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Research Article

Effect of ultraviolet light on viability of liquid formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams with various adjuvants*

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ABSTRACT: The effect of ultraviolet irradiation on the viability of entomopathogenic fungus, *Lecanicillium* (=*Verticillium*) *lecanii* (Zimmerman) Zare and Gams was studied under laboratory conditions at Biocontrol Research Laboratory of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra during 2002-04. The liquid formulation of *L. lecanii* with different adjuvants, *viz.,* glycerol, Tween-80, Triton-x-100, boric acid, indigo, milk, turmeric and arachid oil were exposed to UV light for different exposure periods of 10, 20, 30, 40 and 50 min. The distance between exposed suspension and UV light source was 0.3 m. The UV rays proved detrimental to the growth and development of *L. lecanii*. The detrimental effect increased with increase in exposure period. However, glycerol, boric acid and Tween-80 gave good UV protection to *L. lecanii*, while milk, turmeric and indigo lack the UV protectability.

KEY WORDS: Adjuvant, UV light, *Lecanicillium lecanii*, viability

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INTRODUCTION

Lecanicillium (=*Verticillium*) *lecanii* (Zimmerman) Zare and Gams (Moniliales: Moniliaceae) is a well known pathogen of scale insects, whiteflies and aphids, (Ekbom, 1979; Kanagaratnam *et al.,* 1982; Hall and Papierok, 1982). In India, Sydow and Butler (1911) reported *V. lecanii* infecting coffee scale insect from Karnataka. A liquid suspension formulation of *L. lecanii* developed has given better results in laboratory studies. Sunlight kills the fungi within hours after exposure (Moore *et al*., 1993). *V. lecanii* spores are damaged by ultraviolet radiation (Osman and Valadon, 1979). Inglis *et al.* (1995) noticed negative correlation of persistence of conidia with cumulative total solar radiation in the field conditions. Therefore, the study was carried out to study the impact of UV rays on the radial growth of the fungus with various adjuvants.

MATERIAL AND METHODS

Studies on effect of ultraviolet light on viability of liquid formulation of *L. lecanii* with various adjuvants were carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra during 2002-04.

Culture of L. lecanii

The pure fungus culture available at Biocontrol Research Laboratory of Entomology Department, M. P. K. V., Rahuri, was used. It is the Rahuri *deme* of the fungus isolated from spiralling whitefly, *Aleurodicus dispersus* infesting wild guava plant in 1999. The potato-dextrosem broth medium used for multiplication and growth of the fungus was autoclaved as suggested by Kadam and Jaichakravarthy (2003).

Formulation preparation

The adjuvants tested in the study were glycerol, Tween-80, Triton-x-100, boric acid, indigo, milk, turmeric and arachid oil .

The liquid suspension formulation of *L. lecanii* was tested with different adjuvants, *viz.,* glycerol at 1, 2 and 3 per cent, Tween-80 at 1 and 1.5 per cent, Triton-x-100 at 1 and 1.5 per cent, boric acid at 0.5, 1.0 and 1.5 per cent, milk at 0.25, 0.5 and 1.0 per cent, turmeric at 1, 2 and 3 per cent and indigo at 0.5, 1.0 and 1.5 per cent in completely randomized design with three replications. Each formulation was kept in 250 ml conical flask. The suspension cultures were placed in petri dishes and exposed to UV irradiation. The source of UV light was Philips

TUV 30V/ G 30 T8 lamp with a wavelength of 320 nm. The distance between exposed suspension and UV light source was 0.3 m. Exposure duration was 10, 20, 30, 40 and 50 min. One ml of such exposed formulation was added to 40 ml potato-dextrose broth medium and observed for growth and development up to 30 days. The whole data was then subjected to statistical analysis.

RESULTS AND DISCUSSION

The differences in growth were significant in different treatments with adjuvants after exposure to UV rays for 10 min. In the observations taken one day after exposure, the fungus alone and with milk as adjuvants did not show growth in the culture medium. The fungus culture with adjuvant turmeric and indigo had shown negligible growth. Same trend was observed on later days, indicating loss of viability of the mycoagent. Treatment with adjuvant glycerol exhibited significantly highest growth (27.37 to 45.39%) of the fungus compared to rest of the treatments. Similar trend of growth was observed at 3, 5, 7, 9, 14 and 30 days after exposure (Table 1).

At 30 days, the treatment of *L. lecanii* + glycerol showed significantly highest (86.25 to 96.25%) growth in broth medium with highest growth in 3 per cent glycerol. The mycoagent with Tween-80 (67.50 to 75.00%), Triton-x-100 (56.25 to 68.75%) and boric acid (72.50 to 82.50%) were the next best treatments.

