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Compatibility of biocontrol agent *Verticillium lecanii* (Zimm.) with fungicides, insecticides and herbicides (*in vitro*)

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Abstract

An *in vitro* compatibility studies were conducted with *Verticillium lecanii* and some commonly used chemical pesticides. The experiment was laid out in Completely Randomized Design (CRD) with four replications and seven treatments of fungicides, seven treatments of herbicides and seven treatments of insecticides on biocontrol agents *Verticillium lecanii*. In case of *Verticillium lecanii*, among the fungicides, copper oxychloride and ametoctradin + diamethomorph, were proved moderately toxic while, carbendazim + mancozeb, azoxystrobin + tebuconazole, thiophenate methyl and captan, were proved totally incompatible. Among the herbicides, only one herbicide, carfentrazone ethyl, was found compatible and fomesafen + flauzifop-p-butyl and metribuzin were found slightly toxic. Glyphosate and oxyfloufen were proved moderately toxic and sulfentrazone + clomazone, was totally incompatible. Among the insecticides, chlorantraniliprole, spinosad and emamectin benzoate were found compatible and acetamiprid, imidachloprid and thiamethoxam + lambda cyhalothrin were found slightly toxic. Some chemical pesticides do not affect the growth and development of biocontrol agents. The combination of such chemical pesticides with biocontrol agents can provide an additive or synergistic effect in the control of diseases and pests. These chemical pesticides and biocontrol agents can be used in an integrated pest management programmer.

Keywords: Compatibility, *Verticillium lecanii*, fungicides, insecticides, herbicide

1. Introduction

In agriculture, the use of biocontrol agents is a safe and environmentally acceptable alternative to pesticides (Gampala and Pinnamaneni, 2010) [5]. The effect of entomopathogenic fungi depends not only on the strain and favorable environmental conditions but also on their interaction with other factors such as sprays of pesticides, micronutrients, hormones, etc. used by man in his attempt to increase productivity. *V. lecanii* is easy to mass produce and store and is effective over a wide range of temperatures and humidity levels. It also provides a rapid kill at optimum doses, and the fungus has been recently commercialized as a microbial agent for pest management. It has the additional features to produce extracellular enzymes, such as chitinases, which help promote host colonization (Upadhyay *et al.*, 2014) [14]. It was demonstrated that *Verticillium lecanii* (Zimm) caused over 90% mortality in whiteflies (Schaaf *et al.*, 1990) [16] and coffee green scale *Coccus viridis* (Green) (Reddy *et al.*, 1997) [17] and produced excellent control of thrips in greenhouse-grown crops (Gillespie, 1986; Meyer *et al.*, 2002) [19, 18]. Sprays of synthetic insecticides, botanical insecticides, fungicides, etc. used for the control of other pests and diseases in the same ecosystems may have better chances to interact with *V. lecanii* present in nature and possibly bring down its efficacy on the target pest. Combining a fungicide-tolerant biocontrol agent with respective fungicides has improved the extent of disease control and reduced the quantity of fungicides required for effective management (Buck, 2004) [4]. Hence, an *in vitro* study will be conducted to assess the compatibility of some commonly used, commercially available fungicides, insecticides, and herbicides with the growth of *V. lecanii*.

2. Materials and Methods

The present study was conducted at "Plant Pathology and Agricultural Microbiology" section of Rajarshee Chhatrapati Shahu Maharaj, College of Agriculture, Kolhapur, during the year 2020-21. For this study poisoned food technique is used.

Conical flasks containing PDA medium was sterilized in autoclave at 121 °C temperature and 15 psi pressure for 15 minutes. After sterilization, allowed medium to cool at near the 40 °C and precisely measured doses of chemical pesticides are added into the medium into each conical flask respectively. About 20 ml of PDA medium amended with various chemical pesticides is poured into each 9 cm sterilized Petri plate. Without pesticide amended medium served as control. All the Petri plates allowed to solidifying. Each treatment was performed in four replications.

