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

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ABSTRACT

The field experiment was conducted during winter (*rabi*) 2020–21 and rainy (*kharif*) season of 2021–22 at College of Agriculture (University of Agricultural Sciences, Dharwad), Vijayapura, Karnataka to evaluate efficacy of bio-rationals against citrus leaf miner (*Phyllocnistis citrella* Stainton). The experiment was conducted in a randomized block design (RBD) with 8 treatments, viz. bio digester solution (10%); pongamia leaf extract (5%); *Prosopis juliflora* leaf extract (5%); *Beauveria bassiana* (2×10^8 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 untreated control (water spray), replicated thrice. Among the selected bio-rationals, two application of neem (*Azadirachta indica*)-based insecticide recorded significantly lesser number of live mines/shoot followed by bio-digester solution (10%) and these treatments were found superior in controlling citrus leaf miner on acid lime [*Citrus aurantiifolia* (Christm.) Swingle]. The pongamia leaf extract, *Bacillus thuringiensis* 8 L and *Beauveria bassiana* were proved to be moderately affective against this pest. The bio-rationals found effective can be used in integrated management of citrus leaf miner on acid lime.

Keywords: Acid lime, Bio-rationals, Bio-digester solution, Citrus leaf miner, Neem-based insecticide, *Phyllocnistis citrella*

Acid lime [*Citrus aurantiifolia* (Christm.) Swingle] is an important citrus crop grown in India. The citrus leaf miner, *Phyllocnistis citrella* (Stainton) (Gracillariidae: Lepidoptera) is an economically important pest of citrus and some related ornamental plants (Clausen 1933, Kalshoven 1981, Grafton-Cardwell and Montez 2009). The larvae mine and form serpentine like appearance on the leaves, tender twigs and fruits. The affected leaves start to curl and dry upon infestation. Severe infestation can retard the growth of young plants and may affect the production on mature plant (Grafton-Cardwell *et al*. 2008, Dileepkumar *et al*. 2022). This pest completes single generation in 14–17 days, however environmental variables greatly influence development and infestation levels (Nawaz *et al*. 2021). So, the control of this pest revolves around application of insecticides for multiple times, it may lead to increased cost of plant protection and cause adverse problem of insecticide resistance (Amiri-Besheli 2009).

The rampant use of synthetic insecticides has led to several problems such as insecticide resistance, resurgence, residues in various ecosystems and negative impact on non-target organisms (Aktar *et al*. 2009). The fruits of acid lime possess high demand in European Union and United Arab nations (Veerendrakumar *et al*. 2017). The production of fruits with nil or less pesticide residue is off prime importance in meeting demands of importing nations. The use of bio-rational insecticides composed of natural products including derivatives of plants, animals, microbes and mineral in insect pest management is getting lot of attention worldwide. The bio-rationals offers a safe and effective alternative to synthetic insecticides in control of insect pests. The bio-rational insecticides are well suited for use in organic food production and play a much greater role in the production of pesticide free food (Isman 2006). Since the acid lime fruits having export demand and used for fresh juice and daily consumption purpose, the production of pesticides free fruits assumes paramount importance in the present context. In this regard the present study was carried out to evaluate the efficacy of bio-rationals against citrus leaf miner in acid lime.

MATERIALS AND METHODS

The field experiment was conducted during winter (*rabi*) 2020–21 and rainy (*kharif*) season of 2021–22 at College of Agriculture (University of Agricultural Sciences, Dharwad), Vijayapura (16°49'39.1620" N

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75°43'31.1772" E), Karnataka. The experiment was laid out in randomized block design (RBD) consisted of 8 treatments, viz. bio digester solution (10%); pongamia leaf extract (5%); *Prosopis juliflora* leaf extract (5%); *Beauveria bassiana* (2×10^8 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 untreated control (water spray), replicated thrice. The acid lime crop (var. Kagzi lime) was grown with all the package of practice (except plant protection measures) recommended with row to row and plant to plant geometry of 6 m × 6 m. Two acid lime plants were considered as one replication and tagged. The weekly observations were made to check for incidence of pest. Treatments were imposed when pest reached sufficient incidence levels on plants. Two applications were taken up with the help of knapsack sprayer at fortnightly interval.

