

Bio-efficacy of bio-rationals in acid lime (*Citrus aurantiifolia*) against citrus leaf miner (*Phyllocnistis citrella*)

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ABSTRACT

In order to assess the effectiveness of bio-rationals against citrus leaf miner (*Phyllocnistis citrella* Stainton), a field experiment was carried out at the College of Agriculture (University of Agricultural Sciences, Dharwad), Vijayapura, Karnataka, during the winter (rabi) 2020–21 and the rainy (kharif) season of 2021–22. Eight treatments were used in the randomised block design (RBD) experiment: bio digester solution (10%); pongamia leaf extract (5%); *Prosopis juliflora* leaf extract (5%); *Beauveria bassiana* (2 10⁸ conidia/g) (1 kg/ha); *Bacillus thuringiensis* 8 L (1 L/ha); neem-based insecticide (10000 ppm) (1.5 L/ha); thiamethoxam 25 wg (125 ml/ha) (standard check); and an untreated control (water spray), which was repeated three times. Two applications of neem (*Azadirachta indica*)-based insecticide, followed by bio-digester solution (10%), recorded significantly fewer live mines/shoot among the chosen bio-rationals. These treatments were found to be superior in controlling citrus leaf miner on acid lime [*Citrus aurantiifolia* (Christm.) Swingle]. *Beauveria bassiana*, *Bacillus thuringiensis* 8 L, and pongamia leaf extract were shown to be somewhat effective against this pest. The integrated control of citrus leaf miner on acid lime may make use of the bio-rationals that have been proven to be effective.

Keywords: *Phyllocnistis citrella*, acid lime, bio-rationals, bio-digester solution, citrus leaf miner, and pesticide based on neem

Introduction

Citrus aurantiifolia, or acid lime (Christm.) In India, swingle is a significant citrus crop. An economically significant pest of citrus and certain associated ornamental plants is the citrus leaf miner, *Phyllocnistis citrella* (Stainton) (Gracillariidae: Lepidoptera) (Clausen 1933, Kalshoven 1981, Grafton-Cardwell and Montez 2009). On the leaves, delicate twigs, and fruits, the larvae mine and take on a serpentine look. When an infestation occurs, the afflicted leaves begin to curl and dry. According to Grafton-Cardwell et al. (2008) and Dileepkumar et al. (2022), severe infestation might hinder the development of young plants and have an impact on older plants' productivity. Although environmental factors have a significant impact on growth and infestation levels, this pest completes a single generation in 14–17 days (Nawaz et al. 2021). Therefore, using pesticides repeatedly to suppress this pest may result in higher plant protection costs and the unfavourable issue of insecticide resistance (Amiri-Besheli 2009).

Many issues, including insecticide resistance, resurgence, residues in other habitats, and detrimental effects on non-target creatures, have been brought on by the widespread use of synthetic pesticides (Aktar et al. 2009). Acid lime fruits are highly sought after in the United Arab countries and the European Union (Veerendrakumar et al. 2017). In order to satisfy the needs of importing countries, it is crucial to produce fruits with zero or reduced pesticide residue. Globally, there is a lot of interest in the use of bio-rational pesticides made of natural materials, such as plant, animal, microbial, and mineral derivatives, to control insect pests. When it comes to controlling insect pests, the bio-rationals provide a secure and efficient substitute for synthetic pesticides. The production of pesticide-free food is greatly aided by the use of bio-rational pesticides, which are highly suited for use in organic food production (Isman 2006). The cultivation of pesticide-free fruits is crucial in the current situation since acid lime fruits are in high demand for export and are utilised for both daily consumption and fresh juice. In order to assess the effectiveness of bio-rationals against citrus leaf miner in acid lime, the current investigation was conducted.

