J. Biol. Control, 22(2): 375-381, 2008

Effect of various adjuvants on growth and development of the entomopathogenic fungus, *Verticillium lecanii* (Zimmermann) Viegas

B. P. CHAVAN and J. R. KADAM
Department of Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, Dist. Ahmednagar, Maharashtra, India.
E-mail: bpchavan123@mail.com

ABSTRACT: **Studies on the effect of various adjuvants on growth and development of *Verticillium lecanii* (Zimmermann) Viegas was undertaken with a view to select suitable adjuvant for developing liquid formulation. The study revealed that there was increase in surface area covered (%) and biomass produced (gm) with increase in concentration of the inoculum. Among the different oils tested, significant surface area coverage was recorded in arachid oil @ 1.0 per cent followed by 0.1 to 0.5 per cent, respectively. Similar result was recorded in biomass production. Addition of arachid oil @ 1.0 per cent recorded maximum biomass of 28.40 g, followed by 0.5 per cent (25.2 g) and 0.1 per cent (23.5 g), respectively. Addition of oils and stickers resulted in increased surface area growth significantly. Addition of arachnid oil @ 5 per cent resulted in maximum surface area of biomass. Based on the results it is concluded that arachid oil could be used as adjuvant in the liquid formulation of *V. lecanii* isolated from spiraling whitefly.**

KEY WORDS: Biocfficacy, growth and development, *Maconelicoccus hirsutus*, *Verticillium lecanii*.

INTRODUCTION

Biological control is an important, effective, eco-friendly and economical component of IPM in almost all important crops for the development of sustainable cropping systems. There is ample scope for microbial control of pests of cereals, pulses, vegetables and horticultural crops. Besides viruses and bacteria, some species of entomopathogenic fungi are emerging as potential bio-agents. Among various biocontrol agents recommended for control of sucking pests, *Verticillium lecanii* (Zimmermann) Viegas is one of the highly promising frontline fungal bio-agent. Efforts were made to develop suitable formulations of this mycoagent. Aqueous spray application of conidia or blastospores of *V. lecanii* can be effective in the biological control of aphid, whitefly, mealy bug and red spider mite (Humber and Soper, 1981). Level of infection of insects by *V. lecanii* through direct contact with spores from sprays or sprayed leaves may be very low. Epizootics usually results from insects being infected directly by ariel conidia from sporulating cadavers (Hall, 1976,1979,1980; Hall and Burges, 1979) or mycelial conidia on foliage (Hall, 1982). There are many examples where fungi have been formulated with various adjuvants. The addition of nutrients to a spore spray did improve 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). Glycerol as adjuvant improved the efficacy of spore sprays of *V. lecanii*.

In the present study, a range of adjuvants and vegetable oils were screened for their growth and development of *V. lecanii* on culture medium with a view to select suitable adjuvants for developing liquid formulation of the entomopthogen.

MATERIALS AND METHODS

Studies on the effect of various adjuvants and vegetable oils on growth and develpomnt of *V. lecanii* was carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra State, India during 2002-04.

Culture of *V. lecanii*

The pure fungus culture was available in Biocontrol Research Laboratory of Entomology Department, M.P.K.V., Rahuri. The fungus was isolated from spiralling whitefly, *Aleurodicus dispersus* infesting wild guava plant in 1999 Rahuri, Maharashtra, India.

Medium

The medium used for multiplication and growth of the fungus was autoclaved potato-dextrose broth medium as suggested by Kadam and Jaichakravarthy (2003).

Adjuvant

The adjuvants used for the study were glycerol, tween-80 and triton-x-100 and vegetable oils including arachid, sunflower, safflower, soybean mustard and coconut oil were also tested

METHODOLOGY

Effect of oils on liquid formulation of *V. lecanii*

Sunflower, safflower, soybean, mustard, arachid and coconut oils each at 0.1, 0.5 and 1.0 per cent were added individually to optimum concentration (1 x 10^8 CFU/ml) of aqueous suspension of *V. lecanii* and 18 preparations were made. The bottles with formulated product were kept at ambient temperature. One ml each of the formulated liquid was added individually to 40 ml potato-dextrose broth medium as inoculant in 500 ml capacity conical flask. It was then incubated at 21±1°c for 10 days. Whole process was carried out in laminar flow cabinet. The observations on surface area covered (%) and biomass production (g) were noted by visual observations adopting Completely randomized design replicated thrice. The experimental data was then analyzed statistically.