After the medium has solidified, biocontrol agents were injected aseptically by transferring a circular 5 mm diameter disc produced from sterilized cork-borer from an actively developing 7-day old culture of the fungal biocontrol agent to the middle of the Petri dish. Observations of the mycelial growth of biocontrol agents were recorded by measuring the diameter (mm) of radial growth by using the measuring scale. Observations of four replications were recorded every 24 hours till the end of 7th days.

The growth inhibition of biocontrol agents was estimated by using the following formula given by Vincent (1947) and per cent inhibition of mycelial growth was obtained.

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

2.1 Chemical pesticides and their concentrations

The *in vitro* bio-efficacy of pesticides was determined by the poisoned food technique (Nene and Thapliyal, 1993). Six different fungicides, six herbicides and six insecticides were selected for this study. All about eighteen chemical pesticides were used according to the recommended application rate for field crops.

Table 1: Concentration of fungicides

Sr. no.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T ₁	Captan 50% WP	Caftaf	0.3 g
2	T ₂	Carbendazim 12% + Mancozeb 63% WP	Starlet	0.3 g
3	T ₃	Thiophenate methyl 70% WP	Roko	0.05 g
4	T ₄	sCopper oxychloride 50% WP	Blitox	0.3 g
5	T ₅	Azoxystrobin 11% + Tebuconazole 18.3% W/W SC	Custodia	0.1 ml
6	T ₆	Ametoctradin 22% + Dimethomorph 20% EC	Zampro	0.2 ml
7	T ₇	Control	-	-

Table 2: Concentration of herbicides

Sr. no.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T ₁	Fomesafen 11.1% W/W + flauzifop-p-butyl 11.1% W/W SL	Fusiflex	0.3 ml
2	T ₂	Metribuzin 70% WP	Adrino	0.05 g
3	T ₃	Sulfentrazone 28% + Clomazone 30% WP	Authority	0.4 g
4	T ₄	Glyphosate 41% SL	Touchdown	0.8 ml
5	T ₅	Oxyfluorfen 23.5% EC	Goal	0.2 ml
6	T ₆	Carfentrazone ethyl 40% DF	Affinity	0.01 g
7	T ₇	Control	-	-

Table 3: Concentration of insecticides

Sr. no.	Treatment	Chemical name	Trade name	Application rate/100 ml
1	T ₁	Chlorantraniliprole 18.5% SC	Coragen	0.03 ml
2	T ₂	Imidacloprid 48% SL	Gaicho	0.03ml
3	T ₃	Emamectin benzoate 5% WP	Rilon	0.04 g
4	T ₄	Spinosad	Tracer	0.03 ml
5	T ₅	Thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC	Alika	0.1 ml
6	T ₆	Acetamiprid 20% SP	Manik	0.05 g
7	T ₇	Control	-	-

2.2 Statistical analysis

All laboratory work was carried out in Completely Randomized Design, with four replications and seven treatments each of fungicides, herbicides and insecticides. Data obtained which in per cent format were transformed in arcsine format. Transformed data was subjected to analysis of variance.

3. Results and Discussion

3.1 Compatibility of *Verticillium lecanii* with chemical pesticides

In the present investigation compatibility of *Verticillium lecanii* is tested with six fungicides, six herbicides and six insecticides. According to the reference of Ambethgar (2009) [2], all the tested chemical pesticides were classified in four

categories based on per cent inhibition in mycelial growth of fungal biocontrol agents: 1 = toxic (> 50%), 2 = moderately toxic (35-50%), 3 = slightly toxic (25-35%) and 4 = compatible (< 25% inhibition). The chemical pesticides were toxic or compatible is confirmed by inhibition per cent at 144 hrs.

3.2 Effect of fungicides on *Verticillium lecanii*

Out of six fungicides (Table 4), copper oxychloride proved moderately toxic (23.50 mm) which showed inhibition of 45.19 per cent, followed by ametoctradin + diamethomorph (27.50 mm) which inhibited mycelial growth by 35.86 per cent. Carbendazim + mancozeb, at 24 to 144 hrs, completely inhibited the growth of *Verticillium lecanii* and proved totally incompatible, followed by azoxystrobin + tebuconazole (8.07 mm), thiophenate methyl (9.95 mm) and captan (10.25), which showed inhibition 81.17, 76.79 and 76.09 per cent respectively.

