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Bio-efficacy of liquid bio-pesticides against major insect pests in Citrus nursery under Siang Valley of Arunachal Pradesh

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ABSTRACT: The field experiment on bioefficacy of biopesticides for the management of three major insect pests of citrus nursery *viz.,* citrus butterfly, leaf miner and whitefly was carried out on rough lemon seedlings at College of Horticulture and Forestry, Pasighat, Arunachal Pradesh during 2022-23. The highest efficacy against citrus butterfly caterpillar was observed in *Bacillus thuringiensis* after 4th spray at 7 and 14 days after spraying (DAS) in both parameters of population (0.80 and 0.66/plant) and defoliation percentage (3.00% and 3.90%), respectively. The entomopathogenic fungus *Lecanicillium lecanii* effectively reduced whitefly incidence to 3.10/ plant after the fourth spray at 14 days after sowing (DAS). Additionally, the botanical Azadirachtin at 1500 ppm concentration, applied at a rate of 2ml/ liter of water, significantly decreased leaf miner incidence by 11.40 percent at 7 DAS.

Keywords: Biopesticides, citrus nursery, insect pests, *Metarhizium anisopliae,* rough lemon.

INTRODUCTION

The citrus industry plays a vital role in global agriculture, providing substantial income and nutrition. Arunachal Pradesh stands out among the northeastern states of India for its significant production of Khasi Mandarin. The prosperity of citrus orchards hinges on the availability of high-quality seedlings from healthy nurseries. However, in recent years, citrus nursery pests have seriously threatened successful citrus tree propagation. The Siang Valley, a prominent Khasi Mandarin production area in Arunachal Pradesh, grapples with major incidences of citrus butterfly (*Papilio* spp.), whitefly (*Bemisia tabaci*), and leaf miner (*Phyllocnistis citrella*), resulting in significant crop losses. The *P. citrella* (4.00 to 49.27%) and *Papilio* spp. (3.32 to 27.89%) have been documented to cause substantial damage to Khasi Mandarin in Assam (Deka *et al*., 2016), while Krishna et al. (2021) reported yield losses of 30.70 to 53.20% in East Khasi Hills of Meghalaya due to *P. citrella* infestations.

Although synthetic chemical pesticides have been traditionally used to combat these pests, their widespread application has raised concerns about environmental pollution, pest resistance, and resurgence. Additionally, many farmers practicing natural or organic farming methods favor non-chemical solutions. Consequently, there is growing interest in adopting biopesticides for managing citrus nursery pests. Biopesticides, encompassing microorganisms, botanicals, and biorationals, offer environmentally friendly and sustainable alternatives to chemical pesticides (Rani *et al*., 2021). Derived from natural sources, they often exert minimal impact on non-target organisms and the surrounding ecosystem (Keerthi et al., 2022). With minimal residual effects and reduced harm, they are particularly suitable for pest management, especially in organic ecosystems of the northeastern region of India. Entomopathogenic fungi are considered crucial biological control agents for insect populations (Sharma and Malik, 2012). Moreover, these fungal agents possess biodegradable properties (Mishra et al., 2020). They could serve as viable alternatives to neem-based pesticides for managing pests in organic or natural citrus nursery production systems in Arunachal Pradesh.

