

Bioefficacy and Phytotoxicity Evaluation of *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) Against Mealy Bugs (*Planococcus citri*) on Citrus (Acid lime)

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ABSTRACT

The studies were taken to compare the efficacy and phytotoxicity of bioagent *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) against mealy bugs, *Planococcus citri* on Citrus (Acid lime). Out of seven treatment. Quinalphos 25% EC @ 1400 ml/ha (0.07%) and *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) treatments @ 3000 and 2500 g/ha were effective to control mealy bugs on citrus. All the treatments were non phytotoxic to citrus plants and non toxic to natural enemies. *Verticillium lecanii* 1.15% WP applied @ 2500 g/ha dose was optimum to control mealy bugs on citrus (acid lime). Based on the results the use of *Verticillium lecanii* 1.15% WP @ 2500 g/ha is suggested for the effective management of mealy bugs on citrus.

Key words: *Verticillium lecanii*, Bioefficacy, Phytotoxicity, mealy bugs (*Planococcus citri*) and Citrus.

INTRODUCTION

The productivity and quality of citrus is severely affected by several factors, insect pests being one of them. Among them mealy bug (*Planococcus citri*) is causing large scale damage regularly to citrus cultivar grown across India. Citrus mealy bug is one of them most destructive pest of citrus spp. in India. The citrus mealy bug has been controlled by various chemical pesticides. The insecticides have been found more or less toxic to a number of parasitoids and predators and also to pollinators^{2,3}. The problems of chemicals pesticides resistance as well as the consumer

health and environmental hazards associated with pesticide residues in plant materials (at fresh fruit consumption), have focused attention on alternative methods. *Verticillium lecanis* invades insects by penetrating the cuticle and the fungus rapidly multiplied throughout the body, causes by tissue destruction and occasionally, by toxin produced by the fungus. The study was taken with an objective to determine the bioefficacy and Phytotoxicity evaluation of Biopesticides, Insecticides and Neem formulation against mealy bug (*Planococcus citri*) on citrus crop.

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MATERIALS AND METHOD

Experiment was conducted at farmers field of Village - Manpura, Block – Sehoe (M.P.) during kharif season 2013 and 2014 in randomized block design replicated four times. One plant was taken as one replication. The application of treatments started on appearance of symptoms of pest incidence in the experimental plants. The Citrus *var.* Kagzi Lime. was used in First season–21.06.2013 and 06.07.2013, Second season -12.06.2014 and 27.06.2014 in orchard. The age of plants were taken 3 – 5 years old and each plot measured 20 m² with spacing 4 m x 5. The application of treatments started on appearance of symptoms of pest damage in the experimental crops with seven treatment including control *viz.* *Verticillium lecanii* (1.15% WP) 2000 g, *Verticillium lecanii* 1.15% WP) 2500 g, *Verticillium lecanii* (1.15% WP) 3000 g, Neem oil based EC containing Azadirachtin 0.03% 2500 ml, Quinalphos 25% EC 1400 ml (0.07%), *Verticillium lecanii* 1.15% WP (for phytotoxicity evaluation only) 5000 g. Bioagent *Verticillium lecanii*, Neem oil and Quinalphos 25% EC were obtained from Maharashtra Biocontrol Manufacturers Association. All treatments were sprayed on citrus crop using spray volume @ 500 lit/ha by knapsack sprayer fitted with hollow cone nozzle twice at an interval of 15 days.

Treatment details:

T₁- *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) 1.15% WP @ 2000 g/ha

T₂- *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) 1.15% WP @ 2500 g/ha

T₃- *Verticillium lecanii* 1.15% WP (1 x 10⁸ cfu/g min.) @ 3000 g/ha

T₄-Neem oil based EC containing Azadirachtin 0.03% @ 2500 ml/ha

T₅- Quinalphos 25% EC @ 1400 ml/ha(0.07%)

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T₆- control untreated water

Effect of *Verticillium lecanii* 1.15% WP and others against mealy bugs on citrus-

Pre treatment observations on number of mealy bugs were recorded one day before first spray on randomly selected and tagged 10 twigs per plant. Post treatment observations were recorded after 7 and 14 days of each spray on tagged 10 twigs per plant.

Based on the data per cent reduction in mealy bug population over control was calculated. The dead insects were collected from the treated plants to confirm the insect mortality due to *Verticillium lecanii*. The collected insects were kept in clean and autoclaved Petri dishes and observed under laboratory conditions for 7 days minimum.