MATERIALS AND METHODS

The field experiment was carried out in the College of Agriculture (University of Agricultural Sciences, Dharwad), Vijayapura (16°49'39.1620" N 75°43'31.1772" E), Karnataka, throughout the winter (rabi) of 2020–21 and the rainy (kharif) season of 2021–22. Eight treatments were used in the randomised block design (RBD) experiment: bio digester solution (10%); pongamia leaf extract (5%); *Prosopis juliflora* leaf extract (5%); *Beauveria bassiana* (2 10⁸ conidia/g) (1

kg/ha); *Bacillus thuringiensis* 8 L (1 litre/ha); neem-based insecticide (10000 ppm) (1.5 litre/ha); thiamethoxam 25 wg (125 ml/ha) (standard check); and an untreated control (water spray), which was repeated three times. With a row-to-row and plant-to-plant geometry of 6 m × 6 m, the acid lime crop (var. Kagzi lime) was cultivated using every approved package of practices (apart from plant protection measures). Two acid lime plants were tagged and regarded as a single replication. The purpose of the weekly observations was to determine the prevalence of pests. When pests were sufficiently prevalent on plants, treatments were applied. Using a backpack sprayer, two treatments were made every two weeks. Data collection and analysis: Five randomly chosen juvenile branches from various tree directions were used to capture the observations. The number of leaves with live citrus leaf miner larvae was counted from each shoot to determine the incidence of citrus leaf miner, and the average number of live mines per shoot was calculated. One day before to and three, five, and ten days after the application of treatments, pest density observations were made. Each spray's data was combined, converted, and then put through Duncan's multiple range test and ANOVA (Gomez and Gomez 1984). Additionally, the data was transformed into a percentage decrease in the pest population compared to the untreated control as follows (Henderson and Tilton 1955):

$$\text{Per cent reduction over control} = \left(1 - \frac{\frac{N \text{ in C before treatment} \times N \text{ in T after treatment}}{N \text{ in C after treatment} \times n \text{ in T before treatment}} \right) \times 100$$

where N, Population of live mines/shoot; C, Untreated control plot; T, Treated plot.

RESULTS AND DISCUSSION

Population of live mines per shoot: One day before to spraying during Rabi 2020–21, there was no discernible variation in the number of live mines per shoot between treatments. Table 1 shows that the average number of live mines per shoot varied from 10.17 to 10.54. Ten days after the first spray, plants treated with thiamethoxam 25 wg had much less live mines per shoot (3.36), which was comparable to neem-based insecticide 10000 ppm (3.42 live mines per shoot) and biodigester solution (4.00 live mines per shoot). With populations of 5.92, 6.08, and 6.19 live mines/shoot, respectively, the other treatments—pongamia leaf extract, *B. thuringiensis* 8 L, and *B. bassiana*—were shown to be somewhat successful in controlling citrus leaf miners. *P. juliflora* leaf extract (6.71) was shown to be the least efficient against pests among the chosen biorationals. Similarly, after a second spray, plots treated with neem-based insecticide (1.92) and bio digester solution (2.25) showed a much lower population of citrus leaf miners; these two treatments were shown to be on par with the standard check, thiamethoxam 25 wg (1.50), in controlling citrus leaf miners. Thiamethoxam 25 wg treatment significantly reduced the live mine population compared to the untreated control (70.49%), followed in decreasing order of toxicity by neem-based insecticide (59.77), bio digester solution (56.06), *B. thuringiensis* 8 L (40.37), *B. bassiana* (36.52), *P. juliflora* leaf extract (29.53), and pongamia leaf extract (32.19) treated plots. A consistent population of citrus leaf miners (10.04–10.75 live mines/shoot) was seen in the experimental plots throughout kharif 2021–2022 before treatments were imposed (Table 2). Ten days following the first spray, the number of citrus leaf miners decreased, ranging from 3.08 to 12.71 live mines/shoot. Both the neem-based insecticide-treated plot (3.17 live mines/shoot) and the bio digester solution-treated plot (3.83 live mines/shoot) had much less mines/shoot than the standard check, thiamethoxam 25 wg (3.08 live mines/shoot). Plots treated with pongamia leaf extract, *B. thuringiensis* 8 L, *B. bassiana*, and *P. juliflora* leaf extract showed 5.71, 5.83, 5.96, and 6.29 live mines/shoot, respectively. Similarly, during a second treatment cycle, plots treated with neem-based insecticide and bio digester solution showed 1.83 and 2.13 live mines/shoot, respectively, and the pest was well controlled. Plots treated with *B. thuringiensis* 8 L, pongamia leaf extract, *B. bassiana*, and *P. juliflora* leaf extract showed 3.67, 4.08, 4.34, and 4.96 live mines/shoot, respectively. Overall, following two rounds of treatment imposition, the standard check thiamethoxam 25 wg (70.74%) treatment showed the greatest percentage reduction in the live mine population. Neem-based insecticide (59.21), bio digester solution (54.26), *B. thuringiensis* 8 L (37.37), pongamia leaf extract (33.73), *B. bassiana* (33.71), and *P. juliflora* leaf extract (29.53) treated plots.