Table 1. Efficacy of biopesticides against citrus butterfly and whiteflies

Treatments	Treatments	Dose	Number of Citrus butterfly caterpillar/seedling								Number of Whiteflies/seedling							
			1st spray		2nd spray		3rd spray		4th spray		1st spray		2nd spray		3rd spray		4th spray	
			7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TB_0	Control (Water spray)	-	3.60	4.10	4.40	3.98	3.85	3.68	3.15	2.90	9.85	11.90	13.60	15.10	16.80	18.10	15.80	15.50
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml)	5.0ml/L	1.95	2.02	1.73	1.80	1.41	1.48	1.14	1.08	7.50	7.95	6.20	6.44	5.66	4.96	4.80	4.60
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml)	5.0ml/L	1.96	2.04	1.75	1.86	1.43	1.50	1.16	1.10	6.80	7.15	5.40	5.60	4.10	4.25	3.50	3.65
TB_3	*Lecanicillium lecanii* Vl- 8 (1×10^8 spores/ml)	5.0ml/L	1.80	2.30	2.05	2.50	1.85	2.02	1.80	1.92	7.51	7.60	6.50	6.80	4.80	4.95	3.40	3.10
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10^8 cfu/ml)	5.0ml/L	1.20	1.50	1.10	1.30	1.05	0.95	0.80	0.66	8.90	9.80	10.50	12.60	14.90	14.60	13.40	13.10
TB_5	Azadirachtin 1500 ppm	2ml/L	1.75	1.90	1.30	1.45	1.12	1.08	0.96	0.85	8.01	8.29	7.05	7.50	6.90	7.05	6.05	6.15
TB_6	Thiamethoxam 25WG	0.6ml/L	0.90	1.05	0.60	0.95	0.66	0.45	0.00	0.00	5.20	5.60	4.05	4.18	3.10	3.15	2.50	2.35
SE(m)±			0.02	0.01	0.04	0.02	0.04	0.05	0.02	0.01	0.07	0.06	0.09	0.09	0.09	0.10	0.11	0.09
C.D. (5%)			0.08	0.03	0.14	0.08	0.13	0.15	0.06	0.03	0.21	0.20	0.27	0.29	0.27	0.31	0.34	0.30
CV (%)			2.68	1.20	5.02	2.72	4.79	5.60	2.56	1.52	4.14	3.77	5.45	5.55	5.38	6.04	7.21	6.36

DAS: Days after spraying

MATERIALS AND METHODS

The present investigation was conducted at Fruit Nursery, Department of Fruit Science, College of Horticulture and Forestry, Pasighat located under Siang Valley of Arunachal Pradesh. The study employed a completely randomized design; with each of seven treatments being replicated thrice. The treatments were TB_0 (Control water spray), TB_1 (*Metarhizium anisopliae* NBAIR-Ma 35 ($1×10^8$ spores/ml) @ 5.0ml/L), TB_2 (*Beauveria bassiana* NBAIR Bb-45 ($1×10^8$ spores/ml) @ 5.0ml/L), TB_3 (*Lecanicillium lecanii* Vl- 8 ($1×10^8$ spores/ml) @ 5.0ml/L), TB_4 (*Bacillus thuringiensis* NBAIR BTG4 ($2×10^8$cfu/ml) @ 5.0ml/L), TB_5 (Azadirachtin 1500 ppm @ 2ml/L), TB_6 (Thiamethoxam 25 WG @ 0.6ml/L water). Rough lemon seedlings, with heights ranging from 8 to 10cm, were transplanted into pre-prepared polybags measuring 21×10 cm, with a capacity ranging from 1.20 to 1.30 kg. Various insect pest parameters such as the number of citrus butterfly caterpillars per plant, the number of whiteflies per plant, defoliation caused by citrus butterfly, incidence of leaf miner, number of natural enemies per plant, and plant vegetative parameters like plant height, internodal length, and number of leaves were meticulously recorded. Additionally, biochemical parameters including total chlorophyll (measured according to Arnon, 1949), carotenoids (also measured according to Arnon, 1949), total carbohydrates (following the methods outlined by Hedge and Hofreiter, 1962), and cellulose (analyzed using the procedures described by Updegraff, 1969) were assessed utilizing standard protocols.

Application of biopesticides

Treatments were given within the experimental framework at intervals of 15 days, and observations were carefully planned for the physical parameters for the 7th and 14th day after each treatment application. Observations were recorded 15 days after treatment for vegetative parameters and 25-day intervals for the biochemical parameters. The necessary solution amounts were determined by first spraying with water. A 500 ml solution was carefully made for each treatment plan and then given to a set of thirty plants. The quantity of biopesticide was precisely pipetted into a 500 ml laboratory-grade beaker, and the volume was then adjusted. Afterward, a sprayer was used to distribute the prepared solution evenly throughout the plant population. By ensuring uniform application, the methodological approach preserved the reproducibility and rigor of the experiments. To determine the significance of treatments, the percentage of pest infestation data were transformed into arcsine-transformed values, and the data on the number of pests were transformed into square root transformed values. The statistical analysis was done using OP stats software.