Effect of Phytotoxicity of *Verticillium lecanii* and others 1.15% WP on citrus-

To observe phytotoxicity to citrus plants due to the application of *Verticillium lecanii* 1.15% WP a higher dose 5000 g/ha was also used. Observations were recorded visually for the phytotoxicity parameters *viz.*, leaf injury on tips/ surface, wilting, vein clearing, necrosis, epinasty and hyponasty after 1, 3, 7, 10 and 14 days of each application of treatments.

Effect of *Verticillium lecanii* 1.15% WP and others on the natural enemies on citrus-

To record the effect of *Verticillium lecanii* 1.15% WP on the natural enemies, observations were made for the natural enemies prevailing in the crop ecosystem. The population count of spiders and Coccinellids (dominant natural enemies) was recorded on randomly selected 10 twigs per plant before first application and 7 and 14 days after each application of treatments.

RESULTS DISCUSSION

Effect of *Verticillium lecanii* 1.15% WP and others against mealy bugs on citrus

The population of mealy bugs recorded during two

seasons(2013 and 2014) has been presented in Tables 1 . Pre spray population showed that the pest was uniformly established in all the experimental plants and the difference was non-significant. The mealy bug population significantly declined in various treatments as compared to control at each observation time period. Quinalphos 25% EC a chemical insecticide showed better efficacy than *Verticillium lecanii* 1.15% WP and neem oil based EC containing Azadirachtin 0.03%. However, *Verticillium lecanii* 1.15% WP @ 2500 and 3000 g/ha was superior to neem oil based EC containing Azadirachtin 0.03% @ 2500 ml/ha. *Verticillium lecanii* 1.15% WP @ 3000 g/ha was statistically equally effective to its lower dose @ 2500 g/ha. Singh et al (2011) reported that freshly prepared NSKE(4%) and nembecidine (0.2%)gave significant reductions in mealy bug population.

The percent reduction in pest population over control was also calculated out and presented in Tables 2. The results revealed that all the treatments effectively controlled the pest population, where *Verticillium lecanii* 1.15% WP @ 3000 and 2500 g/ha was more effective than its lower dose @ 2000 g/ha. Neem oil based EC containing Azadirachtin 0.03% @ 2500 ml/ha was also better than *Verticillium lecanii* 1.15% WP @ 2000 g/ha. Considering the effectiveness of *Verticillium lecanii* 1.15% WP @ 3000 g/ha and 2500 g/ha, it is stated that 2500 g/ha dose of the product is optimum to control the mealy bugs in citrus plants.

During both the seasons at the time of experiment, the citrus fruit size was small and also all the plants were not bearing the fruits. The yield data therefore, have not been recorded.

The whitish mycelium growth of fungus, *Verticillium lecanii* on dead insects collected from *Verticillium lecanii* 1.15% WP treated

plants confirmed the mortality due to the application of *Verticillium lecanii* 1.15% WP.

Effect of Phytotoxicity of *Verticillium lecanii* and others 1.15% WP on citrus- The observations recorded visually for the phytotoxicity symptoms after 1, 3, 7, 10 and 14 days after each spray have been presented in Tables 3. The data showed that there was no phytotoxicity to citrus plants when *Verticillium lecanii* 1.15% WP applied up to a level of 5000 g/ha. Hence *Verticillium lecanii* 1.15% WP formulation was non phytotoxic to citrus plants.

Effect of *Verticillium lecanii* 1.15% WP and others on the natural enemies on citrus- The natural enemies population prevailing in the crop ecosystem recorded before first spray and after 7 and 14 days of each spray during both the seasons have been presented in Tables 4. Pre spray population of spiders and Coccinellids observed was more or less uniform in various treatments and the difference was non significant. After application of treatments population of predators declined to some extent but quinalphos showed greater adverse effect. The differences in population was non significant at most of the occasions. A decline in predator population on treated plants may be attributed to the fact that reduction in pest population forced predators to move out to search food. Jyoti and Goud⁶ reported that microbial pesticides (spinosad 45SC, emamctin benzoate 5SG, avermectin 1.9 EC and *Btk*5%) did not affect the natural enemies population (coccinellids, chrysopids and spiders) throughout the crop growth period in brinjal. ecosystem Rajak and Singh⁴ found neem powder @20kg/ha to be effective against *A. foveicollis* infesting muskmelon. Singh and Yadav⁵ also found the efficacy of insecticides biopesticides and neem formulations against *Helicoverpa armigera* on chick pea.

