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Evaluation of synergism / antagonism in the efficacy of entomopathogenic fungi against selected mealybugs through co-administration with some adjuvants under both laboratory and pot conditions

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Abstract

Mealybugs (Hemiptera: Pseudococcidae) are important insect pests in worldwide. Forty one different treatments were tested against *Phenacoccus solenopsis* in laboratory. Among the treatments, lemongrass oil in combination with *Lecanicillium lecanii* (Zimm.) Zare & W. Gams (LIMO2) recorded the highest mortality of 93.10 per cent at 7 DAT, followed by citronella oil in combination with *L. lecanii* (LIMO2) showed the mortality of 89.65 per cent at 7 DAT. As per the results obtained, lemongrass oil, citronella oil, neem oil, pungam oil, mustard oil, sweet flag oil and castor soap shows synergistic effect with *L. lecanii* against meaybugs.

Keywords: Phenacoccus, Lecanicilium, lemongrass oil, citronell oil

1. Introduction

Mealybugs are small, soft-bodied, plant sucking insects, which embrace the second largest family of scale insects (Pseudococcidae) with approximately 2000 species belonging to 300 genera and common name is due to the waxy material which covers the bodies of adult females (Miller and Williams 1997; Downie and Gullan 2004). The mealy bug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) has been observed, damaging cotton crop very seriously from 2004-05 in Gujarat (Jhala *et al.*, 2008). Chemical pesticides are generally used to protect crops and to kill pests. Use of synthetic pesticides causes some unfortunate consequences like environmental pollution, pest resistance and toxicity to other non-target organisms. 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. *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.

2. Materials and methods

2.1 Bioefficacy of effective wax dissolving agents and its combination with Entomopathogenic fungi against different species of Mealybugs.

Experiment was conducted to evaluate the bioefficacy of effective wax dissolving agents *viz.*, lemongrass oil (*Cymbopogon citratus* L.), citronella oil (*Cymbopogon nardus* L. Rendlea), castor soap (*Ricinus communis* L.), neem oil (*Azadirachta indica* L.), mustard oil (*Brassica niger* L.), pungam oil (*Pongamia pinnata* L.), Sweet flag (*Acarus calamus*) oil *viz.*, *L. lecanii* (LIMO2) against *P. solenopsis* on cotton during 2015-2016. The cotton (var. surabhi) was raised in mud pots (30 cm height and 15 cm dia.). In each host plant three plants were maintained. Each treatment was applied to three replicate pots arranged in a complete randomized design (CRD). Pre-treatment observations on *P. solenopsis* population were taken before spraying, while post-treatment observations were taken 3, 7, 10, 15 days after treatment (DAT) in three leaves per plot. Two rounds of application of the treatments were made at 15 days interval. The treatment details are as follows:

All the treatments were imposed @ 20 gm per ml of adjuvant and 10 ml of fungal preparation.

All applications were made using hand sprayer during early morning to take advantage of cool and calm conditions. The per cent mortality of *P. solenopsis* was corrected with that in control as suggested by Henderson and Tilt (1955).

 Table 1: Effect of different solvents, oils, medicinal plant powders and their combinations with Lecanicillium lecanii (LIMO2) against Phenacoccus solenopsis in laboratory.

		Per cent corrected Cumulative data of 7 Days after treatment*											
	T1	T2	Т3	T4	T5	T6	T7	T8	Т9	T10			
Treatment	Clove oil +	Sheekai +	Lemongrass	Surf powder	Citronella oil	Castor oil +	Neem oil +	Eucalyptus oil	Chloroform	Acetone +			
	L. lecanii	L. lecanii	oil+ L. lecanii	+ L. lecanii	+ L. lecanii	L. lecanii	L. lecanii	+ L. lecanii	+ L. lecanii	L. lecanii			
Percent corrected													
mortality of	55.17 ^k	68.96 ^g	93.10 ^a	72.41f	89.65 ^b	62.06 ⁱ	86.20 ^c	62.06 ⁱ	51.72 ¹	65.51 ^h			
Phenacoccus	(48.26)	(56.46)	(75.66)	(58.63)	(71.71)	(52.28)	(68.69)	(52.28)	(46.27)	(54.35)			
solenopsis*													
Percent corrected													
mortality of	58.62 ^j	75.86 ^e	79.31 ^d	41.37°	62.06 ⁱ	44.82 ⁿ	65.51 ^h	48.27 ^m	44.82 ⁿ	31.03 ^q			
Phenacoccus	(50.25)	(60.92)	(63.30)	(40.32)	(52.28)	(42.31)	(54.34)	(44.29)	(42.31)	(34.16)			
solenopsis*													
Percent corrected													
mortality of	55.17 ^k	62.06 ⁱ	51.72 ¹	44.82 ⁿ	55.17 ^k	44.82 ⁿ	41.37°	75.86 ^e	65.51 ^h	62.06 ⁱ			
Phenacoccus	(48.25)	(52.28)	(46.27)	(42.31)	(48.25)	(42.31)	(40.32)	(60.93)	(54.34)	(52.28)			
solenopsis*													
Percent corrected													
mortality of	41.37°	48.27 ^m	86.20°	62.06 ⁱ	62.06 ⁱ	51.72 ¹	58.62 ^j	37.93 ^p	41.37°	44.82 ⁿ	$0.00^{\rm r}$		
Phenacoccus	(40.32)	(44.29)	(68.66)	(52.28)	(52.28)	(46.27)	(50.25)	(38.31)	(40.32)	(42.32)	(4.05)		
solenopsis*								. ,	. ,				
					SEd		CD (P = 0.05)						
					0.9148		1.8198						

