In vitro compatibility and toxicity of *Lecanicillium lecanii* with insecticides against cotton aphids

ABSTRACT

Background: *Lecanicillium lecanii* is the most effective entomopathogenic fungus against all stages of sucking pests like aphids, whiteflies, scale insects, thrips and mealybugs. The compatibility of fungi with commonly used insecticides was investigated in this study.

Aim: To study the *in vitro* compatibility and toxicity of *Lecanicillium lecanii* with insecticides against cotton aphids.

Study design: Completely Randomized Design

Methodology: Six different insecticides were evaluated under *in vitro* conditions against *L. lecanii* by the poison food technique. The results showed that insecticides, viz., Dinotefuran 20 SG (0.006%), Afidopyropen 50 g/L DC (0.1%), Diafenthiuron 50 WP (0.06%) and Buprofezin 25 SC (0.05%), were rated as harmless and proved compatible with *L. lecanii*. Pyriproxyfen 10 EC (0.016%) and Tolfenpyrad 15 EC (0.03%) were moderately harmful to *L. lecanii*. Furthermore, the toxicity of insecticides alone and in combination with the entomopathogenic fungus *L. lecanii* against the cotton aphid, *Aphis gossypii,* was assessed by topical application bioassay under laboratory conditions.

Results: Cent percent mortality of *A. gossypii* was recorded with Diafenthiuron 50 WP (0.06%), Dinotefuran 20 SG (0.006%) and Afidopyropen 50 g/L DC (0.1%) at the recommended dose and their combination at half of the recommended dose with *L. lecanii* (1×10^7 conidia/ml) at 72 h after treatment. While minimum mortality was observed with the individual treatment of *L. lecanii* (1×10^7 conidia/ml).

Conclusion: This study suggests that the most suitable insecticides at half of the recommended dose, when combined with *L. lecanii*, can be effectively integrated into pest management programs.

Keywords: *Aphis gossypii*, Compatibility, Insecticides, *Lecanicillium lecanii*, Toxicity

1. INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a versatile pest known for inducing substantial harm, such as leaf curling and deformation. Additionally, it acts as a carrier for over 76 viral diseases, including potyvirus, cucumber mosaic virus, and zucchini yellow virus, impacting various crops adversely (Kim, 2007). Aphids alone have the potential to inflict yield losses of up to 82% in the case of cruciferous crops when insecticides are not applied (Razaq *et al*., 2011). The intensive use of insecticides to control aphids has led to populations that are now resistant to several classes of insecticides (Tabacian *et al*., 2011). Moreover, the use of pesticides can lead to significant issues related to environmental contamination and can adversely affect beneficial insects like bee populations (Nawaz *et al*., 2014; Van der Sluijs *et al*., 2013). Biopesticides offer a route to protecting the crop while reducing the reliance on synthetic insecticides (Bailey, 2010). Entomopathogenic fungi (EPF) have been found to be effective as a biopesticide (Faria and Wraight, 2007) and have the potential to minimize the target pest populations on multiple crops (Jaber and Enkerli, 2017; Mukherjee, 2020). Another important fact to be considered in favor of these EPF is that, to date, there has been no report of developing resistance (Halder *et al*., 2021).

Entomopathogenic fungi are typically acknowledged for their slower action, requiring more time than traditional methods to achieve adequate insect mortality. Incorporating these fungi into a management strategy along with faster-acting materials could offer a potential solution to this issue. The synergistic action of mycoinsecticides with chemical insecticides can increase mortality and reduce the time until death in insects (Bitsadze, 2013; Sharififard, 2011). In this study, we gauged the compatibility of different insecticides with *L. lecanii* and assessed their toxicity to a prominent aphid pest. The compatibility of EPFs with pesticides could simplify the process of choosing suitable products within IPM programs.

2. MATERIAL AND METHODS

2.1 Fungus culture

Lecanicillium lecanii culture available with Centre for Organic Agriculture Research and Training, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola was used during the present study. This entomopathogen fungus was mass multiplied on Sabouraud's dextrose medium and used for further studies.