RESULTS AND DISCUSSION

The data displayed in the table 1 indicates that, the number of citrus butterfly caterpillar ranges from 0.00 to 4.40. The Thiamethoxam 25 WG @ 0.6ml/L was successful in reduction of citrus butterfly caterpillar to zero occurrence at the 7 and 14 DAS of 4th spray. The treatment *Bacillus thuringiensis* NBAIR BTG4 @ 5.0ml/L (0.80 and 0.66, respectively) recorded with significantly lower caterpillars among the biopesticides during 7 and 14 DAS of the 4thspray. The lower infestation may be attributed to the toxin produced by bacteria (*Bt*), a crystallized protein that creates perforations in the insect's gut lining upon ingestion. The insects eventually cease to feed and ultimately lead to death. Similar results were also documented in the studies conducted by Kalita *et al.* in 2015 and Swadener in 1994. The superiority of *Bt* over other treatments tested is also in agreement with Narayanamma and Savithri (2003).

Meanwhile at 14 DAS of the 4th spray, thiomethoxam 25WG @0.6 ml/L treatment imparted the lowest occurrence of whiteflies (2.35/seedling) (Table 1). Among the biopesticides, the EPF *Lecanicillium lecanii* Vl-8@ 5.0 ml/L was found to be the most successful treatment at 14 DAS with only 3.10 whiteflies per seedling. The *Beauveria bassiana* NBAIR Bb-45@ 5.0 ml/L was shown to be the second most effective treatment at 7 DAS of the fourth spray (3.50/seedling). The lowest population with thiomethoxam treated plants corroborates with the result of Kumar *et al.* (2017) in the study on efficacy of insecticides against whitefly in brinjal and revealed that application of thiomethoxam 25 WG @ 100g/ha gives zero population at the 3rd day of the 4th spray and Yadav *et al.* (2015) studied the efficacy of insecticides and bio-pesticides against sucking pest in black gram and found out thiomethoxam 25 WG @ 0.25g/L resulted best by reducing the whiteflies population from 2.33-3.60 to 0.11/3 leaves after 2nd spray. The result with biopesticides is consistent with research from by Kalita *et al.* (2015), Raghunandan *et al.* (2018) and Javed *et al.* (2019) documented that the enzymes and toxins released

Table 2. Efficacy of biopesticides on defoliation by citrus butterfly and incidence of leaf miner