*Mean of three replications; significant at 1%; figures in parentheses are arc sin transformed value; in a column, means followed by a common letter(s) are not significant different by DMRT (P=0.05)

Table 2: Effect of wax dissolving agents and their combinations with Lecanicillium lecanii (LIMO2) against Phenacoccus solenopsis in pot culture.

		РТС	Percent corrected mortality of Phenacoccus solenopsis*							
Treatment	Dose (g or ml per litre)		First spray				Second spray			
Treatment			Days after treatment				Days after treatment			
			3	7	10	15	3	7	10	15
I = 108 = 100) 20 + 10	10.13	45.39 ^a	69.07 ^a	86.84 ^a	85.33 ^a	44.07 ^a	67.76 ^a	86.18 ^a	88.08 ^a
Lemongrass on + L. lecanti (1 x 10° spores per mi)			(42.64)	(56.52)	(69.16)	(67.94)	(41.88)	(55.72)	(68.61)	(70.29)
Citropalle oil + $I_{accentri}$ (1 x 10 ⁸ spores per ml)	20 ± 10	12.66	40.52 ^b	66.31ª	84.73ª	83.47ª	38.42 ^b	64.73 ^b	85.78 ^a	86.75 ^a
Chronena on $+ L$, <i>lecanti</i> (1 x 10 ⁺ spores per nii)	20 + 10		(39.83)	(54.83)	(67.42)	(66.47)	(38.59)	(53.87)	(68.30)	(69.09)
Castor scop + $L_{1aaanii}$ (1 x 10 ⁸ spores per ml)	20 + 10	11.06	37.95°	62.04b ^c	79.51 ^b	79.25 ^b	35.54 ^c	61.44 ^c	78.31 ^{bc}	80.59 ^b
Castor soap + L. <i>recumi</i> (1 x 10' spores per m)			(38.32)	(52.27)	(63.46)	(63.28)	(36.89)	(51.91)	(62.59)	(64.25)
Norm oil $\downarrow I$ loggiti (1 x 108 spores per ml)	20 + 10	10.53	36.70 ^c	63.29 ^b	80.37 ^b	79.48 ^b	35.44°	62.02 ^c	79.74 ^b	80.25 ^{bc}
Neem on $+ L$. <i>lecanti</i> (1 x 10 ⁺ spores per mi)	20 + 10		(37.58)	(53.01)	(64.08)	(63.45)	(36.83)	(52.25)	(63.62)	(63.98)
Mustard oil $\downarrow L$ leagning (1 x 108 sparses per ml)	20 ± 10	9.73	34.24d ^e	59.58 ^c	75.34°	75.71 ^b	33.56 ^d	58.90 ^d	76.71 ^{cd}	77.94 ^{cd}
Mustard of $+L$. <i>tecunit</i> (1 x 10 ⁻ spores per mi)	20 ± 10		(36.12)	(50.82)	(60.59)	(60.84)	(35.70)	(50.42)	(61.49)	(62.33)
Pup com oil + L loggnii (1 + 108 cnored nor ml)	20 + 10	9.26	32.37 ^d	54.67 ^d	72.66 ^c	69.39 ^c	31.65 ^e	53.95 ^e	74.10 ^d	75.38 ^e
Fungani on $+ L$. <i>tecunit</i> (1 x 10 ^{\circ} spores per nii)			(34.98)	(47.97)	(58.80)	(56.73)	(34.54)	(47.55)	(59.75)	(60.59)
A corres colomus cil + L locarii (1 x 10 ⁸ spores/ml)	20 + 10	8.46	31.49 ^e	55.90 ^d	73.22 ^c	69.69 ^c	30.70 ^e	55.11 ^e	74.80 ^d	75.73 ^{de}
Acarus caranius on $+ L$. <i>recunit</i> (1 x 10 spores/iii)			(34.45)	(48.68)	(59.17)	(56.92)	(33.96)	(48.22)	(60.21)	(60.82)
Control		10.46	0.00^{f}	0.00 ^e	0.00 ^d	0.00 ^d	0.00^{f}	0.00^{f}	0.00 ^e	0.00^{f}
Control			(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
SEd±			0.5334	0.8378	1.0263	1.3746	0.5122	0.5511	0.960	0.7961
CD (P = 0.05)			1.1441	1.7971	2.2014	2.9485	1.0988	1.1820	1.9435	1.7076