The growth response of *Verticillium lecanii* ranging from moderately toxic to toxic with the above fungicides observed in this experiment is in agreement with earlier findings by Hall (1981) [6] who suggested that captan fungicide was toxic and by (Krishnamoorthy *et al.* 2007) [9] who reported that thiophenate methyl and carbendazim totally inhibited the mycelial growth of *Verticillium lecanii* and proved incompatible with *Verticillium lecanii*.

3.3 Effect of herbicides on *Verticillium lecanii*.

In this experiment (Table 5), only one herbicide, carfentrazone ethyl (34.32 mm), was found compatible with *Verticillium lecanii*, which shows mycelial growth 34.32 mm and growth inhibition of 19.94 per cent. Fomesafen +

flauzifop-p-butyl (28.15 mm) and metribuzin (28.05 mm) were found slightly toxic, which showed inhibition 34.34 and 34.58 per cent respectively. The herbicide glyphosate (22.05 mm) reduced the growth by 48.57 per cent and proved moderately toxic, followed by oxyfluorfen (24.40 mm) which reduced the mycelial growth by 43.09 per cent. From 24 hrs to 144 hrs, complete growth inhibition of *Verticillium lecanii* was recorded with the treatment of sulfentrazone + clomazone, which is totally incompatible.

According to these experimental findings, carfentrazone ethyl was compatible with *Verticillium lecanii* and fully incompatible with sulfentrazone + clomazone.

3.4 Effect of insecticides on *Verticillium lecanii*

Among the insecticides (Table 6), chlorantraniliprole was found fully compatible, which shows mycelial growth of 37.40 mm and inhibition of 12.77 per cent, followed by spinosad (37.05 mm) and emamectin benzoate (35.85 mm) which reduce mycelial growth by 13.59 and 16.38 per cent respectively. At 72 hrs, inhibition of mycelial growth is very low, *i.e.* 8.57 per cent. Insecticide acetamiprid (31.02 mm), found slightly toxic, reduced the mycelial growth by 27.64 per cent, followed by imidachloprid (30.82 mm) and thiamethoxam + lambda cyhalothrin (30.25 mm), which reduced the growth by 28.10 and 29.45 per cent respectively.

The present investigation showed that insecticides chlorantraniliprole, spinosad and emamectin benzoate were found completely compatible with *Verticillium lecanii*. Parallel results are obtained as compared to previous reports by (Sword *et al.* 2011) [12], and (Kakati *et al.* 2018) [7]. Alizadeh (2007) [1] also reported that imidacloprid had no effect on *Verticillium lecanii*.

Table 4: Effect of fungicides on the colony growth of *Verticillium lecanii*

Treatments	Average colony diameter of <i>Verticillium lecanii</i> (mm)												Toxicity level
	24 hrs.	% Inhibition	48 hrs.	% Inhibition	72 hrs.	% Inhibition	96 hrs.	% Inhibition	120 hrs.	% Inhibition	144 hrs.	% Inhibition	
T ₁ Captan	1.52*	55.15 (47.91)**	2.30	74.86 (59.94)	3.15	79.97 (63.40)	6.85	81.84 (64.78)	8.10	79.72 (62.23)	10.25	76.09 (60.71)	Toxic
T ₂ Carbendazim + Mancozeb	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	Toxic
T ₃ Thiophenate methyl	1.00	70.59 (57.18)	2.30	74.86 (59.90)	4.05	74.24 (59.52)	8.05	78.66 (62.47)	9.30	76.72 (61.15)	9.95	76.79 (61.19)	Toxic
T ₄ Copper oxychloride	2.50	26.47 (30.87)	4.05	55.74 (48.28)	5.72	63.59 (52.87)	10.95	70.97 (57.38)	15.65	60.83 (51.25)	23.50	45.19 (42.21)	Moderately Toxic
T ₅ Azoxystrobin + Tebuconazole	0.00	100.00 (90.00)	2.20	75.96 (60.63)	2.95	81.24 (64.36)	4.95	86.88 (68.76)	6.52	83.67 (66.20)	8.07	81.17 (64.27)	Toxic
T ₆ Ametoctradin + Dimethomorph	2.00	41.18 (39.87)	4.50	50.82 (45.47)	8.75	44.36 (41.72)	13.00	65.54 (54.04)	20.00	49.94 (44.96)	27.50	35.86 (36.74)	Moderately Toxic
T ₇ Control	3.40	0.00	9.15	0.00	15.725	0.00	37.72	0.00	39.95	0.00	42.87	0.00	
S.Em±	0.02	0.56	0.10	0.59	0.14	0.56	0.32	0.45	0.38	0.61	0.36	0.52	
CD at 1%	0.08	1.65	0.29	1.75	0.42	1.66	0.95	1.33	1.12	1.81	1.07	1.53	