Table 3 displays the combined data (rabi 2020–21 and kharif 2021–22) about the effectiveness of biorationals against citrus leaf miner. Neem-based insecticide (1.88 live mines/shoot) and bio digester solution (2.19 live mines/shoot) were the two biorationals that were most successful in controlling citrus leaf miner on acid lime, according to data from two seasons. The effectiveness of the other treatments in reducing citrus leaf miners was fairly modest. Neem-based pesticide (59.53) and bio digester solution (55.22) were shown to be successful in controlling this pest, according to data on the decrease in live mine population over the untreated control plot. However, there was a considerable decrease in the population of live citrus leaf miner mines for other chosen biorationals, such as *B. thuringiensis* 8 L (38.90), *B. bassiana* (35.04), pongamia leaf extract (32.98), and *P. juliflora* leaf extract (31.06). The current investigation showed that citrus leaf miners on acid lime may be effectively controlled using neem-based pesticide (10,000 ppm) and bio digester solution (10%). Azadirachtin is present in the neem-based formulation as

Table 1 Efficacy of bio-rationals against citrus leaf miner infesting acid lime during *rabi* 2020–21

Treatment	First spray					Per cent ROV /shoot	Second spray					Per cent ROV
	Number of live mines						Number of live mines/shoot					
	1 DBS	3 DAS	5 DAS	10 DAS	Mean		1 DBS	3 DAS	5 DAS	10 DAS	Mean	
Bio digester solution	10.38 (3.30)	7.28 (2.79) ^a	6.08 (2.56) ^a	4.00 (2.10) ^a	5.79	49.13	6.04 (2.56) ^a	4.50 (2.23) ^b	3.17 (1.91) ^a	2.25 (1.65) ^a	3.31	56.06
Pongamia leaf extract	10.25 (3.28)	8.99 (3.08) ^b	7.92 (2.90) ^b	5.92 (2.53) ^b	7.61	32.29	5.83 (2.52) ^a	5.58 (2.47) ^c	4.96 (2.33) ^b	4.25 (2.18) ^{bc}	4.93	32.19
<i>Prosopis juliflora</i> leaf extract	10.54 (3.32)	9.21 (3.12) ^b	8.33 (2.97) ^b	6.71 (2.68) ^b	8.08	30.09	6.44 (2.63) ^a	5.92 (2.53) ^c	5.19 (2.39) ^b	5.08 (2.36) ^c	5.40	32.76
<i>Beauveria bassiana</i> (2 × 10 ⁸ conidia/g)	10.42 (3.30)	9.34 (3.14) ^b	8.71 (3.03) ^b	6.19 (2.59) ^b	8.08	29.28	6.53 (2.65) ^a	5.92 (2.53) ^c	5.08 (2.36) ^b	4.50 (2.24) ^{bc}	5.17	36.52
<i>Bacillus thuringiensis</i> 8 L	10.33 (3.29)	9.17 (3.10) ^b	8.46 (2.99) ^b	6.08 (2.57) ^b	7.90	30.26	6.67 (2.67) ^a	6.08 (2.56) ^c	4.92 (2.32) ^b	3.88 (2.09) ^b	4.96	40.37
Neem based insecticide (10000 ppm)	10.50 (3.32)	6.54 (2.65) ^a	5.29 (2.40) ^a	3.42 (1.98) ^a	5.08	55.88	5.96 (2.54) ^a	4.17 (2.16) ^b	2.88 (1.84) ^a	1.92 (1.55) ^a	2.99	59.77
Thiamethoxam 25 wg	10.17 (3.26)	5.96 (2.54) ^a	5.00 (2.34) ^a	3.36 (1.96) ^a	4.77	57.23	6.25 (2.60) ^a	3.08 (1.89) ^a	2.32 (1.68) ^a	1.50 (1.41) ^a	2.30	70.49
Untreated control	10.46 (3.31)	10.84 (3.37) ^c	11.08 (3.40) ^c	12.50 (3.60) ^c	11.47		11.33 (3.44) ^b	13.08 (3.68) ^d	14.25 (3.84) ^c	15.04 (3.94) ^d	14.13	
S.Em±	0.43	0.43	0.42	0.37			0.28	0.35	0.31	0.29		
CD (<i>P</i> =0.05)	NS	1.32	1.27	1.14			0.86	1.07	0.96	0.88		
CV (%)	7.32	9.01	9.59	10.84			7.17	10.15	10.32	10.49		