Dose: 1×10^8 conidia per ml; PTC: Pre Treatment Count (Mean of *P. solenopsis* per 5 cm twig); *Mean of three replications; significant at 1% level; figures in parentheses are arc

Table 3: Assess the synergistic or antagonistic effect of wax dissolving agents on the efficacy of Lecanicillium lecanii in laboratory conditions

Treatment	Regression	Calculated	LT ₅₀	Fiducia	l limits	LT95	Fiducial limits	
I reatment	equation	χ^2	(Hours)	Lower limit	Upper limit	(Hours)	Lower limit	Upper limit
Clove oil + L. lecanii	y = 1.65x + 1.52	0.3457	153.61	122.53	192.57	406.40	208.07	793.77
Sheekai + L. lecanii	y = 1.85x + 1.37	0.1404	126.28	104.60	152.45	345.40	187.97	634.06
Lemongrass oil+ L. lecanii	y = 3.53x - 1.05	0.3290	80.92	63.24	103.54	200.67	147.43	273.12
Surf powder + L. lecanii	y = 2.16x + 0.82	0.3368	114.36	96.47	135.58	288.41	17935	463.81
Citronella oil + L. lecanii	y = 2.99x - 0.41	0.1494	82.56	63.80	106.85	219.55	151.28	318.62
Castor oil + L. lecanii	y = 1.57x + 1.79	0.0318	140.87	113.34	175.08	402.39	196.48	824.06
Neem oil + L. lecanii	y = 2.80x - 0.11	0.5920	87.74	69.88	110.18	225.40	155.54	326.63
Eucalyptus oil + L. lecanii	y = 1.55x + 1.83	0.3164	144.82	115.52	181.56	415.66	198.77	869.21
Chloroform + L. lecanii	y = 1.38x + 1.99	0.0003	170.32	128.01	226.61	490.79	213.65	1127.39
Acetone + L. lecanii	y = 1.80x + 1.36	0.1410	135.76	111.18	164.74	359.45	195.59	660.57
Soap oil + <i>L. lecanii</i>	y = 1.61x + 1.67	0.0419	147.34	118.07	183.87	404.46	202.39	808.25
Pungam oil + L. lecanii	y = 2.05x + 1.26	0.1567	93.14	72.85	119.09	281.99	161.65	491.93
Mustard oil + L. lecanii	y = 2.37x + 0.66	0.0395	92.46	73.51	116.30	257.31	161.17	410.80

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Mahua oil + <i>L. lecanii</i>	y = 1.21x + 2.11	0.0295	212.87	143.71	315.29	621.90	234.56	1648.86
Gingelly oil + L. lecanii	y = 1.61x + 1.76	0.0423	137.05	111.12	169.03	389.65	194.21	781.18
Rose water + L. lecanii	y = 1.17x + 2.29	0.0292	199.79	137.29	290.74	607.28	224.02	1646.17
Sunflower oil + L. lecanii	y = 1.59x + 1.86	0.0316	130.43	105.91	160.63	386.99	188.20	795.76
Solanum trilobatum + L. lecanii	y = 1.41x + 1.85	0.0003	181.92	134.33	246.38	505.70	222.88	1147.41
Hibiscus powder + L. lecanii	y = 1.45x + 1.67	0.0002	193.47	140.55	266.32	520.09	232.08	1165.27
Amla powder + L. lecanii	y = 1.11x + 2.10	0.0006	259.34	157.56	426.89	764.62	254.35	2298.54
Cynodon dactylon powder + L. lecanii	y = 1.11x + 2.66	0.0284	160.04	117.60	217.78	558.76	192.22	1624.27

3. Results and discussions

3 Evaluation of synergism / antagonism in the efficacy of entomopathogenic fungi against selected mealybugs through co-administration with some adjuvants under both laboratory and pot conditions

3.1.1 Effect of different solvents, oils, medicinal plant powders and their combination with *Lecanicillium lecanii* (LIMO2) for the control of *Phenacoccus solenopsis* in lab conditions.