2.2 *In vitro* compatibility of *L. lecanii* with insecticides

To assess the compatibility, the effect of different treatments on the radial growth of *L. lecanii* was evaluated. The recommended dose of insecticides was added to SDA in a 100 ml conical flask before solidification. Following thorough mixing, the media was transferred to Petri dishes and allowed to solidify with gentle shaking. The plates, after solidification, were inoculated centrally with a 6 mm disc of a young sporulating culture of *L. lecanii* with the help of a sterilized cork borer and a fungal inoculating needle. The experiment on the compatibility of *L. lecanii* with insecticides consisted of seven treatments, each replicated three times. Petry dishes were sealed and placed in an incubator maintained at 27 ± 1 ^{0}C and 80 ± 5% relative humidity. The medium without pesticide was used as a control treatment. The observations on fungal diameter in each plate were recorded after 10 days of inoculation. Percent inhibition of *L. lecanii* was calculated on the basis of the growth diameter of the colony using the formula of Hokkanen and Kutiluoto (1992).

The pesticides were further classified into evaluation categories based on a 1–4 scoring index, i.e., 1-harmless (<50% reduction), 2-slightly harmful (50–79%), 3-moderately harmful (80–90%), and 4-harmful (>90%) according to Hassan's classification (Hassan, 1989).

2.3 Toxicity of *L. lecanii* alone and in combination with insecticides

A population of aphids was collected from the untreated cotton field of the Integrated Farming Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Serial dilutions of the *L. lecanii* isolate, pesticides alone, and mixtures (*L. lecanii* + pesticides) were prepared for each treatment. Once sterilized using sodium hypochlorite (0.5% v/v), detached cotton leaves underwent three washes with distilled water, followed by air drying, before being positioned on 1.5% agar (non-nutritive) in plastic Petri dishes measuring 90 × 20 mm^2. This agar concentration provided moisture essential for sustaining relative humidity during the test. Approximately 30 aphids, comprising a mixture of adult and nymph populations, were allowed to settle on the leaves. A topical spray method was used to treat the aphids with individual and combined applications of pesticides with *L. lecanii*. Mortality data were recorded at 24, 48, and 72 hours post-treatment (Nawaz *et al*., 2022).

2.4 Statistical analysis

A completely randomized design (CRD) was used in all experiments. The data obtained were converted to appropriate transformations, subjected to ANOVA, and means were compared by critical difference (p = 0.01) (Gomez and Gomez, 1984).

3. RESULTS

3.1 *In vitro* compatibility of *L. lecanii* with insecticides

The results on compatibility of *L. lecanii* with insecticides (Table 1) revealed that Dinotefuran 20 SG (0.006%) significantly supported the maximum radial mycelial growth (50.33 mm) of *L. lecanii* over the rest of the insecticides, and growth inhibition was 5.63 percent. This was followed by Afidopyropen 50 g/L DC (0.1%), Diafenthiuron 50 WP (0.06%), and Buprofezin 25 SC (0.05%), which recorded radial mycelial growth of 47, 45.66, and 31.83 mm and 11.87, 14.38, and 40.32 percent mycelial growth inhibition, respectively. The remaining treatments, Pyriproxifen 10 EC (0.016%) and Tolfenpyrad 15 EC (0.03%), with 10.50 and 9.33 mm radial mycelial growth and 80.31 and 82.50 percent mycelial growth inhibition, respectively, were found least compatible with *L. lecanii* in comparison to prior treatments. The insecticides Dinotefuran 20 SG (0.006%), Afidopyropen 50 g/L DC (0.1%), Diafenthuron 50 WP (0.06%), and Buprofezin 25 SC (0.05%) showed less than 50 percent mycelial growth inhibition and are categorized under Grade 1, i.e., harmless, whereas treatments Tolfenpyrad 15 EC (0.03%) and Pyriproxifen 10 EC (0.016%) showed growth inhibition between 80 and 90 percent and were categorized under Grade 3, i.e., moderately harmful (Hassan, 1989).

3.2 Toxicity of *L. lecanii* alone and in combination with insecticides against cotton aphids

The insecticidal treatments showed the same trend of efficacy against aphids on different days of observation (Table 2). The insecticides like Diafenthiuron 50 WP (0.06%), Dinotefuran 20 SG (0.006%), and Afidopyropen 50 g/L DC (0.1%) at the recommended dose and their combination at half of the recommended dose with *L. lecanii* showed a cent percent mortality in cotton aphid at 72 hours after treatment, followed by Buprofezin 25 SC (0.025%) + *L. lecanii*, Buprofezin 25 SC (0.05%), Pyriproxifen 10 EC (0.008%) + *L. lecanii*, Pyriproxifen 10 EC (0.016%), Tolfenpyrad 15 EC (0.015%) + *L. lecanii,* and Tolfenpyrad 15 EC (0.03%) recorded 97.78, 96.67, 94.44, 90.00, 87.78, and 85.56 percent aphid mortality. Comparatively less aphid mortality was recorded in the treatment of *L. lecanii* alone (40.00%).

