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Sustainable practice for the management of guava mealybug, *Ferrisia virgata* with the combined effect of insecticides and entomopathogenic fungi

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ABSTRACT

The study was carried out during 2022 and 2023 at G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand to evaluate the insecticides, entomopathogenic fungi (fungal bioagent) and their tank mixed combinations against mealybug, *Ferrisia virgata* (Cockerell) at fruiting stage of guava, *Psidium guajava* L. A total of 12 treatments including untreated control were taken and were categorized as chemical (insecticides), biological (entomopathogenic fungi) and chemical + biological (tank mixed combinations). It was found that treatment, spinetoram + *Metarhizium anisopliae* was most effective against the pest in both seasons, followed by spinetoram, spinosad + *Metarhizium anisopliae* and spinosad. The experiment was laid out on a randomized block design (RBD) with three replications. However, *M. anisopliae* was observed as the least effective treatment. Considering the groups, cumulative efficacy of two seasons showed that chemical + entomopathogenic fungi was the best with 54.79% control efficacy followed by chemical (CE = 51.10%) and biological (CE = 25.00%).

Keywords: Biological control, Entomopathogenic fungi, Guava, Integrated pest management, Mealybug, *Metarhizium anisopliae*

Guava (*Psidium guajava* L.)*,* is a small monoecious evergreen tree comes under the family Myrtaceae. The fruit is very common among the rich and the poor as well and is popularly known as 'apple of the tropics.' It is native to Mexico (Rios *et al.* 1977) and carried to South America, European, Africa and Asia, therefore, cultivated around the world (Salalzar *et al.* 2006). This fruit was introduced to the Philippines by the Spanish, and to India by the Portuguese (Gill 2016). It thrives in all tropical and subtropical regions worldwide and adapts well to various climates, though it prefers dry conditions (Stone 1970). After flowering, guava fruit matures in approximately 120 days and leaves a characteristic smell and aroma when becomes fleshy. It contains many seeds inside and depending on the variety and the environment, can weigh up to 500 g (Patel *et al.* 2011). Guava crop is cultivating throughout India in an area of 308 thousand ha with the total production of 4582 thousand MT (PIB 2022) and Andhra Pradesh, Uttar Pradesh, Maharashtra, Bihar, West Bengal and Assam are the important cultivators.

Production of guava is affected by several biotic and abiotic factors. Among these, insect pests are the major biotic constraints. Earlier, guava was relatively unaffected by insect pests; however, recent climate changes have altered the pest situation significantly (Beevi *et al.* 1989, Mani and Krishnamoorthy 1996 and Samanta *et al.* 2005). The crop has been reported to be attacked by 80 spp. of insect pests, out of which mealybugs are known to attack guava crop and cause serious damage affecting quantity and quality of the fruits and significantly reducing the yield, showing its economic importance. *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) is one of the most highly polyphagous mealybug species, that infests a diverse range of plant species (Wabale *et al.* 2010). This pest targets over 150 genera across 68 different families of plant (CABI 2002). It has been reported by Baskaran *et al.* (1999) that 95% guava plants were infested by *F. virgata.* Indirect impact on guava as a disease vector has not been reported so far but reported to transmit badna virus from diseased to healthy black pepper plant, [*Piper nigrum* (Lin.)] in Kozhikode and Wayanad (Bhat *et al.* 2003), and presence was also found in south east Asian countries (Lockhart *et al.* 1997). *Badna* is a genus causing piper yellow mottle virus (PYMV) and characterized by the symptoms like chlorosis, vein clearing, leaf distortion, chlorotic mottling, reduced plant vigour and poor fruit set (Duarte *et al.* 2001 and Lockhart *et al.* 1997). The pest feeds on plants by using threadlike mouthparts to pierce various parts of the plant, extract sap and produces honeydew, leading to the growth

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of sooty mold, which severely diminishes the photosynthesis rate of the infested plants. Life cycle of *F. virgata* include a lot of overlapping generations within the year (Nayar *et al.* 1976). The female lays eggs in clusters, with a fecundity rate of 109–185 eggs, which sometimes exceed 500 (Schmutterer 1969), beneath her body, encased in a loosely woven sac made of waxy fibers. After 3–4 hours of incubation, the nymph goes through 3–4 molting with the longevity of 17–57 days. Whereas, adult mealy bug reported the development period of 1–3 days for male and 27–53 days for female. Total lifecycle consisted of 17–110 days depending upon the environmental conditions (Nayar *et al.* 1976, Schmutterer 1969 and Vigneswaran *et al.* 2016).

