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Research Note

Effect of combination of adjuvants on liquid formulations of *Verticillium lecanii* (Zimmermann) Viegas and their efficacy*

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ABSTRACT: Effect of some adjuvant combinations on the growth of *Verticillium lecanii* (Zimmermann) Viegas in culture medium and the subsequent infection of *Maconellicoccus hirsutus* (Green) were evaluated at Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, during 2002-04. The treatment including *V. l.* + glycerol (8%) + Tween 80 (1%) + arachid oil (5%) proved to be the most promising, recording maximum surface coverage (92.00%) and biomass (32.10g) at 10 days of inoculation and bioefficacy at 14 days. However, it was on par with the formulations containing *V. l.* + glycerol 5% + Tween 80 1% + arachid oil 2% and *V. l.* + glycerol 2% + Tween 80 1% + arachid oil 0.5%. In terms of viability and virulence, *V. l.* + glycerol 2% + Tween 80 1% + arachid oil 0.5% and *V. l.* + glycerol 5% + Tween 80 1% + arachid oil 2% emerged as the best combinations.

KEY WORDS: Adjuvants, arachid oil, glycerol, *Maconellicoccus hirsutus*, tween-80, *Verticillium lecanii* viability and virulence.

Verticillium lecanii (Zimmermann) Viegas (Moniliales: Moniliaceae) is a cosmopolitan fungal pathogen of scale insects, whitefly, aphids, larvae of elm bark beetle and colorado potato beetle (Barson, 1976; Hall and Papierok, 1982; Kanagaratnam *et al.,* 1982). So, considering the ecofriendly benefits of biological control, a strain of *V. lecanii* was isolated from spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleurodidae) at the Biocontrol Research Laboratory of Department of Entomology, M. P. K. V., Rahuri. A wettable powder formulation of this strain of *V. lecanii* was developed and branded as Phule Bugicide (Kadam and Jaichakravarthy, 2003). The bioefficacy of this formulation in the laboratory and the farmer's fields was highly encouraging. So, it was felt necessary to develop a liquid formulation of this mycoagent.

Various adjuvants have been used in the formulation of *V. lecanii*. Infection of insects by *V. lecanii* through direct contact with spores from sprays or sprayed leaves can be very low and epizootics usually result from insects being infected directly by aerial conidia from sporulating cadavers or conidia on foliage (Hall, 1976, 1979, 1982; Hall and Burges, 1979). There are many examples where fungi have been formulated with various adjuvants. The addition of nutrients (of unknown composition) to a spore spray did improve the control of aphids and whiteflies in greenhouse cucumber, compared with spores applied in water alone (Hall, 1982). Humectants prolong the viability of *Alternaria cassiae* Jurair and Khan, a fungal pathogen of sicklepod, *Cassia abtusifolia* L. (Shabana *et al*., 1977). *V. lecanii* formulated with arachid oil showed significantly better control of powdery mildew than without the oil (Verhaar *et. al.,* 1999). Use of glycerol as an adjuvant improved the efficacy of spore sprays of *V. lecanii*. In the present study, a range of adjuvants (humectants, nutrients, emulsifiers and vegetable oils, etc.) were screened for their growth and development of *V. lecanii* on culture medium. The efficacy of *V. lecanii* with various adjuvants was also evaluated against *Maconellicoccus hirsutus* (Green) in the laboratory.

The study was carried out at the Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, during 2002-04. The pure fungus culture isolated in 1999 from spiralling whitefly, *A. dispersus,* infesting guava, was available in the Biocontrol Research Laboratory. It was incubated at 21 ± 1°C. The medium used was potato-dextrose broth medium as suggested by Kadam and Jaichakravarthy (2003). Grape mealy bug, *M. hirsutus,* used in the study was collected

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Table 1. Effect of adjuvants in liquid formulations of *V. lecanii* on its growth and development in medium

