1%	4.22	6.01	13.79 x 10^8	17.27	2%	4.19	7.71	13.76 x 10^8	19.67	3%	4.18	7.52	11.82 x 10^8	25.47
Mustard oil	1%	4.10	8.68	12.27 x 10^8	26.39	2%	4.06	10.57	11.18 x 10^8	34.73	3%	4.04	10.61	10.09 x 10^8	36.38
Clove oil	1%	4.06	9.57	9.99 x 10^8	40.07	2%	4.03	11.23	9.89 x 10^8	42.26	3%	4.03	10.84	9.27 x 10^8	41.55
Castor oil	1%	4.01	10.69	8.89 x 10^8	46.67	2%	3.98	12.33	8.48 x 10^8	50.49	3%	4.01	11.28	7.45 x 10^8	53.02
Control	1%	4.49	0.00	16.67 x 10^8	0.00	2%	4.54	0.00	17.13 x 10^8	0.00	3%	4.52	0.00	15.86 x 10^8	0.00
SEd	1%	-	-	-	0.0263	2%	-	-	-	0.0443	3%	-	-	-	0.0320
CD (P = 0.05)	1%	-	-	-	0.0565	2%	-	-	-	0.0953	3%	-	-	-	0.0687

Results and discussion

Development of formulation and optimization of delivery system of most virulent strains obtained by lab studies

Preparation of Micro emulsion

Six oils with three different concentrations (1%, 2% and 3%) were used along with *L. lecanii* (LlMO2) conidia to form micro emulsion formulation. Compatibility of oils with *L. lecanii* were also tested in the laboratory.

Eucalyptus oil

Colony diameter and number of conidia per plate were observed for three different concentrations and by using above observations, "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.01 cm with reduction of 10.69 per cent and number of conidia per plate was 7.93 x 10^8 with reduction of 52.42 per cent (Table 2). "T" value calculated for 1% concentration was 59.20 (MT), which indicates moderately toxic (Table 1). In 2% concentration, colony diameter was 3.99 cm with reduction of 12.11 per cent and number of conidia per plate was 7.96 x 10^8 with reduction of 53.53 per cent (Table 2). "T" value calculated for 2% concentration was 55.19 (MT), which indicates moderately toxic (Table 1). In 3% concentration, colony diameter was 3.94 cm with reduction of 12.83 per cent and number of conidia per plate was 7.21 x 10^8 with reduction of 54.53 per cent (Table 2). "T" value calculated for 3% concentration was 50.38 (MT) which indicates moderately toxic (Table 1).

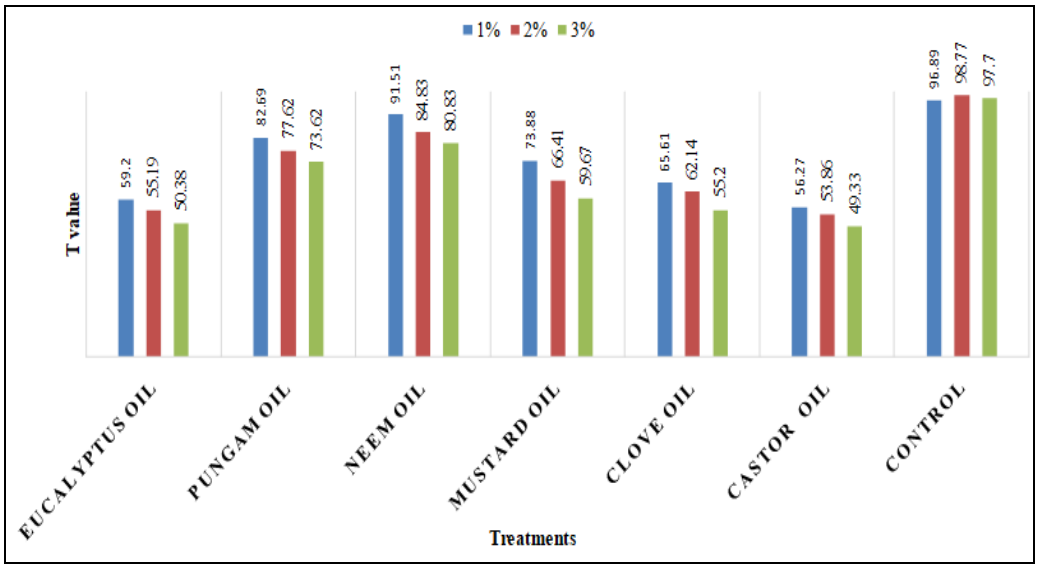

Fig 1: Compatibility studies with different concentrations of oils on L. lecanii (LIMO2)

Pungam oil

Colony diameter and number of conidia per plate was observed for three different concentrations and by using above observations, "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.16 cm with reduction of 7.35 per cent and number of conidia per plate was 12.99 x 10^8 with reduction of 22.07 per cent (Table 2). "T" value calculated for 1% concentration was 82.69 (C), which indicates compatibility (Table 1). In 2% concentration, colony diameter was 4.13 cm with reduction of 9.03 per cent and number of conidia per plate was 12.89 x 10^8 with reduction of 24.75 per cent (Table 2). "T" value calculated for 2% concentration was 77.62 (C), which indicates compatibility (Table 1). In 3% concentration, colony diameter was 4.12 cm with reduction of 8.84 per cent and number of conidia per plate was 10.83 x 10^8 with reduction of 31.71 per cent (Table 2). "T" value calculated for 3% concentration was 73.62 (C), which indicates compatibility (Table 1).