Effect of various adjuvant on liquid formulations of *V. lecanii*

In another experiment, glycerol (G) @ 2, 5, 8 and 10 per cent; tween-80 (T 80) @ 1, 2, 3, 4 and 5 per cent and arachid oil (A) @ 1, 2, 3, 4 and 5 per cent were tested to find out their suitable concentration for liquid formulation of *V. lecanii* for satisfactory growth and development of *V. lecanii* in Completely randomized design with three replications. For this experiment, the optimum concentration (10 x 10^8CFU/ml) of *V. lecanii* was taken in a sterilized 250 ml conical flask and required concentration of adjuvant was added to the liquid suspension of the mycoagent and incubated at ambient temperature.

One ml of each formulation was added individually to 40 ml potato-dextrose broth as inoculant in 500 ml conical flask. It was incubated at 21 ± 1°C for 10 days. Observations on surface area covered (%) by *V. lecanii* growth days after treatment were recorded. The data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Effect of various vegetable oils on growth and development of *V. lecanii*

Various vegetable oils were tested for their effect on growth and development of *V. lecanii* liquid culture and the results are presented in Table 1. In the observations on per cent surface area covered at 3, 7 and 10 days after treatments, the differences were significant among various treatments. At 3rd day the fungus culture with arachid oil covered significantly highest surface area (20.60 to 28.50%) than rest of the treatments. The mycoagent with 1.0 per cent arachid oil exhibited significantly highest growth (28.50%). However, it was on par to the growth in 0.5 per cent (26.30%). Both these treatments were significantly superior to rest of the treatments. Arachid oil 0.1 per cent (26.10%) was next highly promising adjuvant. The fungus culture with 0.1 to 1 per cent coconut oil did not show growth of *V. lecanii* in culture medium.

On 7th day, significantly higher growth (56.80 to 66.00%) was seen in treatment with arachid oil 0.1 to 1 per cent than rest of the treatments. Highest (66.00%) growth of the fungus with arachid oil was observed in 1.0 per cent concentration. But, it was on par with 0.5 per cent arachid oil (62.50 %). The fungus alone covered 35 per cent surface area, which was significantly more than that in other vegetable oils.

On 10th day after treatment, arachid oil 0.1, 0.5 and 1 per cent as adjuvant in liquid *V. lecanii* culture registered 72.20, 76.70 and 82.50 per cent surface coverage of the mycoagent. It was significantly higher than the growth in rest of the treatments. The growth of *V. lecanii* liquid culture alone was 52 per cent which was superior over all concentrations of rest of the treatments (17.40 to 47.30%) with sunflower, safflower, soybean, mustard and coconut oil. Addition of arachid oil at one per cent was most promising with significantly highest growth (82.50%) of the fungus.

The differences were significant for biomass produced on 10th day in various treatments. The fungus culture with arachid oil maintained its superiority over rest of the treatments producing 23.50 to 28.40g biomass. Arachid oil at one per cent with the mycoagent culture resulted in highest (28.40 g) biomass production, which was significantly superior to 0.5 per cent (25.20g) and 0.1 per cent (23.50 g) concentrations. *V. lecanii* without vegetable oil had only 12.5 g biomass per 40 ml medium on 10th day after treatment. These results established that arachid oil at 0.1,0.5 and 1 per cent was found to be superior oil adjuvant for liquid *V. lecanii*. All the other listed oils *viz.*, sunflower, safflower, soybean, mustard and coconut oil at all test concentrations were proved to be antagonistic as evidenced from lesser growth and biomass than in *V. lecanii* alone.