Use of insecticides has become the prime method for the management of insect pests. These, however affect the natural enemy population and create concern regarding environment. Repetitive and intensive use of insecticides is a main cause of pest resurgence (Lou *et al*. 2013) and it also has been observed that these chemicals sometimes do not maintain their desired efficacy (Matsumura and Sanadamorimura 2010). Hence, need to reduce the use of insecticides is necessary. The prospect of integrating chemical and fungal insecticides through combination offers a promising strategy to reduce environmental impacts as part of an Integrated Pest Management (IPM) approach (Sain *et al.* 2019). Therefore, this study aimed to design and assess the effectiveness of management strategies that combine chemical and fungal insecticides (Biological) for the management of *F. virgata* in guava.

MATERIALS AND METHODS

The study was carried out during 2022 and 2023 at G B Pant University of Agriculture and Technology, Pantnagar (29°N latitude and 79.3°E longitude; 243.84 m amsl), Uttarakhand. Commercially available five insecticides [Spinosad 45 SC (Tracer) (0.4 ml/L), Spinetoram 11.7 SC (Delegate) (1 ml/L), Emamectin benzoate 5 SG (Proactive) (0.4 g/L), Novaluron 5.25 + Indoxacarb 4.5 SC (Plethora) (2 ml/L) and Flubendiamide 39.35 SC (Fame) (0.75 ml/L)], one biological (Entomopathogenic fungi, EPF) [*M. anisopliae* 1 WP (Kalichakra) (7 g/L)] and their five combinations [Spinosad 45 SC (Tracer) + *M. anisopliae* 1 WP (Kalichakra) (0.4 ml/L + 7 g/L), Spinetoram 11.7 SC (Delegate) + *M. anisopliae* 1 WP (Kalichakra) (1 ml/L + 7 g/L), Emamectin benzoate 5 SG (Proactive) + *M. anisopliae* 1 WP (Kalichakra) (0.4 g/L + 7 g/L), Novaluron 5.25 + Indoxacarb 4.5 SC (Plethora) + *M. anisopliae* 1 WP (Kalichakra) (2 ml/L + 7 g/L) and Flubendiamide 39.35 SC (Fame) + *M. anisopliae* 1 WP (Kalichakra) (0.75 ml/L + 7 g/L)] summing up 12 treatments including untreated control were taken for the experiment. The experiment was laid out on a randomized block design (RBD) with three replications. A total number of 36 guava trees were selected, and each of them were sprayed with different treatments during the study. Two sprays were taken at fruiting stage; when fruits attain marble size and second 15 days after 1st application. Observations on pest incidence were recorded one day prior and 1, 3, 7, 10 and 14 days after spray (DAS). To assess the mealy bug infestation, 10 shoots of 30 cm were taken randomly in each replication from four directions, viz. east, south, west and north for counting of mealybug nymph and adult population. After each observation the control efficacy in each treatment was calculated using the Abbott equation (Abbott 1925):

$$CE(\%) = \left(1 - \frac{Pt}{Pc}\right) \times 100$$

Where CE, Control efficacy (%); Pc, Average number of mealybug nymph and adult in control and Pt, Average number of mealybug nymph and adult in the treatment. Data were subjected to One Way Analysis of Variance (ANOVA) following Snedecor and Cochran (1967) and the means were compared using Tukey's HSD (honestly significant difference) test (Tukey 1949) using SPSS 26.0. Cumulative control efficacy (%) of cropping season 2022 and 2023 for each management practice was also calculated.

RESULTS AND DISCUSSION

Considering the objective of the experiment to find out the best strategy for the management of *F. virgata* in guava, mean number of nymph and adult of the pest were observed. In first cropping season (2022), 1st application of treatments at marble fruit stage was done when mealybugs/10 shoots ranged between 43.40 and 54.30 (Table 1). Treatments spinetoram + *M. anisopliae* was the most effective recording minimum population of mealybugs/ 10 shoots (21.23) with the control efficacy of 57.98%, although this treatment showed its statistical parity with spinosad + *M. anisopliae,* spinetoram and spinosad. Combination insecticides though recorded decrease in mealybug population but were found to be statistical at par with their individual treatment, this trend sustained till the end of observations in both first and second spray. Fungal bioagent, *M. anisopliae* on first spray recorded its performance at par with control at first day after spray, maintained its efficacy at lowest among the treatments till 10th day and surpassed the treatment flubendiamide at 14th day of observation. Whereas, on 2nd application of treatments (Table 2), despite of having statistical parity with flubendiamide and its combination treatments, fungal bioagent, *M. anisopliae* emerged as less effective treatment at 1st day, identified as the second less effective treatment after flubendiamide at 3rd and 7th day of observation and after securing the least effective position at 10th day of observation, the treatment again retained its second least effective spot at the end of observations. Overall, flubendiamide, novaluron + indoxacarb and their combinations with the fungal agent, *M. anisopliae* showed poor control efficacy even lower than the fungal agent alone. Reason behind this effectiveness may be establishment of bioagents in the field when applied alone and incompatibility with insecticides (Neves and Santoro 2011, Joshi 2014, Khun *et al.* 2021 and Hirapara *et al.* 2023) when used as a tank mixed solution. This type of pathogenic activity may be due to environmental factors (Butt and Copping 2000).