Data recording and analysis: The observations were recorded from 5 randomly selected young shoots per tree from different direction of the tree. To record the incidence of citrus leaf miner, from each shoot, number of leaves having live citrus leaf miner larvae were counted, and average number of live mines per shoot was worked out. The observations on pest density were recorded one day before and three, five and ten days after imposition of treatments. The data of each spray was pooled and later transformed data was subjected to ANOVA and Duncan's multiple range test (Gomez and Gomez 1984). Further, obtained data were converted into per cent reduction of pest population over untreated control as (Henderson and Tilton 1955):

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

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

RESULTS AND DISCUSSION

Population of live mines per shoot: During *rabi* 2020–21, at one day before spray a non-significant difference was observed among treatments in terms of number of live mines/shoot. The average number of live mines ranged from 10.17–10.54/shoot (Table 1). At 10 days after first spray, a significantly lesser number of live mines/shoot were recorded in thiamethoxam 25 WG treated plants (3.36 live mines/shoot) and which was found on par with neem-based insecticide 10000 ppm (3.42 live mines/shoot) and bio digester solution (4.00 live mines/shoot). The other treatments, viz. pongamia leaf extract, *B. thuringiensis* 8 L and *B. bassiana* were found to be moderately effective in control of citrus leaf miner with population of 5.92, 6.08 and 6.19 live mines/shoot, respectively. Among the selected biorationals, *P. juliflora* leaf extract (6.71) was found to be least effective against pest. Similarly, after second spray, significantly less population of citrus leaf miner was observed in neem-based insecticide (1.92) and bio digester solution (2.25) treated plots, and these two treatments were found on par with standard check, thiamethoxam 25 WG (1.50) in controlling citrus leaf miner. The data on per cent reduction in live mine population over untreated control indicated a significantly higher reduction in thiamethoxam 25 WG (70.49%) treatment, which was followed 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 in a decreasing order of toxicity.

During *kharif* 2021–22, prior to the imposition of treatments, a uniform population of citrus leaf miner was observed in the experimental plots (10.04–10.75 live mines/shoot) (Table 2). The application of treatments resulted in reduction in population of citrus leaf miner ranging from 3.08–12.71 live mines/shoot at 10 days after first spray. A significantly less mines/shoot was observed in neem-based insecticide treated plot (3.17 live mines/shoot) and bio digester solution treated plot (3.83 live mines/shoot), and these were found on par with standard check, thiamethoxam 25 WG (3.08 live mines/shoot). The pongamia leaf extract, *B. thuringiensis* 8 L, *B. bassiana* and *P. juliflora* leaf extract treated plots recorded 5.71, 5.83, 5.96 and 6.29 live mines/shoot, respectively. Similarly, after second round of application, neem-based insecticide and bio digester solution treated plots recorded 1.83 and 2.13 live mines/shoot, respectively and resulted in good control of the pest. Other treatments, viz. *B. thuringiensis* 8 L, pongamia leaf extract, *B. bassiana* and *P. juliflora* leaf extract treated plots recorded 3.67, 4.08, 4.34 and 4.96 live mines/shoot respectively. Overall, after two rounds of imposition of treatments, the higher per cent reduction in live mine population was found in standard check thiamethoxam 25 WG (70.74%) treatment, which was followed by 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.

The pooled data (*rabi* 2020–21 and *kharif* 2021–22) on efficacy of biorationals against citrus leaf miner is presented in Table 3. It is clearly evident from two seasons data that, among the selected biorationals, neem-based insecticide (1.88 live mines/shoot) and bio digester solution (2.19 live mines/shoot) were more effective in managing citrus leaf miner on acid lime. The other treatments recorded comparatively moderate efficacy in controlling citrus leaf miner. The data on reduction of live mine population over untreated control plot also depicted that; neem-based insecticide (59.53) and bio digester solution (55.22) were proved to be effective in reducing this pest. However, other selected biorationals, viz. *B. thuringiensis* 8 L (38.90), *B. bassiana* (35.04), pongamia leaf extract (32.98) and *P. juliflora* leaf extract (31.06) were recorded moderate reduction in population of live mines of citrus leaf miner.