* Data is average of four replications

()** = Figures in parenthesis are arcsine transformed value

Table 5: Effect of herbicides on the colony growth of *Verticillium lecanii*

Treatments	Average colony diameter of <i>Verticillium lecanii</i> (mm)												Toxicity level
	24 hrs.	% Inhibition	48 hrs.	% Inhibition	72 hrs.	% Inhibition	96 hrs.	% Inhibition	120 hrs.	% Inhibition	144 hrs.	% Inhibition	
T ₁ Fomesafen + Flauzifop-p-butyl	1.50*	55.88 (48.34)**	3.30	63.93 (53.06)	6.62	57.94 (49.54)	9.65	74.42 (59.59)	22.25	44.31 (41.72)	28.15	34.34 (35.70)	Slightly Toxic
T ₂ Metribuzin	2.30	32.35 (34.52)	3.05	66.67 (54.69)	5.00	68.25 (55.72)	9.05	76.01 (60.65)	20.05	49.81 (44.91)	28.05	34.58 (35.83)	Slightly Toxic
T ₃ Sulfentrazone+ Clomazone	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	Toxic
T ₄ Glyphosate	1.20	64.71 (53.50)	3.95	56.83 (48.88)	7.72	50.95 (45.53)	15.62	58.58 (49.91)	18.82	52.88 (46.64)	22.05	48.57 (44.06)	Moderately Toxic
T ₅ Oxyfluorofen	2.10	38.24 (38.14)	3.75	59.02 (50.23)	6.72	57.30 (49.19)	9.40	75.08 (60.06)	19.37	51.50 (45.86)	24.40	43.09 (40.84)	Moderately Toxic
T ₆ Carfentrazone Ethyl	1.65	51.47 (45.78)	5.52	39.62 (38.99)	11.20	28.89 (32.45)	18.30	51.49 (45.83)	28.50	28.66 (32.34)	34.32	19.94 (26.15)	Compatible
T ₇ Control	3.40	0.00	9.15	0.00	15.75	0.00	37.72	0.00	39.95	0.00	42.87	0.00	
S.Em±	0.03	0.86	0.12	0.55	0.18	0.87	0.30	0.46	0.54	0.78	0.66	1.41	
CD at 1%	0.10	2.55	0.35	1.61	0.55	2.56	0.90	1.37	1.60	2.31	1.95	4.15	