Second cropping season (2023) witnessed overall slightly lower number of mealybugs than the previous

Table 1 Field efficacy of different treatments against mealybug after 1st spray on guava during the year 2022

Treatment	1 DBS	1 DAS		3 DAS		7 DAS		10 DAS		14 DAS		Mean CE%
	P*	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	
T_1	47.80	25.57^{a}	49.41	19.10^{a}	63.03	14.60^{a}	74.39	13.30^{a}	75.93	16.10^{a}	72.26	67.00
T_2	43.70	24.30^{a}	51.91	17.80^{a}	65.55	13.83^{a}	75.73	12.00^{a}	78.29	15.83^{a}	72.72	68.84
T_3	52.10	38.07^{bc}	24.67	32.57^{b}	36.97	28.00^{bc}	50.88	28.93^{bc}	47.65	30.33^{bc}	47.73	41.58
T_4	51.70	41.10^{bc}	18.67	40.17^{c}	22.26	35.43^{cd}	37.84	34.23^{cd}	38.06	37.40^{cd}	35.55	30.47
T_5	54.03	43.43^{cd}	14.05	41.43^{c}	19.81	39.30^{de}	31.05	38.37^{d}	30.58	43.53^{d}	24.99	24.09
T_6	51.90	48.50^{de}	4.02	42.80^{c}	17.16	43.50^{e}	23.68	39.60^{d}	28.35	40.73^{d}	29.81	20.61
T_7	48.50	23.43^{a}	53.63	16.57^{a}	67.94	11.83^{a}	79.24	10.43^{a}	81.12	14.67^{a}	74.73	71.33
T_8	43.40	21.23^{a}	57.98	15.30^{a}	70.39	10.87^{a}	80.94	9.80^{a}	82.27	12.93^{a}	77.71	73.86
T_9	44.13	37.30^{b}	26.19	29.90^{b}	42.13	26.83^{b}	52.92	25.57^{b}	53.74	27.17^{b}	53.19	45.63
T_{10}	48.70	40.77^{bc}	19.33	40.10^{c}	22.39	36.07^{de}	36.73	35.23^{d}	36.25	36.93^{cd}	36.36	30.21
T_{11}	51.83	41.57^{bc}	17.74	38.63^{c}	25.23	36.33^{de}	36.26	35.17^{d}	36.37	40.07^{d}	30.96	29.31
T_{12}	54.30	50.53^{e}	-	51.67^{d}	-	57.00^{f}	-	55.27^{e}	-	58.03^{e}	-	-
SEM	11.76	3.91		3.68		6.49		4.00		7.38		
CV	7.26	7.35		6.06		8.72		5.79		9.10		

*Means followed by the same letter in the column do not differ among themselves by the Tukey's HSD test; DBS, Days before spray; DAS, Days after spray; P, Number of mealybugs/10 shoots; CE, Control efficacy; CV, Coefficient of variation; SEM, Standard error mean. Treatment details are given under Materials and Methods.

Table 2 Field efficacy of different treatments against mealybug after 2nd spray on guava during the year 2022