Forty one different treatments were tested against *P. solenopsis.* Among the treatments, lemongrass oil in combination with *L. lecanii* (LIMO2) recorded the highest mortality of 93.10 per cent at 7 DAT, followed by citronella oil in combination with *L. lecanii* (LIMO2) showed the mortality of 89.65 per cent at 7 DAT and neem oil in combination with *L. lecanii* (LIMO2) and castor soap in combination with *L. lecanii* (LIMO2) recorded the mortality of 86.20 per cent at 7 DAT. Mustard oil with *L. lecanii* (LIMO2) recorded the mortality of 86.20 per cent at 7 DAT. Mustard oil with *L. lecanii* (LIMO2) recorded a mortality of 79.31 per cent and both pungam oil with *L. lecanii* (LIMO2) and sweet flag (*Acarus calamus*) oil with *L. lecanii* (LIMO2) recorded the mortality of 75.86 per cent at 7 DAT (Table 1).

3.2 Effect of wax dissolving agents and their combination with *Lecanicillium lecanii* (LIMO2) for the control of *Phenacoccus solenopsis* in pot conditions.

Based on the above results, treatments were designed to carry out the pot experiment (Location: Insectary, Crop: Cotton) to assess the efficacy of oils in combination with entomopathogenic fungi.

3.2.1 Lemongrass oil + L. lecanii (1 x 10⁸ spores per ml)

In the lemongrass oil + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and

15 days of first spraying, mortalities of 45.39 per cent, 69.07 per cent, 86.84 per cent and 85.33 per cent were recorded, respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 44.07 per cent, 67.76 per cent, 86.18 per cent and 88.08 per cent were recorded, respectively (Table 2) (Plate 1).

3.2.2 Citronella oil + L. lecanii (1 x 10⁸ spores per ml)

In the citronella oil + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and

15 days of first spraying, mortalities of 40.52 per cent, 66.31 per cent, 84.73 per cent and 83.47 percent were recorded, respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 38.42 per cent, 64.73 per cent, 85.78 per cent and

86.75 per cent were recorded, respectively (Table 2) (Plate 1).

3.2.3 Neem oil + L. lecanii (1 x 10⁸ spores per ml)

In the neem oil + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and 15 days of first spraying, mortalities of 36.70 per

cent, 63.29 per cent, 80.37 per cent and

79.48 percent were recorded, respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 35.44 per cent, 62.02 per cent, 79.74 per cent and

80.25 per cent were recorded, respectively (Table 2) (Plate 1).





Neem oil + L. lecanii

Plate 1: Mealy bug affected due to combination of oil with L. lecanii

3.2.4 Mustard oil + L. lecanii (1 x 10⁸ spores per ml)

In the mustard oil + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and 15 days of first spraying, mortalities of 34.24 per cent, 59.58 per cent, 75.34 per cent and 75.71 percent were recorded. respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 33.56 per cent, 58.90 per cent, 76.71 per cent and 77.94 per cent were recorded, respectively (Table 2).

3.2.5 Pungam oil + *L. lecanii* (1 x 10⁸ spores per ml)

In the pungam oil + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and 15 days of first spraying, mortalities of 32.37 per cent, 54.67 per cent, 72.66 per cent and 69.39 percent were recorded, respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 31.65 per cent, 53.95 per cent, 74.10 per cent and 75.38 per cent were recorded, respectively (Table 2).

3.2.6 Sweetflag oil + L. lecanii (1 x 10⁸ spores per ml)

In the Sweetflag (*Acarus calamus*) + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and 15 days of first spraying, mortalities of 31.49 per cent, 55.90 per cent,

73.22 per cent and 69.69 percent were recorded, respectively. After 3 days, 7 days,

10 days and 15 days of second spraying, mortalities of 30.70 per cent, 55.11 per cent, 74.80 per cent and 75.73 per cent were recorded, respectively (Table 2).



Fig 1: Effect of wax dissolving agents and their combinations with Lecanicillium lecanii (LIMO2) against P. solenopsis in pot cluture

3.2.7 Castor soap + L. lecanii (1 x 10⁸ spores per ml)

In the castor soap + *L. lecanii* treated pots, after 3 days, 7 days, 10 days and 15 days of first spraying, mortalities of 37.95 per cent, 62.04 per cent, 79.51 per cent and 79.25 percent were recorded, respectively. After 3 days, 7 days, 10 days and 15 days of second spraying, mortalities of 35.54 per cent, 61.44 per cent, 78.31 per cent and

80.59 per cent were recorded, respectively (Table 2).

As per the results obtained, lemongrass oil, citronella oil, neem oil, pungam oil, mustard oil, sweet flag oil and castor soap shows synergistic effect with *Lecanicillium lecanii* against meaybugs. (Table 3).

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