Table 1: Effect of *Verticillium lecanii* 1.15% WP and others against mealy bugs on citrus during 2013 and 2014

S. No.	Treatment	Dose formulation/ha	Mealy bugs population/10 twigs 2013					Mealy bugs population/10 twigs 2014				
			Pre spray	After first spray		After second spray		Pre spray	After first spray		After second spray	
				7 days	14 days	7 days	14 days		7 days	14 days	7 days	14 days
1.	<i>Verticillium lecanii</i> 1.15% WP	2000 g	42.75 (6.54)	31.00 (5.57)	23.50 (4.85)	61.25 (7.83)	19.25 (4.39)	61.25 (7.83)	52.00 (7.21)	45.75 (6.76)	31.25 (5.59)	27.50 (5.24)
2.	<i>Verticillium lecanii</i> 1.15% WP	2500 g	40.25 (6.34)	25.50 (5.05)	18.00 (4.24)	58.75 (7.66)	12.00 (3.46)	58.75 (7.66)	43.75 (6.61)	30.75 (5.55)	21.50 (4.64)	18.25 (4.27)
3.	<i>Verticillium lecanii</i> 1.15% WP	3000 g	38.50 (6.20)	23.50 (4.85)	17.50 (4.18)	60.75 (7.79)	11.25 (3.35)	60.75 (7.79)	41.25 (6.42)	28.00 (5.29)	19.75 (4.44)	17.00 (4.12)
4.	Neem oil based EC containing Azadirachtin 0.03%	2500 ml	43.75 (6.61)	34.25 (5.85)	31.75 (5.63)	67.00 (8.19)	21.25 (4.61)	67.00 (8.19)	56.25 (7.50)	48.25 (6.95)	33.75 (5.81)	25.50 (5.05)
5.	Quinalphos 25% EC	1400 ml	37.25 (6.10)	15.00 (3.87)	10.25 (3.20)	55.75 (7.47)	6.25 (2.50)	55.75 (7.47)	29.25 (5.41)	18.00 (4.24)	13.25 (3.64)	10.00 (3.16)
6.	Control	–	41.50 (6.44)	48.75 (6.98)	66.25 (8.14)	62.50 (7.91)	67.25 (8.20)	62.50 (7.91)	71.75 (8.47)	76.75 (8.76)	85.25 (9.23)	79.50 (8.92)
	S.E. ±		(0.37)	(0.23)	(0.19)	(0.45)	(0.27)	(0.45)	(0.24)	(0.22)	(0.29)	(0.31)
	CD at 5%		(NS)	(0.69)	(0.58)	(NS)	(0.81)	(NS)	(0.72)	(0.66)	(0.88)	(0.92)

NS = Non significant

Figures in parentheses are square root transformed values

Table 2: Per cent reduction in mealy bugs population over control in citrus during 2013 and 2014

S. No.	Treatment	Dose formulation/ha	Per cent reduction in mealy bugs population over control 2013					Per cent reduction in mealy bugs population over control 2014				
			After first spray		After second spray		Mean	After first spray		After second spray		Mean
			7 days	14 days	7 days	14 days		7 days	14 days	7 days	14 days	
1.	<i>Verticillium lecanii</i> 1.15% WP	2000 g	36.41 (37.11)	64.53 (53.45)	73.50 (59.02)	71.38 (57.66)	61.46 (51.63)	27.53 (31.65)	40.39 (39.46)	63.34 (52.74)	65.41 (53.98)	49.17 (44.52)
2.	<i>Verticillium lecanii</i> 1.15% WP	2500 g	47.69 (43.68)	72.83 (58.58)	80.92 (64.10)	82.16 (65.02)	70.90 (57.35)	39.02 (38.66)	59.93 (50.73)	74.78 (59.85)	77.04 (61.37)	62.69 (52.35)
3.	<i>Verticillium lecanii</i> 1.15% WP	3000 g	51.79 (46.03)	73.58 (59.07)	84.10 (66.50)	83.27 (65.86)	73.19 (58.82)	42.51 (40.69)	63.52 (52.84)	76.83 (61.23)	78.62 (62.46)	65.37 (53.95)
4.	Neem oil based EC containing Azadirachtin 0.03%	2500 ml	29.74 (33.05)	52.08 (46.19)	62.54 (52.26)	68.40 (55.80)	53.19 (46.83)	21.60 (27.70)	37.13 (37.54)	60.41 (51.01)	67.92 (55.50)	46.77 (43.15)
5.	Quinalphos 25% EC	1400 ml	69.23 (56.31)	84.53 (66.84)	87.63 (69.41)	90.71 (72.25)	83.03 (65.67)	59.23 (50.32)	76.55 (61.04)	84.46 (66.78)	87.42 (69.23)	76.92 (61.29)
6.	Control	–	-	-	-	-	-	-	-	-	-	-
	S.E. ±		(1.17)	(0.97)	(1.12)	(1.27)	(1.08)	(1.01)	(1.16)	(0.94)	(1.19)	(0.87)
	CD at 5%		(3.61)	(2.99)	(3.46)	(3.92)	(3.33)	(3.12)	(3.58)	(2.90)	(3.67)	(2.68)