* Data is average of four replications

(**) = Figures in parenthesis are arcsine transformed value

Table 6: Effect of insecticides on the colony growth of *Verticillium lecanii*

Treatments	Average colony diameter of <i>Verticillium lecanii</i> (mm)												Toxicity Level
	24 hrs.	% Inhibition	48 hrs.	% Inhibition	72 hrs.	% Inhibition	96 hrs.	% Inhibition	120 hrs.	% Inhibition	144 hrs.	% Inhibition	
T ₁ Chlorantra Niliprole	2.10*	38.24 (38.22)**	7.05	22.95 (28.37)	13.75	12.70 (19.32)	23.42	37.91 (38.01)	30.20	24.41 (29.60)	37.40	12.77 (19.97)	Compatible
T ₂ Imidacloprid	2.30	32.35 (34.53)	4.85	46.99 (43.14)	8.35	46.98 (43.06)	17.67	53.15 (46.77)	28.10	29.66 (32.97)	30.82	28.10 (31.91)	Slightly Toxic
T ₃ Emamectin benzoate	2.40	29.41 (32.65)	7.92	13.39 (18.44)	14.40	8.57 (15.86)	23.52	37.64 (37.77)	28.50	28.66 (32.36)	35.85	16.38 (21.78)	Compatible
T ₄ Spinosad	1.92	43.38 (41.09)	6.52	28.69 (32.10)	12.30	21.90 (27.80)	23.05	38.90 (38.54)	31.15	22.03 (27.96)	37.05	13.59 (21.21)	Compatible
T ₅ Thimethoxam+ Lambda cyhalothrin	2.27	33.09 (35.06)	7.87	13.93 (20.79)	13.05	17.14 (23.43)	17.62	53.28 (46.85)	21.37	46.50 (42.98)	30.25	29.45 (32.63)	Slightly Toxic
T ₆ Acetamiprid	1.42	58.09 (49.65)	5.20	43.17 (40.93)	10.10	35.87 (36.67)	15.00	60.24 (50.88)	24.72	38.11 (38.01)	31.02	27.64 (31.59)	Slightly Toxic
T ₇ Control	3.40	0.00	9.15	0.00	15.75	0.00	37.72	0.00	39.95	0.00	42.87	0.00(0.00)	
S.Em±	0.05	1.16	0.19	3.16	0.36	2.65	0.58	0.94	0.71	1.12	0.97	2.69	
CD at 1%	0.16	3.42	0.57	9.23	1.07	7.80	1.71	2.76	2.11	3.31	2.87	7.91	

* Data Average of four replications

(**) = Figures in parenthesis are arcsine transformed value

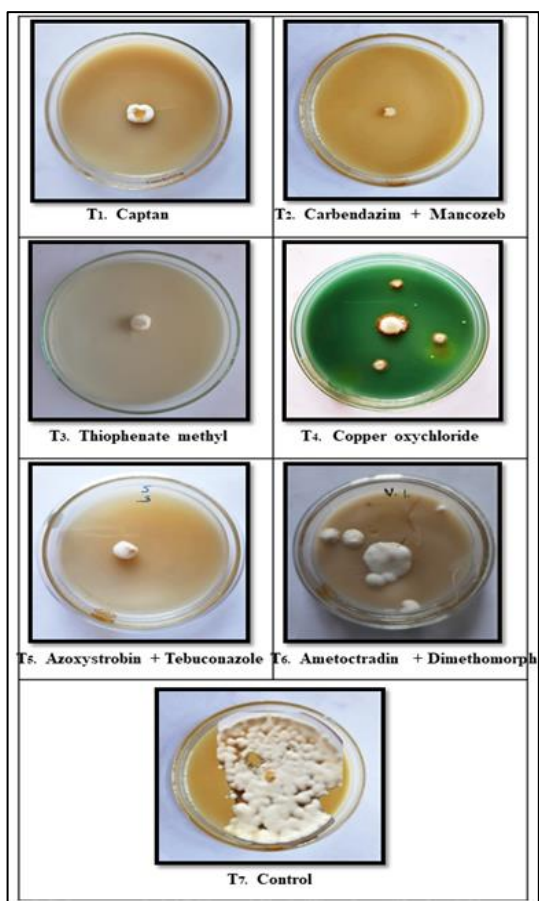


Fig 1: Colony growth inhibition of *Verticillium lecanii* by fungicides.