Neem oil

Colony diameter and number of conidia per plate was observed for three different concentrations and by using above observations "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.22 cm with reduction of 6.01 per cent and number of conidia per plate was 13.79 x 10^8 with reduction of 17.27 per cent (Table 2). "T" value calculated for 1%

concentration was 91.51 (C), which indicates compatibility (Table 1). In 2% concentration, colony diameter was 4.19 cm with reduction of 7.71 per cent and number of conidia per plate was 13.76 x 10^8 with reduction of 19.67 per cent (Table 2). "T" value calculated for 2% concentration was 84.83 (C), which indicates compatibility (Table 1). In 3% concentration, colony diameter was 4.18 cm with reduction of 7.52 per cent and number of conidia per plate was 11.82 x 10^8 with reduction of 25.47 per cent (Table 2). "T" value calculated for 3% concentration was 80.83 (C), which indicates compatibility (Table 1).

Mustard oil

Colony diameter and number of conidia per plate was observed for three different concentrations and by using above observations "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.10 cm with reduction of 8.68 per cent and number of conidia per plate was 12.27 x 10^8 with reduction of 26.39 per cent (Table 2). "T" value calculated for 1% concentration was 73.88 (C), which indicates compatibility (Table 1). In 2% concentration, colony diameter was 4.06 cm with reduction of 10.57 per cent and number of conidia per plate was 11.18 x 10^8 with reduction of 34.73 per cent (Table 2). "T" value calculated for 2% concentration was 66.41 (C), which indicates compatibility (Table 1). In 3% concentration, colony diameter was 4.04 cm with reduction of 10.61 per cent and number of conidia per plate was 10.09 x 10^8 with reduction of 36.38 per cent (Table 2). "T" value calculated for 3% concentration was 59.67 (MT), which indicates moderately toxic (Table 1).

Clove oil

Colony diameter and number of conidia per plate was observed for three different concentrations and by using above observations, "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.06 cm with reduction of 9.57 per cent and number of conidia per plate was 9.99 x 10^8 with reduction of 40.07 per cent (Table 2). "T" value calculated for 1% concentration was 65.61 (C), which indicates compatibility (Table 1). In 2% concentration, colony diameter was 4.03 cm with reduction of 11.23 per cent and number of conidia per plate was 9.89 x 10^8 with reduction of 42.26 per cent (Table 2). "T" value calculated for 2% concentration was 62.14 (C), which indicates compatibility (Table 1). In 3% concentration, colony diameter was 4.03 cm with reduction of 10.84 per cent and number of conidia per plate was 9.27 x 10^8 with reduction of 41.55 per cent (Table 2). "T" value calculated for 3% concentration was 55.20 (MT), which indicates moderately toxic (Table 1).

Castor oil

Colony diameter and number of conidia per plate was observed for three different concentrations and by using above observations "T" value was calculated for three different concentrations. In 1% concentration, colony diameter was 4.01 cm with reduction of 10.69 per cent and number of conidia per plate was 8.89 x 10^8 with reduction of 46.67 per cent (Table 2). "T" value calculated for 1% concentration was 56.27 (MT), which indicates moderately toxic (Table 1). In 2% concentration, colony diameter was 3.98 cm with reduction of 12.23 per cent and number of conidia per plate was 8.48 x 10^8 with reduction of 50.49 per cent (Table 2). "T" value calculated for 2% concentration was 53.86 (MT), which indicates moderately toxic (Table 1). In 3% concentration, colony diameter was 4.01 cm with reduction of 11.28 per cent and number of conidia per plate was 7.45 x 10^8 with reduction of 53.02 per cent (Table 2). "T" value calculated for 3% concentration was 49.33 (MT), which indicates moderately toxic (Table 1).

References

1. Alves SB, Moino A, Almeida JEM. Produtos fitossanitários entomopatóg. In- Controle microbiano de insetos. Fealq, São Paulo, 1998, 217-238
2. Bell JV. Mycoses. In Cantwell G.E. (Ed.) Insect Disease. New York, Marcel Dekker, Inc. 1974; 1:185-236.
3. Brown S. Entomogenus fungi. In CRC Hand book of natural Pesticides. Microbial Insecticides. CRC. 1971; 5:151-236.
4. David MB, Nguya KM, Manrkus K, Hamadi IB. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* strains to tetranychus evansi. Exp. Appl. Acarol. 2008; 46:275-285.
5. Ferron P. Biological control of insect pests by entomogenous fungi. Ann. Rev. Entomol. 1978; 23:409.
6. Keller S. Control of *Melolontha* with *Beauveria brongniartii*- Comparision of two methods. 25thAnnual meeting, Society for Invertebrate Pathology, 1992, 174.
7. Kerwin JL. EPA resister *Lagenidium giguntum* for mosquito control. Society for Invertebrate Pathology Newsletter. 1992; 24:8-9.
8. McCoy CW, Samson RA, Boucias DG. Entomogenous fungi. In Handbook of Natural Pesticides, Boca, Ra-ton, Fla: Mr ic Press. Vol. 5, Microbial Insecticides, Part A, entomogenous Protozoa and Fungi, C. M. Ignoffo and N. B. Mandava, eds. CRC Press, Boca Raton, FL, 1988, 151-236.
9. Reddy NP, Pathan A, Khan A, Uma Devi K, Victor JS. Sharma HC. Assessment of the suitability of Tinopal as an enhancing adjuvant in formulations of the insect pathogenic fungus, *Beauveria bassiana* (Bals.) Vuillemin. Pest Manag Sci, 2008, 1526-498X.