L. lecanii treated with glycerol covered highest surface area (25.77 to 32.99%) one day after UV exposure for 20 min (Table 2). The growth pattern was similar at 3 to 14 days after treatment. At 30 days after exposure, the mycoagent without any adjuvant had no growth indicating loss of viability after the exposure to UV rays. *L. lecanii* with glycerol recorded highest growth (80.95 to 92.85%), followed by that with boric acid (67.85 to 79.76%), Tween-80 (57.14 to 71.42%) and Triton-x-100 (45.24 to 59.52%). *L. lecanii* with glycerol 2 and 3 per cent registered significantly highest surface radial growth of 88.90 and 92.85 per cent than rest of the treatments, respectively.

L. lecanii + glycerol maintained its superiority over other adjuvants after exposure to UV rays for 30 min

Table 1. Influence of UV irradiation for 10 minutes on viability of *Lecanicillium lecanii* suspension formulation with various adjuvants

Treatments*	Conc. (%) of adjuvant	Surface area covered (%) by *V. lecanii* growth at days after UV exposure						
		1	3	5	7	9	14	30
V.l. + G	3.0	45.39	68.82	78.88	81.82	84.52	95.00	96.25
	2.0	35.79	60.22	71.11	77.27	78.57	90.00	90.0
	1.0	27.37	52.90	64.44	69.32	72.62	85.00	86.25
V.l. + T 80	1.5	22.10	36.56	46.66	40.25	61.90	73.50	75.00
	1.0	16.84	30.11	42.22	46.64	52.76	66.25	67.50
V.l. + T-x-100	1.5	15.79	29.03	40.00	39.77	53.17	66.25	68.75
	1.0	10.53	23.66	35.55	67.05	42.86	56.20	56.25
V.l. + B	1.5	34.74	50.54	58.88	59.09	76.79	81.25	82.50
	1.0	23.16	40.86	51.11	54.54	66.66	76.25	77.50
	0.5	18.95	37.63	46.95	14.77	61.90	72.50	72.50
V.l. + M	1.0	0.00	4.30	12.22	6.81	11.90	10.0	6.25
	0.5	0.00	0.00	5.11	2.27	4.76	1.25	0.00
	0.25	0.00	0.00	1.11	0.00	0.00	0.00	0.00
V.l. + T	3.0	0.00	1.07	1.11	0.00	0.00	0.00	0.00
	2.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	5.68	0.00	0.00	0.00
V.l. + I	1.5	0.00	0.00	6.66	3.40	2.38	0.00	0.00
	1.0	0.00	0.00	3.33	1.14	0.00	0.00	0.00
	0.5	0.00	0.00	2.22	0.00	0.00	0.00	0.00
V.l	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE ±		0.82	1.51	1.05	0.96	1.85	1.11	1.13
P = 0.05		2.36	4.32	3.01	2.76	5.30	3.18	3.25

Table 2. Influence of UV irradiation for 20 minutes on viability of *Lecanicillium lecanii* suspension formulation with various adjuvants

Treatments*	Conc. (%) of adjuvant	Surface area covered (%) by *V. lecanii* growth at days after UV exposure						
		1	3	5	7	9	14	30
V.l. + G	3.0	32.99	56.84	61.54	67.41	70.93	92.77	92.85
	2.0	28.87	48.42	50.55	55.06	59.30	89.56	88.09
	1.0	25.77	40.00	45.05	50.56	54.61	81.93	80.95
V.l. + T 80	1.5	23.71	32.63	40.66	44.94	52.32	71.08	71.42
	1.0	15.53	27.37	34.06	39.33	45.35	57.83	57.14
V.l. + T-x-100	1.5	14.43	24.21	28.57	37.08	43.48	59.04	59.52
	1.0	10.31	20.00	24.17	33.07	38.37	49.40	45.24
V.l. + B	1.5	29.90	46.32	53.85	58.43	67.44	79.20	79.76
	1.0	22.68	37.89	47.25	55.06	61.62	71.08	70.24
	0.5	20.62	33.68	40.66	48.31	54.61	68.67	67.85
V.l. + M	1.0	0.00	3.16	4.40	6.74	8.14	3.60	4.76
	0.5	0.00	0.00	0.00	1.12	1.16	2.40	0.00
	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + T	3.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + I	1.5	0.00	0.00	2.20	3.37	2.32	0.00	0.00
	1.0	0.00	0.00	0.00	1.12	0.00	0.00	0.00
	0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE. ±		0.85	0.96	1.06	1.20	1.28	1.42	1.31
P = 0.05		2.43	2.74	3.04	3.45	3.68	4.08	3.75

* *V.l.* = *Verticillium lecanii*; G = Glycerol; T 80 = Tween-80; T-x-100 = Triton-x-100; T = Turmeric; B = Boric acid; M = Milk; I = Indigo

(Table 3). At 30 days after exposure of the UV rays, treatments of the mycoagent with glycerol showed high growth (78.41 to 88.64%) in culture medium than rest of the treatments. The fungus with glycerol at 3 per cent showed significantly highest growth (88.64%) among all treatments. Boric acid (64.77 to 77.27%), Tween-80 (52.27 to 60.22%) and Triton-x-100 (40.90 to 55.68%) were the next best adjuvants to protect the mycoagent from the UV effect.