Treatments*	Surface area covered (%) by *V. lecanii* growth days after treatment			Biomass on 10th day grams-40ml medium
	3	7	10	
V.l. + G 2 + A 0.5	26.80	55.30	81.00	21.20
V.l. + G 5 + A 2	28.50	57.00	83.10	23.60
V.l. + G 8 + A 5	31.70	58.70	85.50	25.70
V.l. + T 80 1 + A 0.5	22.40	52.30	72.30	15.80
V.l. + T 80 1 + A 2	23.30	52.00	74.20	16.20
V.l. + T 80 1 + A 5	24.60	54.10	75.00	18.60
V.l. + G 2 + T 80 1 + A 0.5	28.50	60.40	88.60	31.50
V.l. + G 5 + T 80 1 + A 2	31.20	62.10	91.20	32.20
V.l. + G 8 + T 80 1 + A 5	32.40	63.80	92.00	32.50
V.l. + A 0.5	24.00	55.00	74.60	23.10
V.l. + A 2.0	26.00	57.20	77.90	25.00
V.l. + A 5.0	29.20	61.50	82.10	28.40
V.l. + G 2.0	24.50	56.30	78.00	21.50
V.l. + G 5.0	27.20	61.80	79.20	24.30
V.l.+G 8.0	30.30	62.50	82.30	26.10
V.l.+T 80 1.0	21.70	50.80	72.00	17.00
U.C (*V.l.only)*	15.50	42.50	65.00	18.00
SEM ±	1.02	2.02	1.19	1.07
P = 0.05	2.93	5.82	3.43	3.22

* *V.l. = Verticillium lecanii*; T 80-1 = Tween-80 1%; G2 = Glycerol 2%; G5= Glycerol 5%; G8 = Glycerol 8%; A 0.5 = Arachid oil 0.5%; A 2.0 = Arachid oil 2%; A 5 = Arachid oil 5%

from infested fields and reared in the laboratory on sprouted potatoes. First instar mealy bug nymphs were used for the study. The adjuvants used in the study were glycerol, Tween 80 and arachid oil.

The adjuvants in combination were added to the liquid culture of *V. lecanii* and incubated at ambient temperature. The resultant formulations were tested with aqueous suspension of inoculum for growth and development and bioefficacy against *M. hirsutus*. For this, the optimum spore load (10×10^8 CFUml^{-1}) of *V. lecanii* was taken in sterilized 250ml conical flasks and the required concentration of the adjuvant was added to the liquid suspension. The conical flask was then closed with cotton wool and incubated at ambient temperature. The whole process was carried out in a laminar flow cabinet.

One ml each of the formulated liquid was added individually to 40ml potato-dextrose broth medium as inoculant in 500ml capacity conical flask. It was then incubated at 21 ± 1°C for 10 days. The whole process was carried out in a laminar flow cabinet. The observations on the surface area covered (%) and biomass production (g) were noted by visual observations in a completely randomized design replicated thrice. The experimental data were then analyzed statistically.

The bioefficacy against *M. hirsutus* was tested in a completely randomized design with three replications. Thirty mealy bug nymphs (1st instar) were released on each sprouted potato by a soft hair brush. One ml of each formulation was diluted in 99ml of water and sprayed on mealy bug. The observations on mortality were taken for up to 14 days after treatment. The data were first corrected by Abbott's formula (Abbott, 1925) and then subjected to arc sin square root transformation to improve homogeneity (Gomez and Gomez, 1984) before statistical analysis.

Observations on surface area covered (%) at 3 days after treatment revealed that all treatment combinations were significantly superior (21.70 to 32.40%) to *V. lecanii* alone (15.50%) for coverage of surface growth (Table 1). Treatment 9 (*V. l.* + Glycerol 8% + Tween 80 1% + Arachid oil 5%) proved to be the best by recording maximum surface coverage (32.40%). However, T8 (*V. l.* + Glycerol 5 % + Tween 80 1% + Arachid oil 2%), T3 (*V. l.* + Glycerol 8% + Arachid oil 5%) and T 15 (*V. l.* + Glycerol 8%) were on par with it (30.30-31.70 per cent surface coverage). The growth in the rest of the treatments ranged from 21.70 to 29.20 per cent against that of 15.50 per cent in *V. lecanii* alone. At 7 days post-treatment, T_9 proved to be superior among all the treatments recording 63.80 per cent surface coverage. However, it was on par with treatments

Table 2. Effect of adjuvants in liquid formulations of *V. lecanii* on the mortality of mealybug, *M. hirsutus*