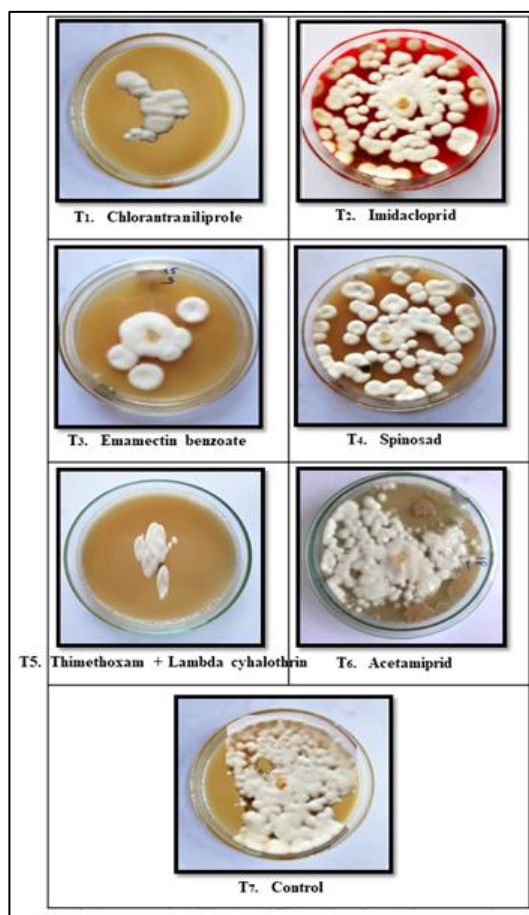


Fig 3: Colony growth inhibition of *Verticillium lecanii* by insecticides.

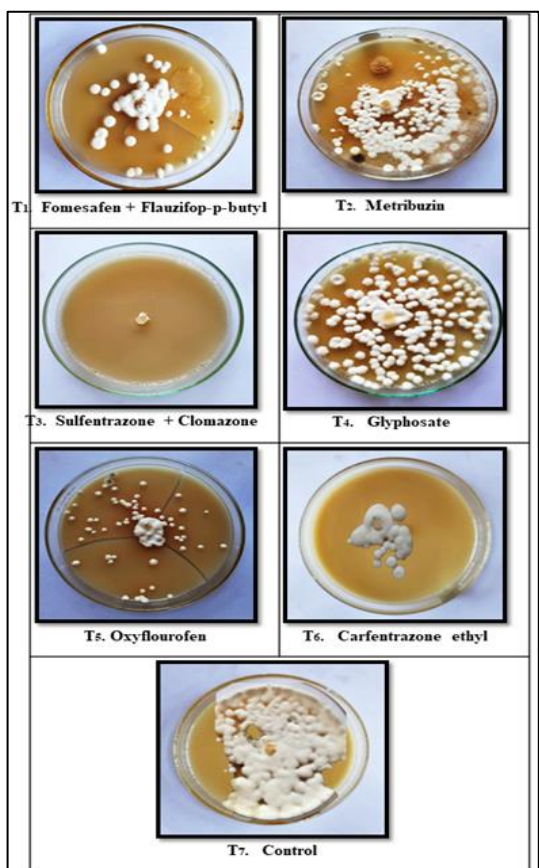


Fig 2: Colony growth inhibition of *Verticillium lecanii* by herbicides.

4. Conclusion

The results obtained in this study clearly showed that some chemical pesticides severely affected the growth and development of biocontrol agents. These pesticides can cause harmful effects on biocontrol agents when they are applied together or in an integrated pest management programme. The use of such chemicals with biocontrol agents cannot provide satisfactory control against diseases and pests and can cause economic loss to farmer.

Some chemical pesticides do not affect the growth and development of biocontrol agents. The combination of such chemical pesticides can provide an additive or synergistic effect in the control of diseases and pests. These chemical pesticides and biocontrol agents can be used in an integrated pest management programme.

Among six fungicides, copper oxychloride and ametoctradin + diamethomorph, were proved moderately toxic, so these fungicides shall be avoided with *Verticillium lecanii*. Fungicides, carbendazim + mancozeb, azoxystrobin + tebuconazole, thiophenate methyl and captan, were proved totally incompatible, hence not suitable to use with *Verticillium lecanii*, just before or just after application of IPDM.

Among the herbicides, only one herbicide, carfentrazone ethyl, was found compatible and fomesafen + flauzifop-p-butyl and metribuzin were found slightly toxic, so they can be applied safely in combination with *Verticillium lecanii*. The herbicide glyphosate and oxyfluorfen were proved moderately toxic and caution must be taken when applied in

IPM with *Verticillium lecanii*. Herbicide, sulfentrazone + clomazone, was totally incompatible, so it shall be avoided with *Verticillium lecanii*.

Among the insecticides, chlorantraniliprole, spinosad and emamectin benzoate were found compatible and insecticides, acetamiprid, imidachloprid and thiamethoxam + lambda cyhalothrin were found slightly toxic, so all the insecticides under study can be considered safe to *Verticillium lecanii*, in an integrated pest management programmer.

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