The present study demonstrated that neem-based insecticide (10000 ppm) and bio digester solution (10%) were effective in control citrus leaf miner on acid lime. The neem-based formulation contains azadirachtin as

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

Treatment	First spray					Per cent ROV	Second spray					Per cent ROV
	Number of live mines/shoot						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
Prosopis juliflora 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
Beauveria bassiana (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
Bacillus thuringiensis 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 (P=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
Prosopis juliflora 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
Beauveria bassiana (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
Bacillus thuringiensis 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)^{e}	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 (P=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	First spray					Per cent ROV	Second spray					Per cent ROV
	Number of live mines/shoot						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
Prosopis juliflora 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
Beauveria bassiana (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
Bacillus thuringiensis 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)^{e}	14.15 (3.83)^{d}	14.96 (3.93)^{e}	14.02	
S.Em±	0.35	0.42	0.27	0.26			0.26	0.27	0.27	0.25		
CD (P=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 principle component which is known to have strong insecticidal property (Kilani-Morakchi *et al.* 2021). The triterpenoid, azadirachtin would induce mortality of insect by various modes of actions, viz. repellent, anti-feedant, disrupting normal growth and development by interfering with moulting, make the insects sterile and deter adults from laying eggs (Chaudhary *et al.* 2017, Michel *et al.* 2023). The repellent and anti-feedant action of azadirachtin might have helped in improving efficacy of neem-based insecticide over other selected bio-rationals in the current study. The present findings are supported by Borad *et al*. (2001), Rao (2004) and Kumari (2006), as they suggested that application of azadirachtin as prophylactic and curative measure was effective in inducing mortality of citrus leaf miner. Similarly, Shareef *et al*. (2016), Gawhale *et al*. (2018), Ananda (2021) and Kumbhar *et al*. (2022) also found that azadirachtin (10000 ppm) highly effective in controlling citrus leaf miner on citrus crops. Jallow *et al*. (2018) and Buragohain *et al*. (2021) reported, application of neem-based formulation caused significant mortality of other lepidopteran leaf miner pests of crops.

The bio-digester solution was also found significantly effective in control of citrus leaf miner on acid lime. The bio digester solution was prepared with cow dung, cow urine, mixtures of organic wastes and leaves of neem, vitex, lantana, custard apple and agave (Babalad *et al*. 2010). The organic mixers were allowed for decomposition for 2–3 weeks before use, during the process of preparation it may undergo fermentation and led to production of alcohol, so it is may be the possible reason for improved efficacy of bio digester solution over other bio-rationals. In addition, the leaves of neem and vitex are known to have strong insecticidal properties, so it may also provide strong evidence for efficacy of bio digester solution against citrus leaf miner on acid lime. Similarly, Biradar (2014) and Kattebennuru (2017) found bio digester solution can reduce leaf miner infestation on acid lime.

The foliar spray of *Bacillus thuringiensis* 8 L was found moderately effective in control of citrus leaf miner on acid lime. The present findings are in close conformity with Besheli (2007) who reported that *B. thuringiensis* recorded 49.08% larval mortality of citrus leaf miner after 96 h of exposure under laboratory condition. Bhut *et al*. (2013) also reported that *B. thuringiensis* (0.2%) was more effective against citrus leaf miner infesting Kagzi lime. Rana (2020) recorded 20.27–20.44% mortality of larvae of *P. citrella* when *B. thuringiensis* was applied as curative measure for control of citrus leaf miner. On the contrary, Kumari (2006) found that *B. thuringiensis* was highly effective and recorded 96.76% reduction in larval population of citrus leaf miner under laboratory condition. The large variation in efficacy of *B. thuringiensis* on citrus leaf miner may be due to strength of spores present in formulation used during the study. *B. thuringiensis* also requires ideal climatic conditions mainly relative humidity for better control of insect pests. Under laboratory conditions, existence of uniform conditions may have enhanced the efficacy in the earlier reports, while in field condition various factor are found to influence which may affect the efficacy of *B. thuringiensis* in controlling citrus leaf miner.

It is very much essential to search for eco-friendly products for the management of insect pest to overcome the problems of insecticide resistance, resurgence, residues and pollution of various environmental compartments. Acid lime is one of the important fruit crops having enormous export opportunities, the use of bio-rationals in management of citrus leaf miner helps to overcome problems of residues and at the same time one can harvest good production of the fruits.

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