Treatment	1 DBS	1 DAS		3 DAS		7 DAS		10 DAS		14 DAS		Mean CE%
	P	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	
T_1	-	11.37^{a}	79.80	9.97^{a}	82.51	9.20^{a}	83.98	9.53^{a}	83.65	10.97^{ab}	81.44	82.28
T_2	-	10.50^{a}	81.34	6.63^{a}	88.36	6.63^{a}	88.45	6.33^{a}	89.14	8.77^{a}	85.17	86.49
T_3	-	21.60^{b}	61.61	19.00^{b}	66.67	19.17^{bc}	66.63	18.90^{b}	67.58	19.20^{c}	67.51	66.00
T_4	-	33.63^{cd}	40.23	28.40^{cd}	50.18	26.37^{de}	54.09	26.60^{c}	54.37	29.20^{d}	50.59	49.89
T_5	-	36.47^{cd}	35.19	35.77^{e}	37.25	35.27^{f}	38.60	34.77^{d}	40.37	37.40^{e}	36.72	37.62
T_6	-	38.40	31.75	35.00^{e}	38.60	34.83^{f}	39.35	34.97^{d}	40.02	35.47^{de}	39.99	37.94
T_7	-	10.00^{a}	82.23	8.37^{a}	85.32	7.70^{a}	86.59	7.70^{a}	86.79	8.27^{a}	86.01	85.39
T_8	-	8.03^{a}	85.72	6.60^{a}	88.42	5.37^{a}	90.66	5.20^{a}	91.08	7.53^{a}	87.25	88.63
T_9	-	20.70^{b}	63.21	18.23^{b}	68.01	18.07^{b}	68.54	18.37^{b}	68.50	18.60^{bc}	68.53	67.36
T_{10}	-	31.53^{c}	43.96	23.23^{bc}	59.24	24.43^{cd}	57.46	25.47^{c}	56.32	29.03^{d}	50.87	53.57
T_{11}	-	35.43^{cd}	37.03	31.17^{de}	45.32	30.13^{ef}	47.53	30.57^{cd}	47.57	33.80^{de}	42.81	44.05
T_{12}	-	56.27^{e}	-	57.00^{f}	-	57.43^{g}	-	58.30^{e}	-	59.10^{f}	-	-
SEM		4.181		4.712		3.311		4.054		7.162		
CV		7.97		9.16		7.62		6.38		10.06		

*Means followed by the same letter in the column do not differ among themselves by the Tukey's HSD test; DBS, Days before spray; DAS, Days after spray; P, Number of mealybugs/10 shoots; CE, Control efficacy; CV, Coefficient of variation; SEM, Standard error mean. Treatment details are given under Materials and Methods.

season. For this cropping season, spray was done when mealybug population per 10 shoots ranged between 30.50 and 38.30 (Table 3). At 1st day of observation, plants treated with spinetoram + *M. anisopliae* recorded minimum number of mealybugs (14.63 mealybugs/10 shoots, CE = 58.74%) and managed its statistical similarity with spinetoram (CE = 52.07%), spinosad (CE = 50.09%) and spinosad + *M. anisopliae* (CE = 54.04%). Whereas, fungal bioagent, *M. anisopliae*, which showed its inferiority among the treatments and recorded statistically at par with untreated control at 1st day of observation, was found to be statistically at par with novaluron + indoxacarb, flubendiamide and their combinations with fungal bioagent till 14th day of observation. Overall, fungal bioagent showed the lowest control efficacy (20.74%). After 2nd application of treatments, the trend of efficacy was continued and spinetoram + *M. anisopliae* remained the most effective treatment with control efficacy of 86.41% (Supplementary Table 1).

Considering the different management practices, chemical + entomopathogenic fungi provided the maximum efficacy against mealybugs (CE = 54.79%), followed by chemical group (CE = 51.10%) and biological (entomopathogenic fungi) (CE = 25.00%) (Fig. 1). There was a slight difference in control efficacy recorded between chemical and chemical + biological group as fungal agent used as biological practice is known to have slower action and takes days to show their results (Faria and Wraight 2001).

Combining all methods of insect pest management as an integrated pest management, improve the control efficiency and reduced the use of chemical insecticides (Cook *et al.* 2007, Hendrichs *et al.* 2007, Rutledge *et al.* 2004). Following this practice, not only hazardous impact of chemical on environment can be minimized but also the development of resistance in insects can be slowed down. Resistance development to a new insecticide is rare but using common mode of action insecticides with the repeated application can increase the possibility (Georghiou and Taylor 1986). Combining insecticides with the biological agents, viz. entomopathogenic fungi is one of the improved methods to combat these problems (Srivastava *et al.* 2009) and it also reduce the problem of non-selectivity in pest control (Ramaraje *et al.* 1967). Fargues (1975) has outlined that insecticide and entomopathogenic fungi combinations are responsible for the delay in the development of resistance in

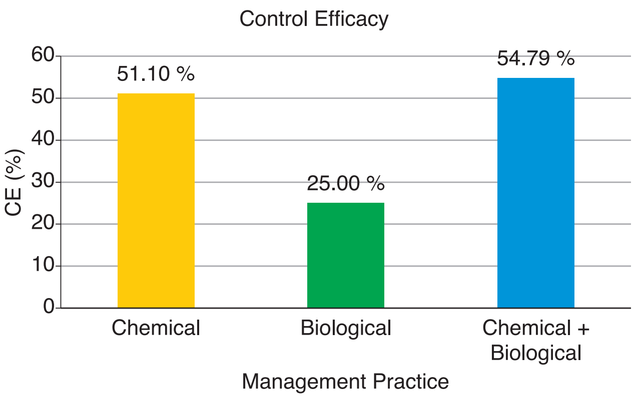

Fig. 1 Cumulative control efficacy (%) of each management practice against mealybug (2022 and 2023).