One-day after exposure to UV rays for 40 minutes, the fungus culture with adjuvant glycerol covered 18 to 25 per cent surface area in culture medium, followed by that of 15 to 23 per cent with boric acid and 17 to 22 per cent with Tween-80 and 13 to 16 per cent with Triton-x-100. More or less similar growth pattern was seen at 3, 5, 7, 9 and 14 days after exposure to UV rays (Table 4).

At 30 days after exposure of the fungus to UV rays, the fungus with adjuvant milk, turmeric, indigo and without adjuvants resulted in zero per cent growth of the fungus in culture medium. The mycoagent with adjuvant glycerol covered 75.00 to 85.87 per cent surface area, followed by boric acid (60.88 to 75.00%), Tween-80 (43.48 to 53.61%) and Triton-x-100 (36.96 to 51.08%).

The growth of mycoagent with glycerol was 24.00 to 38.00, 29.59 to 42.86, 75.00 to 85.42 and 71.87 to 82.29 per cent growth at 3, 7, 14 and 30 days after 50 min of UV rays exposure, respectively (Table 5). The fungus culture with adjuvants Tween-80 and Triton-x-100 showed 46.87 to 47.91 and 29.17 to 43.75 per cent growth at 30 days after the UV exposure, respectively. At 30 days of exposure, the fungus culture with adjuvant glycerol 3 per cent covered significantly highest surface area (82.29%). However, it was at par with 2 per cent glycerol recording 81.25 per cent growth of the fungus in culture medium. Glycerol at 1 per cent was the next best treatment recording 71.87 per cent growth of the fungus. The fungus with

Table 3. Influence of UV irradiation for 30 minutes on viability of *Lecanicillium lecanii* suspension formulation with various adjuvants

Treatments*	Conc. (%) of adjuvant	Surface area covered (%) by *V. lecanii* growth at days after UV exposure						
		1	3	5	7	9	14	30
V.l. + G	3.0	30.30	49.48	53.19	60.21	62.92	90.69	88.64
	2.0	26.26	42.27	44.68	50.54	53.93	84.88	84.09
	1.0	23.23	35.05	37.23	44.09	46.06	80.23	78.41
V.l. + T 80	1.5	22.22	29.90	37.23	41.93	42.69	61.62	60.22
	1.0	18.18	26.80	32.98	33.33	34.83	54.65	52.27
V.l. + T-x-100	1.5	16.16	23.71	27.66	36.56	39.33	53.48	55.68
	1.0	13.13	18.56	22.34	33.33	34.83	43.02	40.90
V.l. + B	1.5	27.27	39.17	47.87	53.76	62.92	76.14	77.27
	1.0	24.24	35.05	41.50	49.46	57.30	68.60	68.18
	0.5	20.20	27.83	36.17	45.16	50.56	66.28	64.77
V.l. + M	1.0	0.00	2.06	3.19	5.37	7.86	1.16	2.27
	0.5	0.00	0.00	0.00	2.15	1.12	0.00	0.00
	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + T	3.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + I	1.5	0.00	0.00	1.06	3.22	2.24	0.00	0.00
	1.0	0.00	0.00	0.00	1.07	0.00	0.00	0.00
	0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE ±	–	1.13	1.23	1.25	1.05	1.19	1.12	1.13
P = 0.05	–	3.24	3.53	3.59	3.02	3.41	3.21	3.25

milk, turmeric and indigo observed negligible growth, while, the fungus without adjuvant failed to grow. It indicated loss of viability of the fungus and poor UV protect ability of these substrates.