ROV, Reduction over control; DBS, Days before spray; DAS, Days after spray. The values in the parentheses are square root transformed.

Table 2 Efficacy of bio-rationals against citrus leaf miner infesting acid lime during *kharif* 2021–22

Treatment	Number of live mines/shoot					Per cent ROV	Number of live mines/shoot					Per cent ROV
	1 DBS	3 DAS	5 DAS	10 DAS	Mean		1 DBS	3 DAS	5 DAS	10 DAS	Mean	
	First spray						Second spray					
Bio digester solution	10.50 (3.32)	7.08 (2.75) ^a	5.67 (2.48) ^a	3.83 (2.08) ^a	5.53	52.38	6.28 (2.60) ^a	4.92 (2.33) ^{bc}	3.08 (1.89) ^b	2.13 (1.62) ^a	3.38	54.26
Pongamia leaf extract	10.04 (3.25)	8.92 (3.07) ^b	7.79 (2.88) ^b	5.71 (2.49) ^b	7.47	32.73	6.04 (2.56) ^a	5.38 (2.42) ^{cd}	4.67 (2.27) ^c	4.08 (2.14) ^b	4.71	33.73
<i>Prosopis juliflora</i> leaf extract	10.71 (3.35)	9.04 (3.09) ^b	8.21 (2.95) ^b	6.29 (2.61) ^b	7.85	33.73	6.38 (2.62) ^a	5.84 (2.52) ^{cd}	5.08 (2.36) ^c	4.96 (2.34) ^c	5.29	29.53
<i>Beauveria bassiana</i> (2×10 ⁸ conidia/g)	10.38 (3.30)	9.25 (3.12) ^b	8.33 (2.97) ^b	5.96 (2.54) ^b	7.85	31.62	6.50 (2.65) ^a	5.92 (2.53) ^{cd}	4.96 (2.34) ^c	4.34 (2.20) ^{bc}	5.07	33.71
<i>Bacillus thuringiensis</i> 8 L	10.46 (3.31)	9.04 (3.09) ^b	8.42 (2.99) ^b	5.83 (2.51) ^b	7.76	32.93	6.54 (2.65) ^a	6.00 (2.55) ^d	4.79 (2.30) ^c	3.67 (2.04) ^b	4.82	37.37
Neem based insecticide (10000 ppm)	10.63 (3.33)	6.42 (2.62) ^a	5.00 (2.34) ^a	3.17 (1.91) ^a	4.86	58.66	6.00 (2.54) ^a	4.08 (2.14) ^{ab}	2.71 (1.79) ^{ab}	1.83 (1.51) ^a	2.88	59.21
Thiamethoxam 25 wg	10.17 (3.27)	6.17 (2.58) ^a	4.92 (2.33) ^a	3.08 (1.89) ^a	4.72	58.04	6.42 (2.63) ^a	3.25 (1.92) ^a	2.08 (1.60) ^a	1.29 (1.34) ^a	2.21	70.74
Untreated control	10.75 (3.35)	11.42 (3.45) ^c	11.54 (3.47) ^c	12.71 (3.63) ^c	11.89		11.83 (3.51) ^b	12.83 (3.65) ^c	14.04 (3.81) ^d	14.88 (3.92) ^d	13.92	
S.Em±	0.48	0.53	0.44	0.35			0.33	0.35	0.31	0.27		
CD (<i>P</i> =0.05)	NS	1.61	1.35	1.06			1.01	1.07	0.96	0.84		
CV (%)	8.06	10.96	10.31	10.44			8.27	10.20	10.65	10.41		