4. Discussion

4.1. *In vitro* compatibility of *L. lecanii* with insecticides

The findings regarding compatibility of *L. lecanii* with insecticides align with previous research by Kim (2007), who observed no impact on the mycelial growth of *L. attennuatum* when exposed to neonicotinoid (Imidachloprid). Similarly, in this study, dinotefuran (also a neonicotinoid) demonstrated no harm to *L. lecanii*. Patel *et al*. (2020) reported that dinotefuran and difenthiuron showed heightened compatibility with entomopathogenic fungi at recommended doses. Reddy *et al*. (2020) corroborated that buprofezin posed no harm to *L. lecanii* at both recommended and half-recommended doses. Gowda and Rani (2022) revealed the toxicity of pyrazole insecticides to *L. lecanii*, a result consistent with the decreased compatibility observed with Tolfenpyrad, another pyrazole insecticide, in the current study. Additionally, Barari *et al*. (2012) demonstrated a significant reduction in conidial germination, vegetative growth, and spore production of *B. bassiana* when exposed to Pyriproxifen 10 EC.

4.2. Toxicity of *L. lecanii* alone and in combination with insecticides against cotton aphids

The findings from experiments testing toxicity of *L. lecanii* alone and in combination with insecticides against cotton aphids are supported by the research of Nawaz *et al*. (2022) discovered that combining *M. anisopliae* with Dinotefuran and Pyriproxifen had a synergistic effect, resulting in increased mortality of cotton aphids. They concluded that the insecticides exhibited consistent toxicity aligned with their compatibility with *M. anisopliae*, ranking in efficacy exactly as they did for compatibility. Whereas, Reddy *et al*. (2020) found Buprofezin 25 SC, at recommended and varied concentrations, to be compatible with *L. lecanii*. Combining Buprofezin with entomopathogenic fungi increased Brown Plant Hopper (BPH) mortality compared to using Buprofezin alone, with similar outcomes reported by Ijaz *et al*. (2021) under laboratory and field conditions against *Sogatella furcifera*, *Akanthomyces lecanii*, and buprofezin. Halder *et al*. (2019) noted that combining *L. lecanii* with neonicotinoid insecticides resulted in co-toxicity coefficient values (CTC) >1 and lower LT_{50}

values than using each independently, indicating compatibility and synergistic action. Combining *L. lecanii* with appropriate sub-lethal concentrations of neonicotinoids in a two-in-one tank mixture against sucking insect pests alleviates pressure on insecticide selection and curbs simultaneous resistance development in targeted pests. These findings align with Goto *et al*. (2019) and Horikoshi *et al*. (2022), reporting the highest insecticidal activity of the pyripyropene derivative, Afidopyropen, against aphids. On a different note, Shreekanth and Reddy (2011), Zala *et al*. (2014), and Bajya *et al*. (2016) highlighted high effectiveness of diafenthiuron in suppressing sucking pests without adverse effects on natural enemies. Conversely, Kumar *et al*. (2016) ranked Diafenthiuron>Pyriproxyfen>Tolfenpyrad in terms of toxicity against cotton's sucking pests, while Wang *et al*. (2017) observed Tolfenpyrad's highest toxicity against sucking pests in laboratory conditions.

Table 1. Compatibility of *L. lecanii* with insecticides by Poison Food Technique

Sr. No	**Treatment**	Concentration **(%)**	**10 days after inoculation of *L. lecanii***		**Grade***
			Mean of radial mycelial growth (mm)	**% mycelial growth inhibition**	
1	Buprofezin 25 SC	0.05	31.83 d	40.32	1
2	Diafenthiuron 50 WP	0.06	45.66 bc	14.38	1
3	Dinotefuran 20 SG	0.006	50.33 ab	5.63	1
4	Pyriproxifen 10 EC	0.016	10.50 e	80.31	3
5	Tolfenpyrad 15 EC	0.03	9.33 e	82.50	3
6	Afidopyropen 50 g/L DC	0.1	47.00 bc	11.87	1
7	*L. lecanii*(Control)	-	53.33 a	-	-
‘F’ test		Sig.			
SE(m) ±		1.30			
CD (p = 0.01)		5.47			

Note: Different letter within the treatments denote significant differences in the same column.
*Grade: 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50–79%), 3 = moderately harmful (80–90%), 4 = harmful (>90%) in *in vitro* toxicity tests (Hassan, 1989).