Earlier few workers documented the superiority of vegetable oils in the mycoagent formulation. Bateman *et al.* (1992) opined that oil formulations assist by disrupting the waxy layer of insect cuticle as well as protecting the conidia from desiccation. Quimby *et al.* (1989) noted that oil mixtures enable germination even in the absence of moisture. Boyette (1994) pointed out that the oil mixtures of mycoherbicides enable the conidia to tolerate periods of low humidity and continues development when favourable condition returns. However, Erkilic (1992) observed that oil mixtures against aphids have been less successful. In the present investigations, various vegetable oils were studied for their effect on formulation of *V. lecanii*. Arachid oil was found to be superior in this study. The observations made in the study were in agreement with those reported by Verhaar *et al.* (1999) who reported that *V. lecanii* with arachid oil gave best development and showed significantly better control of powdery mildew.

Effect of various adjuvants on viability of *V. lecanii*

Arachid oil, glycerol and tween-80 were added individually to liquid culture of *V. lecanii* and results with respect to their effects on growth and development of *V. lecanii* are presented in Table 2. There is increase in surface area covered (%) and biomass produced (g) with the increase in concentration of the adjuvants. Addition of arachid

Table 1. Effect of vegetable oils on growth and development of *V. lecanii* in liquid formulation

Sr. No.	Treatments	Concentration (%)	Surface area covered (%) by *V. lecanii* growth days after treatment			Biomass on 10th day/ 40ml
			3	7	10	
1	*V.l.**+Sunflower oil	0.1	10.50	21.20	40.50	14.20
		0.5	12.60	23.50	42.90	15.80
		1.0	15.20	27.00	47.30	16.40
2	*V.l.*+Safflower oil	0.1	8.20	16.60	31.00	8.50
		0.5	10.00	19.00	36.20	10.20
		1.0	12.80	22.20	41.50	13.60
3	*V.l.*+Soyabean oil	0.1	0.00	12.40	27.00	5.10
		0.5	5.75	16.60	32.50	6.90
		1.0	8.45	18.50	38.80	9.40
4	*V.l.*+Mustard oil	0.1	0.00	12.20	27.20	6.30
		0.5	0.00	15.90	31.60	7.00
		1.0	10.00	17.10	35.30	8.90
5	*V.l.*+Arachid oil	0.1	20.60	56.80	72.20	23.50
		0.5	26.30	62.50	76.70	25.20
		1.0	28.50	66.0	82.50	28.40
6	*V.l.*+Coconut oil	0.1	0.00	5.20	17.40	5.20
		0.5	0.00	10.80	21.30	7.60
		1.0	0.00	14.40	27.80	9.40
7	U.C.*(V.l. only)*		12.00	35.00	52.00	12.5
	S.E.±		0.79	1.41	1.16	0.92
	P = 0.05		2.28	4.05	3.34	2.63

* *V.l. = Verticillium lecanii*

oil at five per cent covered highest surface area (33.60%), however it was at par to 4 (32.20%) and 3 per cent (31.40%) at 3 days after treatment. In the treatment of fungus culture with all tween-80 test concentrations (1 to 5 per cent) showed at par growth (22.50 to 24.20%). There was significant increase in surface area covered (26.00 to 36.50%) with the increase in concentration of glycerol.

At 7 days after treatment, growth of the fungus culture with arachid oil, tween-80 and glycerol was 64.70 to 73.40, 55.20 to 65.00 and 57.17 to 71.60 per cent, respectively. The fungus culture alone covered only 32.00 per cent surface area.

All the treatments were significantly superior (68.60 to 86.20%) to the fungus culture alone (47.00 %) at 10 days after treatment considering the surface area by the fungal growth. The fungus culture with arachid oil allowed maximum surface coverage compared to other adjuvants. It resulted in 80.50, 81.00, 85.00, 85.50 and 86.20 per cent surface coverage at 1,2,3,4 and 5 per cent concentrations, respectively. The treatment with 5 per cent arachid oil concentration was at par with 3 and 4 per cent concentrations. The mycoagent