ROV, Reduction over control; DBS, Days before spray; DAS, Days after spray. The values in the parentheses are square root transformed.

Table 3 Pooled data on efficacy of bio-rationals against citrus leaf miner infesting acid lime (*rabi* 2020–21 and *kharif* 2021–22)

Treatment	Number of live mines		First spray		Per cent ROV /shoot		Second spray					Per cent ROV
							Number of live mines/shoot					
	1 DBS	3 DAS	5 DAS	10 DAS	Mean	1 DBS	3 DAS	5 DAS	10 DAS	Mean		
Bio digester solution	10.44 (3.31)	7.18 (2.77) ^a	5.88 (2.52) ^b	3.92 (2.09) ^a	5.66	50.75	6.16 (2.58) ^a	4.71 (2.28) ^{bc}	3.13 (1.90) ^b	2.19 (1.64) ^b	3.34	55.22
Pongamia leaf extract	10.15 (3.26)	8.95 (3.07) ^b	7.86 (2.89) ^c	5.81 (2.51) ^b	7.54	32.52	5.94 (2.54) ^a	5.48 (2.44) ^{cd}	4.81 (2.30) ^c	4.17 (2.16) ^c	4.82	32.98
<i>Prosopis juliflora</i> leaf extract	10.63 (3.34)	9.13 (3.10) ^b	8.27 (2.96) ^c	6.50 (2.65) ^b	7.97	31.89	6.41 (2.63) ^a	5.88 (2.53) ^d	5.14 (2.37) ^c	5.02 (2.35) ^d	5.35	31.06
<i>Beauveria bassiana</i> (2 × 10 ⁸ conidia/g)	10.40 (3.30)	9.29 (3.13) ^b	8.52 (3.00) ^c	6.08 (2.56) ^b	7.96	30.47	6.51 (2.65) ^a	5.92 (2.53) ^d	5.02 (2.35) ^c	4.42 (2.22) ^{cd}	5.12	35.04
<i>Bacillus thuringiensis</i> 8 L	10.40 (3.30)	9.11 (3.10) ^b	8.44 (2.99) ^c	5.96 (2.54) ^b	7.83	31.61	6.61 (2.66) ^a	6.04 (2.55) ^d	4.86 (2.31) ^c	3.77 (2.07) ^c	4.89	38.90
Neem based insecticide (10000 ppm)	10.56 (3.33)	6.48 (2.64) ^a	5.15 (2.38) ^{ab}	3.29 (1.94) ^a	4.97	57.25	5.98 (2.54) ^a	4.13 (2.15) ^b	2.79 (1.81) ^{ab}	1.88 (1.54) ^{ab}	2.93	59.53
Thiamethoxam 25 wg	10.17 (3.27)	6.06 (2.56) ^a	4.96 (2.34) ^a	3.22 (1.93) ^a	4.75	57.57	6.33 (2.61) ^a	3.17 (1.91) ^a	2.20 (1.64) ^a	1.40 (1.37) ^a	2.26	70.51
Untreated control	10.61 (3.33)	11.13 (3.41) ^c	11.31 (3.44) ^d	12.61 (3.62) ^c	11.68		11.58 (3.48) ^b	12.96 (3.67) ^c	14.15 (3.83) ^d	14.96 (3.93) ^c	14.02	
S.Em±	0.35	0.42	0.27	0.26			0.26	0.27	0.27	0.25		
CD (<i>P</i> =0.05)	NS	1.29	0.83	0.81			0.79	0.84	0.82	0.76		
CV (%)	5.96	8.80	7.35	7.89			6.54	8.01	8.94	9.26		