Table 2. Toxicity of *L. lecanii* alone and in combination with insecticides against cotton aphids

Note: Different letter within the treatments denote significant differences in the same column.
*Figures in parentheses are corresponding arcsine transformed values

Sr. No.	Treatment	Concentration (%)	Per cent aphid mortality		
			24 hrs	**48 hrs**	**72 hrs**
1	Buprofezin 25 SC	0.05	26.67 f (31.06)*	63.33 e (52.75)	96.67gh (81.32)
2	Diafenthuron 50 WP	0.06	47.78 e (43.73)	77.78 cd (61.89)	100.00abcdef (89.43)
3	Dinotefuran 20 SG	0.006	76.67 b (61.15)	100.00 a (89.43)	100.00 ab (89.43)
4	Pyriproxifen 10 EC	0.016	8.89gh (17.28)	40.00fg (39.22)	90.00ij (71.73)
5	Tolfenpyrad 15EC	0.03	2.22i (7.19)	23.33 h (28.85)	85.56jk (67.69)
6	Afidopyropen 50 g/L DC	0.1	63.33 cd (52.75)	93.33 b (75.36)	100.00abcd (89.43)
7	Buprofezin 25 SC + *L. lecanii*	0.025 + 1×10^7 (conidia/ml)	31.11 f (33.90)	67.78 de (55.42)	97.78abcdefg (82.80)
8	Diafenthuron 50 WP + *L. lecanii*	0.03 + 1×10^7 (conidia/ml)	56.67 de (48.84)	84.44 c (66.80)	100.00abcde (89.43)
9	Dinotefuran 20 SG + *L. lecanii*	0.003 + 1×10^7 (conidia/ml)	85.56 a (67.69)	100.00 a (89.43)	100.00 a (89.43)
10	Pyriproxifen 10 EC + *L. lecanii*	0.008 + 1×10^7 (conidia/ml)	14.44 g (22.31)	45.56 f (42.45)	94.44 hi (76.52)
11	Tolfenpyrad 15EC + *L. lecanii*	0.015 + 1×10^7 (conidia/ml)	6.67 h (14.64)	34.44fgh (35.93)	87.78ijk (69.58)
12	Afidopyropen 50 g/L DC + *L. lecanii*	0.05 + 1×10^7 (conidia/ml)	70.00bc (56.81)	95.56 b (80.16)	100.00abc (89.43)
13	*L. lecanii*	1×10^7 (conidia/ml)	0.00 j (0.52)	6.67i (14.64)	40.00 l (39.19)
14	Untreated Control	-	0.00 k (0.52)	2.22 j (7.19)	5.56 m (13.48)
'F' test			Sig.	Sig	Sig
SE(m)±			1.40	1.99	1.77
CD (p = 0.01)			5.47	7.77	6.92

The value of 0% is substituted by (1/4n) and the value of 100% by (100-1/4n), where n is the number of units upon which the percentage data is based (i.e., the denominator used in computing the percentage).

5. Conclusion

The findings suggest that insecticides such as Dinotefuran 20 SG, Diafenthiuron 50 WP, and Afidopyropen 50 g/L DC, when used in combination with *L. lecanii* at half the recommended dose, exhibit equal efficacy to their solo application at the recommended dose. These combinations resulted in a cent percent mortality rate and were deemed compatible with *L. lecanii*. Utilizing these combined applications can enhance control efficacy by decreasing the quantities applied, lowering the risk of environmental pollution, and mitigating the development of pest resistance.

CONSENT FOR PUBLICATION

All authors have agreed to publish this paper.

AVAILABILITY OF DATA AND MATERIALS

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

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ABBREVIATIONS

DC, Dispersible Concentrate; EC, Emulsifiable Concentrate; SC, Soluble Concentrate; SG, Soluble Granules; SDA, Sabouraud Dextrose Agar; WP, Wettable Powders