According to Osman and Valadon (1981), the harmful wavelength of UV rays (320-450 nm with a peak of 370 nm) affected the germination of conidia in 24 to 48 hrs at 25°C. Moore *et al.* (1996) also pointed out that UVB light rapidly reduced the survival of conidia (90%) of *Verticillium agaricinum* within 15-60 minutes in water. UV light delayed the germination of many conidia from 24 to 48 hrs or more at 25°C (Zimmermann, 1982). Braga *et al*. (2002) observed decreased culturability with increased UV-B exposure for all strains of *L. lcecanii*. Rangel *et al.* (2004) and Wraight *et al.* (2001) also reported the harmful effect of UV rays on entomopathogenic fungi. These findings are in line with the present investigation. However, the work on effect of UV rays exposure on *L. lecanii* liquid suspension culture with adjuvants tested here is meager. So, it cannot be compared for paucity of literature on this aspect.

The UV rays proved detrimental to the growth and development of *L. lecanii*. The detrimental effect increased with increase in exposure period to UV rays. However, glycerol, boric acid and Tween-80 were emerged as best UV protectants for *L. lecanii*, while milk, turmeric and indigo lack the UV protectability for the mycoagent.

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Table 4. Influence of UV irradiation for 40 minutes on viability of *Lecanicillium lecanii* suspension formulation with various adjuvants

Treatments*	Conc. (%) of adjuvant	Surface area covered (%) by *V. lecanii* growth at days after UV exposure						
		1	3	5	7	9	14	30
V.l. + *G*	3.0	25.0	43.0	45.92	50.00	54.83	88.04	85.87
	2.0	22.0	40.0	40.82	43.75	47.31	81.52	78.26
	1.0	18.0	34.0	35.71	48.96	41.93	78.26	75.00
V.l. + T-80	1.5	22.0	28.0	34.69	36.46	38.70	48.91	53.61
	1.0	17.0	23.0	27.55	27.08	30.10	50.00	43.48
V.l. + T-x-100	1.5	16.0	24.0	26.53	34.37	36.55	38.45	51.08
	1.0	13.0	20.0	21.43	32.29	33.33	76.08	36.96
V.l. + B	1.5	23.0	35.0	41.84	48.96	56.98	70.65	75.00
	1.0	22.0	30.0	36.73	43.75	53.76	64.13	67.39
	0.5	15.0	24.0	29.59	36.46	47.31	52.50	60.88
V.l. + M	1.0	0.00	3.00	3.06	2.08	4.30	0.00	0.00
	0.5	0.00	1.00	0.00	0.00	1.07	0.00	0.00
	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + T	3.0	0.00	2.00	0.00	0.00	0.00	0.00	0.00
	2.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + I	1.5	0.00	2.00	1.02	1.04	0.00	0.00	0.00
	1.0	0.00	1.00	0.00	0.00	0.00	0.00	0.00
	0.5	0.00	1.00	0.00	0.00	0.00	0.00	0.00
V.l	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE .±		0.86	1.03	1.24	1.04	1.09	1.05	1.00
P = 0.05		2.47	2.95	3.54	2.97	3.12	3.01	2.87

* *V.l.* = *Verticillium lecanii*; G = Glycerol; T 80 = Tween-80; T-x-100 = Triton-x-100; T = Turmeric; B = Boric acid; M = Milk; I = Indigo

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Table 5. Influence of UV rays irradiation for 50 minutes on viability of *Lecanicillium lecanii* suspension formulation with various adjuvants

Treatments*	Conc. (%) of adjuvant	Surface area covered (%) by *V. lecanii* growth at days after UV exposure						
		1	3	5	7	9	14	30
V.l. + G	3.0	18.00	38.00	40.82	42.86	46.87	85.42	82.29
	2.0	15.00	31.00	34.69	37.75	41.66	81.25	81.25
	1.0	12.00	24.00	24.49	29.59	34.37	75.00	71.87
V.l. + T-80	1.5	16.00	21.00	25.51	27.55	31.25	48.96	47.91
	1.0	14.00	18.00	19.39	21.43	25.00	45.83	46.87
V.l. + T-x-100	1.5	12.00	21.00	23.47	32.62	36.46	46.87	43.75
	1.0	11.00	19.00	19.39	27.55	30.20	35.41	29.17
V.l. + B	1.5	18.00	29.00	34.69	42.85	50.00	90.83	69.79
	1.0	14.00	22.00	26.53	39.79	41.66	66.66	60.42
	0.5	10.00	16.00	21.43	33.67	35.42	60.42	55.20
V.l. + M	1.0	0.00	0.00	0.00	0.00	2.08	0.00	0.00
	0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + T	3.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l. + I	1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V.l	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE ±		0.66	0.75	0.84	1.20	1.02	1.05	1.12
P = 0.05		1.88	2.15	2.41	3.45	2.93	3.00	3.22

* *V.l.* = *Verticillium lecanii*; G = Glycerol; T 80 = Tween-80; T-x-100 = Triton-x-100; T = Turmeric; B = Boric acid; M = Milk; I = Indigo

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