Treatments*	Nymphal mortality (%) at days after treatment					
	1	3	5	7	9	14
V.l. + G 2 + A 0.5	7.5 (15.89)*	16.22 (23.73)	25.84 (30.53)	45.71 (42.53)	58.23 (49.72)	73.49 (59.02)
V.l. + G 5 + A 2	10.0 (18.44)	18.92 (25.77)	29.21 (32.71)	47.42 (43.51)	61.18 (51.47)	77.10 (61.41)
V.l. + G 8 + A 5	12.5 (20.70)	22.70 (28.45)	31.46 (34.14)	50.85 (45.46)	62.35 (52.12)	78.92 (62.65)
V.l. + T 80 1 + A 0.5	5.0 (12.92)	15.68 (23.34)	20.78 (32.90)	40.00 (39.23)	54.12 (47.41)	69.88 (56.73)
V.l. + T 80 1 + A 2	7.5 (15.89)	17.83 (24.95)	23.59 (29.06)	43.43 (41.21)	55.88 (48.39)	72.89 (58.63)
V.l. + T 80 1 + A 5	10.5 (18.91)	18.92 (25.77)	26.96 (31.24)	45.71 (42.53)	57.65 (49.37)	75.30 (60.20)
V.l. + G 2 + T 80 1 + A 0.5	7.5 (15.89)	21.08 (27.35)	29.20 (32.01)	49.36 (42.88)	63.62 (51.47)	81.28 (63.08)
V.l. + G 5 + T 80 1 + A 2	12.5 (20.70)	27.03 (31.31)	31.60 (33.40)	49.60 (44.20)	63.90 (53.91)	81.93 (64.82)
V.l. + G 8 + T 80 1 + A 5	15.0 (22.79)	31.35 (34.02)	32.50 (36.15)	50.50 (46.15)	64.70 (55.30)	82.50 (65.80)
V.l. + A 0.5	5.0 (12.92)	14.59 (22.46)	29.77 (33.09)	36.57 (37.23)	61.76 (51.83)	65.66 (54.15)
V.l. + A 2.0	7.5 (15.89)	15.68 (23.34)	21.91 (27.90)	39.43 (38.88)	53.53 (47.01)	67.46 (55.24)
V.l. + A 5.0	10.0 (18.44)	18.92 (25.77)	23.59 (29.06)	42.28 (40.57)	55.29 (48.04)	71.08 (56.85)
V.l. + G 2.0	7.5 (15.89)	16.76 (24.12)	24.71 (29.80)	42.86 (40.92)	57.65 (49.37)	73.49 (59.02)
V.l. + G 5.0	12.0 (20.27)	20.00 (26.56)	26.97 (31.24)	45.14 (42.19)	59.41 (50.42)	75.30 (60.20)
V.l. + G 8.0	15.5 (23.19)	23.78 (32.90)	31.46 (34.14)	46.28 (42.88)	61.18 (51.47)	77.10 (61.41)
V.l. + T 80 1.0	7.5 (15.89)	11.89 (20.18)	23.59 (29.06)	36.00 (36.87)	49.41 (44.66)	65.06 (53.79)
V. l .only	12.5 (20.70)	15.68 (23.34)	26.97 (31.24)	32.57 (34.82)	43.53 (41.27)	57.83 (49.49)
Untreated control (only water spray)**	0.00	0.00	0.00	0.00	0.00	0.00
SEM ±	0.72	0.81	0.91	1.21	1.15	1.27
P = 0.05	2.18	2.45	2.72	3.65	3.51	3.80

* Figures in parentheses indicate Arcsin transformed values; **The corrected mortality at 3rd, 5th,7th,9thand 14th day using Abbott's formula when actual mortality in untreated control (only water spray) was 7.50,11.00,12.50,15.00 and 17.00 %, respectively; ***V.l. = *Verticillium lecanii*; T 80 1 = Tween-80 1 %; G2 = Glycerol 2%; G5= Glycerol 5%; G8 = Glycerol 8%; A 0.5 = Arachid oil 0.5%; A2 = Arachid oil 2%; A 5 = Arachid oil 5%

8, 7, 12, 14 and 15, which recorded 62.10, 60.40, 61.50, 61.80 and 62.50 per cent growth, respectively. The fungus culture alone covered 42.50 per cent surface area and it was significantly less than that in the rest of the treatments.

At 10 days after inoculation, treatment 9 maintained its superiority over the rest of the treatments, recording 92.00 per cent surface coverage. However, it was on par with treatment 8 (91.20%) and 7 (88.60%). Other combinations

showed 72.0-85.55 per cent coverage of surface area. The fungus alone covered only 65.00 per cent surface of the culture medium and all the combinations were significantly superior to it.