Treatments	Treatments	Dose	Defoliation by citrus butterfly caterpillar (%)								Incidence of leaf miner (%)							
			1st spray		2nd spray		3rd spray		4th spray		1st spray		2nd spray		3rd spray		4th spray	
			7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TB_0	Control (Water spray)	-	23.70 (29.1)	25.00 (29.9)	27.50 (31.6)	29.80 (33.0)	33.10 (35.1)	32.05 (34.4)	29.50 (32.8)	26.10 (30.7)	35.10 (36.32)	36.90 (30.97)	28.50 (32.25)	31.40 (30.64(	29.20 (32.69)	30.00 (26.69)	24.70 (29.79)	26.20 (23.08)
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10⁸ spores/ml)	5.0ml/L	13.30 (21.3)	15.80 (23.4)	10.80 (19.1)	12.10 (20.3)	11.50 (19.8)	13.70 (21.7)	12.05 (20.3)	11.75 (20.0)	22.70 (37.39)	23.90 (29.72)	21.8 (34.06)	23.50 (29.52)	15.30 (33.19)	16.40 (25.39)	13.80 (22.37)	12.50 (17.25)
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10⁸ spores/ml)	5.0ml/L	15.50 (23.1)	15.90 (23.4)	11.50 (19.8)	13.50 (21.5)	11.75 (20.0)	14.50 (22.3)	13.15 (21.2)	14.50 (22.3)	25.20 (28.43)	26.10 (30.51)	23.50 (27.81)	25.90 (30.38)	19.50 (23.01)	20.10 (25.90)	14.50 (30.78)	15.10 (19.99)
TB_3	*Lecanicillium lecanii* Vl- 8 (1×10⁸ spores/ml)	5.0ml/L	15.70 (23.2)	18.00 (25.0)	16.50 (23.9)	20.75 (27.0)	18.60 (25.5)	22.50 (28.3)	18.10 (25.1)	20.10 (26.6)	20.70 (30.71)	22.60 26.69)	17.60 (30.58)	19.20 (24.64)	18.90 (26.62)	16.75 (21.95)	13.70 (20.35)	12.10 (19.71)
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10⁸ cfu/ml)	5.0ml/L	11.20 (19.5)	11.70 (19.9)	7.10 (15.3)	8.70 (17.1)	4.20 (11.8)	5.30 (13.2)	3.00 (9.93)	3.90 (11.3)	25.60 (29.25)	26.50 (27.05)	23.40 (28.98)	26.00 (24.79)	19.60 (23.87)	20.20 (25.76)	14.70 (22.85)	15.40 (22.53)
TB_5	Azadirachtin 1500 ppm	2ml/L	11.50 (19.8)	11.90 (20.1)	8.40 (16.8)	9.20 (17.6)	5.30 (13.2)	6.00 (14.1)	3.30 (10.7)	4.10 (11.6)	24.60 (30.12)	25.80 (28.37)	24.30 (28.98)	25.60 (25.96)	18.40 (26.19)	19.10 (24.14)	11.40 (21.80)	11.70 (16.53)
TB_6	Thiamethoxam 25WG	0.6ml/L	10.20 (18.61)	10.40 (18.78)	6.80 (15.1)	7.20 (15.5)	3.80 (11.1)	4.20 (11.7)	1.50 (6.95)	2.00 (8.06)	20.20 (30.38)	22.70 (28.44)	17.40 (28.91)	16.50 (23.95)	14.00 (26.25)	10.50 (18.89)	8.80 (21.71)	6.50 (14.76)
SE(m)±			0.67	0.58	0.60	0.62	0.56	0.62	0.57	0.56	0.43	0.40	0.43	0.44	0.48	0.47	0.28	0.41
C.D(5%)			2.06	1.78	1.85	1.90	1.73	1.92	1.74	1.73	1.32	1.24	1.34	1.36	1.49	1.449	0.877	1.26
CV (%)			5.28	4.393	5.17	4.94	5.02	5.22	5.45	5.23	2.36	2.44	2.51	2.85	3.07	3.39	2.04	3.72

DAS: Days after spraying, Figures in the parentheses are arc sine transformed values

Table 3. Efficacy of biopesticides on natural enemies in citrus nursery

Number of natural enemies (spiders) per seedling										
Trt. No.	**Treatments**	**Dose**	**1st spray**		**2nd spray**		**3rd spray**		**4th spray**	
			7 DAS	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**	**7 DAS**	**14 DAS**
TB_0	Control (Water spray)	-	1.90	2.20	2.40	2.70	2.80	2.40	2.10	1.90
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml)	5.0ml/L	1.45	1.53	1.66	1.85 (1.5	1.90	1.74	1.53	1.35
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml)	5.0ml/L	1.56	1.66	1.53	1.83	1.75	1.66	1.41	1.29
TB_3	*Lecanicillium lecanii* Vl-8 (1×10^8 spores/ml)	5.0ml/L	1.48	1.63	1.33	1.45	1.65	1.60	1.45	1.33
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10^8 cfu/ml)	5.0ml/L	1.43	1.51	1.33	1.75	1.85	1.75	1.66	1.51
TB_5	Azadirachtin 1500 ppm	2ml/L	1.35	1.50	1.43	1.66	1.53	1.38	1.33	1.21
TB_6	Thiamethoxam 25WG	0.6ml/L	0.70	0.80	0.90	0.70	0.40	0.30	0.10	0.00
SE(m)±			0.03	0.06	0.06	0.06	0.06	0.04	0.04	0.03
C.D (5%)			0.11	0.19	0.18	0.20	0.19	0.13	0.13	0.10
CV (%)			4.35	7.09	6.21	7.481	6.28	5.26	4.87	4.10