Figures in parentheses are angular transformed values

Table 3: Effect of Phytotoxicity of *Verticillium lecanii* and others 1.15% WP on citrus during 2013 and 2014

S. No.	Treatment	Dose Formulation/ ha	Phytotoxicity parameters observed (Mean observations recorded after 1, 3, 7, 10 and 14 days of each spray) (2013)						Phytotoxicity parameters observed (Mean observations recorded after 1, 3, 7, 10 and 14 days of each spray) (2014)					
			Leaf injury on tips/ surface*	Wilting	Vein clearing	Necrosis	Epinasty	Hyponasty	Leaf injury on tips/ surface*	Wilting	Vein clearing	Necrosis	Epinasty	Hyponasty
			1.	<i>Verticillium lecanii</i> 1.15% WP	2000 g	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil
2.	<i>Verticillium lecanii</i> 1.15% WP	2500 g	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
3.	<i>Verticillium lecanii</i> 1.15% WP	3000 g	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
4.	Neem oil based EC containing Azadirachtin 0.03%	2500 ml	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
5.	Quinalphos 25% EC	1400 ml	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
6.	Control	–	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
7.	<i>Verticillium lecanii</i> 1.15% WP	5000 g	1	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil

* Scale 1-10% = 1, 11-20% = 2, 21-30% = 3, 31-40% = 4, 41-50% = 5, 51-60% = 6, 61-70% = 7, 71-80% = 8, 81-90% = 9, 91-100% = 10

Table 8: Effect of *Verticillium lecanii* 1.15% WP on natural enemies on citrus plants during 2013 and 2014

S. No.	Treatment	Dose Formulation/ha	Mean population of natural enemies/ 10 twigs(in 2013)										Mean population of natural enemies/ 10 twigs(2014)									
			Spiders				Coccinellids						Spiders				Coccinellids					
			Pre	7 DAFS	14 DAFS	7 DASS	14 DASS	Pre spray	7 DAFS	14 DAFS	7 DASS	14 DASS	Pre spray	7 DAFS	14 DAFS	7 DASS	14 DASS	Pre spray	7 DAFS	14 DAFS	7 DASS	14 DASS
1.	<i>Verticillium lecanii</i> 1.15% WP	2000 g	2.50	2.25	2.75	1.50	1.25	2.50	1.75	2.00	1.75	1.00	1.25	1.25	1.75	2.00	1.50	2.25	1.75	1.75	2.00	1.50
2.	<i>Verticillium lecanii</i> 1.15% WP	2500 g	3.75	2.75	3.00	2.00	0.75	2.75	2.00	1.75	1.50	0.75	2.00	1.50	1.50	1.25	1.00	2.50	2.00	1.25	1.50	1.00
3.	<i>Verticillium lecanii</i> 1.15% WP	3000 g	2.75	2.25	2.75	1.50	1.00	1.75	2.00	1.25	1.00	0.50	1.75	1.25	1.75	1.00	0.75	2.75	2.25	1.50	1.00	0.50
4.	Neem oil based EC containing Azadirachtin 0.03%	2500 ml	3.50	3.00	2.50	2.25	1.50	2.25	1.75	2.25	2.50	1.50	1.25	1.50	2.25	3.25	1.75	2.00	2.25	1.75	2.25	1.25
5.	Quinalphos 25% EC	1400 ml	3.25	2.25	1.75	1.00	0.25	2.00	1.25	1.00	0.50	0.25	2.25	1.00	0.75	0.25	0.50	1.75	1.00	0.75	1.00	0.50
6.	Control	–	3.00	3.50	3.75	3.50	3.00	2.75	3.00	3.50	3.25	2.25	1.75	2.25	2.75	2.50	3.25	1.75	2.50	2.75	2.25	1.75
	S.E. ±		0.53	0.58	0.27	0.36	0.28	0.54	0.52	0.26	0.55	0.27	0.49	0.53	0.55	0.23	0.28	0.58	0.51	0.31	0.56	0.24
	CD at 5%		NS	NS	0.82	1.10	0.85	NS	NS	0.79	NS	0.82	NS	NS	NS	0.70	0.85	NS	NS	0.93	NS	0.73

DAFS – Days after first spray DASS – Days after second spray NS – Non significant

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