ROV, Reduction over control; DBS, Days before spray; DAS, Days after spray. The values in the parentheses are square root transformed.

a key element with a potent insecticidal effect (Kilani-Morakchi et al. 2021). According to Chaudhary et al. (2017) and Michel et al. (2023), azadirachtin is a triterpenoid that causes insect death via a variety of mechanisms, including repellent, anti-feedant, interrupting normal growth and development by interfering with moulting, making the insects sterile, and discouraging adults from laying eggs. Azadirachtin's repellent and anti-feedant properties may have contributed to the neem-based insecticide's increased effectiveness over other chosen bio-rationals in the present investigation. According to Borad et al. (2001), Rao (2004), and Kumari (2006), using azadirachtin as a preventative and therapeutic strategy was successful in causing citrus leaf miner mortality, which supports the current results. Azadirachtin (10000 ppm) was also reported to be very efficient in reducing citrus leaf miner on citrus crops by Shareef et al. (2016), Gawhale et al. (2018), Ananda (2021), and Kumbhar et al. (2022). According to Jallow et al. (2018) and Buragohain et al. (2021), various lepidopteran leaf miner pests of crops significantly died when a neem-based formulation was applied. Additionally, it was discovered that the bio-digester solution was very successful in controlling citrus leaf miner on acid lime. Cow dung, cow urine, mixes of organic wastes, and leaves from neem, vitex, lantana, custard apple, and agave were used to make the bio digester solution (Babalad et al. 2010). Before being used, the organic mixers were given two to three weeks to decompose. During this time, they may have undergone fermentation and produced alcohol, which might explain why the bio digester solution is more effective than other bio-rationals. Furthermore, neem and vitex leaves are known to have potent insecticidal qualities, which may provide compelling proof of the bio digester solution's effectiveness against citrus leaf miner on acid lime. In a similar vein, Biradar (2014) and Kattebennuru (2017) discovered that a bio digester solution may lessen the infestation of leaf miners on acid lime.

On acid lime, *Bacillus thuringiensis* 8 L foliar spray was shown to be somewhat successful in controlling citrus leaf miner. The current results closely match those of Besheli (2007), who found that after 96 hours of exposure in a lab setting, *B. thuringiensis* caused 49.08% of citrus leaf miner larvae mortality. Additionally, *B. thuringiensis* (0.2%) was shown to be more efficient against citrus leaf miners infesting Kagzi lime by Bhut et al. (2013). When *B. thuringiensis* was used as a therapeutic strategy to reduce citrus leaf miner, Rana (2020) found that 20.27–20.44% of *P. citrella* larvae died. In contrast, Kumari (2006) discovered that *B. thuringiensis* was very successful in reducing the number of citrus leaf miner larvae by 96.76% in a lab setting. The wide range in effectiveness of *B. thuringiensis* may be the cause of *B. thuringiensis* on citrus leaf miner. In order to effectively manage insect pests, *B. thuringiensis* also needs perfect environmental conditions, particularly relative humidity. While many factors are discovered to impact the effectiveness of *B. thuringiensis* in reducing citrus leaf miners in field circumstances, the presence of consistent conditions under laboratory conditions may have improved the efficacy in previous publications.

To solve the issues of pesticide resistance, resurgence, residues, and contamination of different environmental compartments, it is essential to look for environmentally benign insect pest control solutions. One of the major fruit crops with a lot of export potential is acid lime. By using bio-rationals to control citrus leaf miners, residual issues may be resolved and excellent fruit production can be achieved.

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