DAS: Days after spraying

by *L. lecanii* and *B. bassiana* spores penetrate the body of the insect through spiracles and cause internal organ and tissue damage. The population of infected whiteflies often declined as a result of the infected individuals' reduced ability to reproduce.

Data pertaining to defoliation by citrus butterfly and incidence of leaf miner are shown in table 2. From the table it is confirmed that the most effective treatment for defoliation by citrus butterfly was observed in thiamethoxam 25WG @ 0.6ml/L with 1.50 and 2.00 percent at 7 and 14 DAS of the 4^{th} spray, respectively. With regards to biopesticides the *B. thuringiensis* NBAIR BTG4 @ 5.0ml/L (3.00% and 3.90%) was recorded with least defoliation followed by TB_5 (Azadirachtin 1500 ppm @ 2ml/L) (3.30% and 4.10%) at 7 and 14 DAS of the 4^{th} spray. This parameter correlates with the number of citrus butterfly caterpillar per plant. Since the population of caterpillar reduces due to the effect of Bt the percentage of defoliation eventually reduces.

Regarding the leaf miner the most effective treatment was in chemical treatment thiamethoxam 25WG @ 0.6ml/L with 8.80 and 6.50 percent incidence respectively, at 7 and 14 DAS of 4^{th} spray followed by the treatment Azadirachtin 1500 ppm @ 2ml/L *i.e.,* 11.40 and 11.70 percent incidence. Among all the treatments tested, thiomethoxam was found the most superior and this results are in agreement with Shinde *et al.* (2017) which evaluated different insecticides against citrus leaf miner in Nagpur Mandarin and reveals that thiomethoxam 25 WG (0.06%) recorded lowest leaf infestation percentage (5.47) and likewise Farmanullah and Gul (2005) evaluated six different insecticides for the control of citrus psylla and reveals that the percent decrease in population of *D. citri* was highest in thiomethoxam 25 WG (72.20 %) in first spray and (83.54 %) in the 2nd spray. Similar outcomes regarding biopesticides are also noted by Kalita *et al.* (2015) and Abebe (2019) while using azadirachtin and neem oil, both of which have insect-repelling qualities. These materials coat plants with oil particles, which create a barrier that discourages female moths from choosing to lay their eggs. This reduces infestation and population. Moreover, Azadirachtin hinders insect larvae's ability to moult and develop, which keeps them from maturing and reproducing, which stops the pest population from growing.

The data in the table 3 depicts the occurrence of natural enemies mainly spiders present per seedlings. During 7 and 14 DAS of last spray application the maximum number of spiders was recorded in control treatment of water spray (2.10 and 1.90, respectively) followed by *B. thuringiensis* NBAIR BTG4@ 5.0 ml/L (1.66 and 1.51, respectively) and *M. anisopliae* NBAIR-Ma 35@ 5.0 ml/L (1.53 and 1.35, respectively). Considering the aforementioned findings during the investigation, it was noticed that the literature on this aspect was found negligible. However, increased activity of natural enemies, which often follows the pest building pattern under normal agro-ecosystems, can be related to the decreased occurrence of pests during the fourth spray. This study demonstrates that Bacillus thuringiensis NBAIR BTG4 (at a concentration of 2×10^8 cfu/ml) at 5.0ml/L, *Lecanicillium lecanii* Vl-8 (with 1×10^8 spores/ml) at 5.0ml/L, and Azadirachtin at 1500 ppm (applied at 2ml/L) were effective in reducing the populations of citrus butterfly caterpillars, whiteflies, and leaf miners, respectively.

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