Table 2. Effect of addition of some adjuvants on growth of *V. lecanii*

Sr. No.	Treatments	Concentration (%)	Surface area covered (%) by *V. lecanii* growth days after treatment			Biomass on 10th day grams / 40ml medium
			3	7	10	
1	*V.l.**+ *A*	1	27.50	64.70	80.50	27.00
		2	29.60	66.20	81.00	29.20
		3	31.40	68.80	85.00	30.60
		4	32.20	70.30	85.50	32.40
		5	33.60	73.40	86.20	33.10
2	*V.l.* + T 80	1	22.50	55.20	68.60	21.70
		2	23.10	56.00	71.00	22.50
		3	23.40	57.50	72.00	23.00
		4	24.00	61.20	72.00	23.60
		5	24.20	65.00	72.50	24.30
3	*V.l.* + G	2	26.00	57.17	77.00	26.70
		5	29.50	63.50	82.50	28.30
		8	32.20	68.40	83.60	30.90
		10	36.50	71.60	85.10	31.20
4	U.C.(*V.lecanii*)	-	14.00	32.00	47.00	14.00
	S.E.±		0.94	1.30	1.59	1.28
	P = 0.05		2.70	3.80	4.56	3.66

* *V.l.* = *Verticillium lecanii*
T 80 = Tween-80
G = Glycerol
A=Arachid oil

culture with tween-80 showed 68.60 to 72.50 per cent surface coverage at one to five per cent concentrations. All these concentrations growth was significantly differing. The fungus culture with glycerol covered 77.00 to 85.10 per cent surface at 2 to 10 per cent concentrations. The treatment of the fungus culture with 10 per cent glycerol produced highest surface area covered (85.10%). However, it was at par with 5 (82.50%) and 8 per cent (83.60%) concentrations, respectively.

Among all the treatments, addition of arachid oil at five per cent was the most promising for the growth of *V. lecanii* recording 86.2 per cent surface coverage. The next highly promising treatment were arachid oil 4 per cent, glycerol 10 per cent, arachid oil 3 per cent and glycerol 8 and 5 per cent, respectively in descending order. The fungal growth in these treatments covered 82.50 to 85.00 per cent medium surface. Although, appreciable surface coverage (71.00 to 81.00%) appeared in rest of the concentrations of adjuvants, the growth was less than 50 per cent in *V. lecanii* alone during the period of 10 days.

The corresponding observations on biomass produced 10 days after treatment revealed that all the treatments were significantly superior to untreated control (*V. lecanii* liquid culture alone). All the treatments with adjuvants produced significantly higher biomass than *V. lecanii* alone. Arachid oil produced 27.00 to 33.10g biomass. The

treatment with five per cent arachid oil registered highest biomass (33.10g). However, it was on par with three (30.60g) and four per cent concentrations (32.40g). The fungus culture with tween-80 had 21.70 to 24.30g biomass and all its concentrations were at par to each other. The mycoagent with glycerol had 26.70 to 31.20g biomass per 40 ml culture medium. Glycerol at 10 per cent concentration resulted in highest biomass (31.20g), but it was at par with its 5 per cent (28.30g) and 8 per cent concentration (30.90g).

It is very explicit from the results that there is increase in surface area covered (%) and biomass produced (gm) with the increase in concentration of adjuvants. The effectiveness of arachid oil is well documented by Verhaar *et al.* (1999), Bateman *et al.* (1992), Quimby *et al.* (1989) and Boyette (1994).

The effectiveness of glycerol as adjuvant in mycoagent formulation was reported earlier without mentioning concentration 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. The utility of tween-80 as effective adjuvant has been documented by Easwaramoorthi and Jayaraj (1977) and Prior *et al.* (1988). Arachid oil was found to be a good adjuvant in the mycoagent formulation. According to Verhaar *et al.* (1999) arachid oil (0.5 %) gave the best development of *V. lecanii* on mildewed cucumber leaves.

Considering overall performance of the adjuvants with the mycoagent, it is concluded that addition of glycerol 5 to 10 per cent and arachid oil 2 to 5 per cent were the most promising for the growth and development of *V. lecanii*, followed by tween-80 1 to 5 per cent.

REFERENCES

Abbott, W. S. 1925. Method of computing effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.

Bateman, R. P., Godonou, I. and Kpindu, D. 1992. Development of novel technique for assessing mycoinsecticide ULV formulations. In: (Eds. C. J. Lomer and Prior, C) *Biological Control of Locusts and Grasshoppers*, CAB International, Wallingford, pp. 255-262.

Boyette, C. D. 1994. Unrefined corn oil improves the mycoherbicidal activity of *Colletotrichum truncatum* for Hemp sesbanic (*Sesbania exaltata*) control. Weed Technology, **8**: 526-529.