Corresponding observations on biomass produced in gram^{-40ml} liquid medium indicated the superiority of treatment 9 (32.50g biomass). However, it was on par with treatments 8 and 7 (32.20 and 31.50g biomass, respectively). Other combinations developed 15.80 to 28.40g biomass. The fungus culture without any adjuvant produced 18.00g biomass.

The effectiveness of glycerol as an adjuvant in *V. lecanii* formulations was reported earlier by Santharam *et al.* (1977) who noted that glycerol improved the efficacy of *V. lecanii* spores. The findings of Easwaramoorthi and Jayaraj (1977) are also in agreement with the present findings. Easwaramoorthi and Jayaraj (1978) and Prior *et al.* (1988) and reported that tween-80 could be used as an effective adjuvant in mycoagent formulations.

Arachid oil emerged as a good adjuvant in the formulation. According to Verhaar *et al.* (1999), arachid oil (0.5%) gave the best development of *V. lecanii* on mildewed cucumber leaves. The effectiveness of vegetable oils in mycoagent formulations is well documented by Bateman *et al.* (1992) and Boyette (1994).

Arachid oil or maize oil with tween-80 stimulated the germination of *V. lecanii* and considerably improved the biocontrol potential of *V. lecanii* at reduced humidities (Verhaar *et al.,* 1999). According to Curtis *et al.* (2003), spray mixtures of glycerol and egg powder resulted in high germination of conidia on leaves at 70% RH. The highest level of aphid infection also occurred in EGP: GLY treatments.

However, the effectiveness of combination of glycerol, tween-80 and arachid oil could not be compared for want of literature on combination of adjuvants in mycoagent formulations.

The formulations containing glycerol, tween-80 and arachid oil with liquid culture of *V. lecanii* were tested for their bioefficacy against *M. hirsutus* for up to 14 days. The bioefficacy data re vealed that the mortality among the treatments ranged from 5.00 to 15.50, 11.89 to 31.35, 20.78 to 32.50, 32.57 to 50.85, 43.53 to 64.70 and 57.83 to 82.50 per cent at 1,3,5,7,9 and 14 days after treatment, respectively (Table 2). The lowest mortality (zero) appeared in untreated control (*V. lecanii* alone).

At one day after treatment, treatment 15 (*V.l.* + G 8) caused the highest (15.50%) mortality, however, it was on par with treatment 9 (*V.l.* + G8 + T 80 1 + A5) (15.00% kill).

In the observations taken at 3 days after treatment, treatment 9 inflicted significantly highest (31.35%) mortality among all the treatments. On 5 and 7 days after treatment, a similar trend was seen. In the observations taken at 9 days after treatment, treatment 9 (*V. l.* + G8 + T 80 1 + A 5) proved to be the best (64.70%), followed by treatments 8 and 7 (63.90 and 63.62 per cent mortality, respectively).

At 14 days after treatment, all the combinations were significantly superior (65.06 to 82.50%) to the fungus culture alone (57.83%). Treatment 9 (*V.l.* + G8 + T 80 1 + A 5) proved significantly superior (82.50%) to the rest of the combinations in controlling the pest. However, treatments 8 (81.93%) and 7 (81.28%) were on par with it. As such, these three treatment combinations proved to be highly promising. So, T_9, T_8 and T_7 were considered as formulations C, B and A, respectively. In view of on par results for virulence, viability and cost of formulation, formulations A and B were selected for further studies.
Easwaramoorthi and Jayaraj (1977) noted increased mortality of bugs from 47.5 to 62.6 per cent by the fungus with glycerol (0.1%). Tween 80 (1.00%) also emerged as good adjuvant. Easwaramoorthi and Jayaraj (1978) found that Tween 20 at 0.05 per cent in spray suspension increased the mortality (92.60%) of *Coccus viridis* by *V. lecanii.* Easwaramoorthi and Jayaraj (1977) observed increased mortality of *Coccus viridis* by addition of Tween 20 (0.05%) in *V. lecanii*. The effectiveness of arachid oil (0.5 and 2.00%) in *V. lecanii* as adjuvant is in conformity with that of Verhaar *et al.* (1999) who noticed that *V. lecanii* formulated with arachid oil (0.5%) showed significantly better control of powdery mildew than without the oil. In this study, in terms of viability and virulence, the treatments *V. l.* + glycerol 2% + Tween 80 1% + arachid oil 0.5% and *V. l.* + glycerol 5% + Tween 80 1% + arachid oil 2% emerged as the best combinations.

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