Table 3 Field efficacy of different treatments against mealybug after 1st spray on guava during the year 2023

Treatment	1 DBS	1 DAS		3 DAS		7 DAS		10 DAS		14 DAS		Mean CE%
	P*	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	P	CE (%)	
T_1	33.57	17.70^{a}	50.09	13.43^{a}	63.10	10.13^{a}	74.79	9.20^{a}	76.41	11.07^{a}	72.96	67.47
T_2	30.70	17.00^{a}	52.07	12.23^{a}	66.39	9.43^{a}	76.53	8.27^{a}	78.80	10.83^{a}	73.53	69.47
T_3	36.63	26.67^{bc}	24.81	22.93^{b}	37.00	19.33^{b}	51.91	20.07^{bc}	48.55	21.27^{bc}	48.05	42.06
T_4	36.40	29.10^{bc}	17.95	28.37^{c}	22.07	24.80^{c}	38.31	23.87^{cd}	38.80	26.20^{cd}	35.99	30.63
T_5	38.07	30.50^{cd}	14.00	29.10^{c}	20.05	27.67^{cd}	31.18	26.90^{d}	31.03	30.70^{d}	25.00	24.25
T_6	36.63	34.27^{de}	3.38	30.03^{c}	17.49	30.50^{cd}	24.13	27.87^{d}	28.55	28.60^{d}	30.13	20.74
T_7	34.00	16.30^{a}	54.04	11.30^{a}	68.96	8.00^{a}	80.10	7.07^{a}	81.88	9.90^{a}	75.81	72.16
T_8	30.50	14.63^{a}	58.74	10.37^{a}	71.52	7.30^{a}	81.84	6.57^{a}	83.16	8.97^{a}	78.09	74.67
T_9	30.97	26.20^{b}	26.13	20.77^{b}	42.95	18.63^{b}	53.65	17.70^{b}	54.62	18.87^{b}	53.91	46.25
T_{10}	34.27	28.60^{bc}	19.36	28.10^{c}	22.80	25.27^{cd}	37.15	24.57^{d}	37.01	25.73^{cd}	37.13	30.69
T_{11}	36.43	29.10^{bc}	17.95	27.17^{c}	25.37	25.77^{cd}	35.90	24.57^{d}	37.01	28.13^{d}	31.27	29.50
T_{12}	38.30	35.47^{e}	-	36.40^{d}	-	40.20^{e}	-	39.00^{e}	-	40.93^{e}	-	-
SEM	5.94	2.03		1.94		3.23		2.14		3.82		
CV (%)	7.33	4.45		6.26		8.82		5.96		9.36		

*Means followed by the same letter in the column do not differ among themselves by the Tukey's HSD test; DBS, Days before spray; DAS, Days after spray; P, Number of mealybugs/10 shoots; CE, Control efficacy; CV, Coefficient of variation; SEM, Standard error mean.

Treatment details are given under Materials and Methods.

insects. Literature pertaining specifically to the combinations of selected chemical insecticides with fungal bioagent (*M. anisopliae*) is not available. However, studies showed that *M. anisopliae* with thiamethoxam recorded the highest control efficiency against *Nilaparvata lugens* and *Sogatella furcifera* in paddy (Tang *et al.* 2019) and *Mahanarva fimbriolata* in sugarcane (Kassab *et al.* 2014) belonging to same order as of *F. virgata* i.e. Hemiptera. Nawaz *et al.* (2022) observed that the combined effect of *M. anisopliae* and insecticides was significantly superior and more toxic to *Aphis gossypii* as compared to individual treatments and lowest efficacy was seen in the treatment *M. anisopliae.* According to Rachappa *et al.* (2007) *M. anisopliae* is safe to use with spinosad and leads to enhancement in efficacy.

The results demonstrated that chemical + biological (*M. anisopliae*) consistently outperformed the individual treatments against mealybug populations. Although the differences in efficacy between these were not statistically significant, the trend towards improved performance with the combinations was evident. The combined approach may potentially reduce the overall cost of pest management, the environmental impact is minimized, additionally, entomopathogenic fungi are naturally occurring and pose minimal environmental risks compared to chemical insecticides and this approach can mitigate the development of resistance in pest populations by employing multiple modes of action. Moreover, the insecticides provide immediate knockdown effects, while the fungal bioagents offers longer-lasting residual control through its colonization of the pest.

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