Curtis, J. E., Price, T. V. and Ridland, P. M. 2003. Initial development of spray formulation which promotes germination and growth of the fungal entomopathogen, *Verticillium lecanii* (Zimm.) Viegas (Deuteromycotina: Hyphomycetes) on capsicum leaves (*Capsicum annum* grossum Sendt. Var. California Wonder) and infection of *Myzus persicae* Sulzer (*Homoptera: Aphididae*). *Biocontrol Science and Technology*, **13**: 35-46.

Easwaramoorthi, S. and Jayaraj, S. 1977. The effect of temperature, pH and media on the growth of the fungus, *Cephalosporium lecanii*. *Journal of Invertebrate Pathology*, **29**: 399-400.

Erkilic, L. 1992. Studies on the use of oil based formulations of *Verticillium lecanii* against green peach aphid, *Myzus persicae*. Masters thesis, Plant Protection Research Institute, Adana, Turkey.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedure for Agricultural Research, Wiley and Sons, New York.

Hall, R.A.1976. *Verticillium lecanii* on the aphid *Macrosiphoniella sanborni*. *Journal of Invertebrate Pathology*, **28**: 389-391.

Hall, R.A.1979. Pathogenicity of *Verticillium lecanii* conidia and blatospores against the aphid, *Macrosiphoniella sanborni*. *Entomophaga*, **24**:191-198.

Hall, R. A.1980. Effect of relative humidity on survival of washed and unwashed conidiospores of *Verticillium lecanii*. *Acta Oecologia*, **1**:265-273.

Hall, R. A. 1982. Control of whitefly, *Trialeurodes vaporariorum* and the cotton aphid, *Aphis gossypii*, in glasshouses by *Verticillium lecanii*. *Annals of Applied Biology*, **101**: 1-11.

Hall, R. A and Burges, H. D. 1979. Control of aphids in glass houses by *Verticillium lecanii*. *Annals of Applied Biology*, **93**: 239-246.

Humber, R. A. and Soper, R. S. 1981. Isolation, preservation and identification of entomopathogenic fungi. In: (Ed. Rogerts, D.W.) Entomopathogenic Fungi Allenheld Osmum, Montclair, New Jersey.

Kadam, J. R. and Jaichakravarthy, G. 2003. Bioefficacy of *Verticillium lecanii* (Zimm.) Viegas against nymphs of *Maconellicoccus hirsutus* Green. Proc. of the State Level Seminar on Pest Management for Sustainable Agriculture, Parbhani, Feb. 6-7, pp. 12-15.

Prior, C., Jollands, P. and Le Patourel, G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (*Deuteromycotina*: Hyphomycetes) on the cocoa weevil pest, *Pantorhytes plutus* (*Coleoptera*: Curculionidae). *Journal of Invertebrate Pathology*, **52**: 66-72.

Quimby, P. C., Fulgham, F. E., Boyette, C. D. and Connick, W. J. 1989. An invert imulsion replaces dew in biocontrol of sicklepod- a preliminary study. In: (Eds. Hovde, D. A. and Bateman, G. B.) Pesticide Formulations and Application Systems, Vol. 8, ASTM STP 980 American Society of Testing Materials, Philadelphia, PA, pp. 264-270.

Santharam, G., Easwaramoorthi, S., Regupathy, A. and Jayaraj, S. 1977. Possibility of increasing the pathogenicity of the white halo fungus, *Cephalosporium lecanii* on the coffee green bug, *Coccus viridis* during summer. *Journal of Plantation Crops*, **5**: 121-122.

Shabana, Y.M., Charudattan, R., Devalerio, J.T. and Elwakil, M.A.1977. An evaluation of hydrophillic polymers for formulating the bioherbicide agents, *Alternaria cassiae* and *A. eichhorniae*. *Weed Technology*, **11**:212-220.

Verhaar, M. A., Hijwegen, T. and Zadoks, J. C. 1999. Improvement of the efficacy of *Verticillium lecanii* used in biocontrol of *Sphaerotheca fuliginea* by addition of oil formulations. *Biocontrol*, **44**: 73-87.

(Received: 28.01.2008; Revised: 01.04.2008; Accepted: 30.06.2008)