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Compatibility and enhanced efficacy of mixtures of insectopathogenic fungi and neonicotinoid insecticides: an ecofriendly strategy to combat *Aphis craccivora*

Jaydeep Halder* **and Suraj Soni**[1]

ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India

Abstract

The black bean aphid, *Aphis craccivora,* is a sap sucking insect that feeds on leguminous vegetable crops. Both nymphs and adults suck the sap from young shoots, tender fruits, apical buds and devitalizing the plants. To control this sucking pests, different entomopathogenic fungi, *viz. Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii*, were tested alone and their half of their recommended doses with neonicotinoids insecticides (Imidacloprid 17.8% SL, Thiamethoxam 25% WG and Acetamiprid 20% SP) against *A. craccivora* during 2022 and 2023. For both consecutive years (2022 and 2023), *L. lecanii* had the lowest median lethal time (44.29 and 46.60 h) to kill 50% of the test population, followed by *B. bassiana* (45.77 and 47.89 h) and *M. anisopliae* (46.45 and 48.27 h). Similarly, among neonicotinoids examined, Acetamiprid was the most toxic (LT_{50} = 26.39 and 28.78 h). The combination of *L. lecanii* + Acetamiprid at half of their recommended doses was found to be the most effective, leading to the lowest median lethal time of 21.85 and 22.67 h during 2022 and 2023, respectively, followed by *B. bassiana* + Acetamiprid (22.82 and 23.29 h). Combination of EPF like *B. bassiana*, *M. anisopliae*, and *L. lecanii* with neonicotinoids at half of their recommended concentrations are not only compatible but also synergistic in action and could be a viable ecofriendly option in the management of *A. craccivora*.

Key words: Entomopathogenic fungi, Neonicotinoids, *Aphis craccivora,* Median lethal time, Co-toxicity coefficient

Leguminous vegetables are important economic crops globally, cowpea [*Vigna unguiculata* (L.)], peas (*Pisum sativum* L.), French beans (*Phaseolus vulgaris* L.), Indian beans [*Lablab purpureus* (L.) Sweet] etc. being important ones. In India, potential yield of legume vegetables is not fully achieved. One of the key causes of its reduced output and productivity is occurrence of various biotic stresses thorough out its growth period. Insect pests alone cause 10-30 percent yield losses in vegetables (Halder *et al.,* 2018a). The black bean aphid, *Aphis craccivora* Koch (Aphididae: Hemiptera) is an important polyphagous sap-sucking insect that exclusively feed on the leguminous vegetable crops. Both nymphs and adults of aphids sip the sap from young shoots, apical buds, flowers and flower buds and even from tender fruits and devitalizing the plants. The leaves became yellowish in colour, curled, and had a leathery texture (Rai and Halder, 2023). To manage this nefarious sucking pest, Indian farmers mostly use chemical pesticides, which are frequently employed indiscriminately, and excessively. This results in development of pesticide resistance, resurgence of target insects and secondary pest outbreaks, residues in food and beverages, groundwater contamination, health risks for people, and widespread killing and decimation of non-target organisms (Halder *et al.,* 2019 and 2023).

***Corresponding author :** jaydeep.halder@gmail.com

[1]Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

Due to their target specificity, self-perpetuity, and apparent environmental safety, biological insect pests control method of using various entomopathogenic microorganisms, as well as predator and parasitoid insects, are currently gaining importance in light of the negative effects of these synthetic insecticides (Roy *et al.,* 2017). The pest control potential of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae), *Metarhizium anisopliae* (Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) and *Lecanicillium* (= *Verticillium*) *lecanii* R. Zare & W. Gams (Hypocreales: Clavicipitaceae) (Rai *et al.,* 2014a,b; Halder *et al.,* 2016 and 2018b) have been proved beyond doubt over the past decades.

There is not much information on the interactions between various entomopathogenic fungus and neonicotinoid pesticides against this pest. In order to determine whether there was any compatibility between various entomopathogenic fungi and neonicotinoid pesticides, an attempt was undertaken to determine the median lethal time (LT_{50}). In an analogous way, relative lethal toxicity was estimated for various entomopathogenic fungi and neonicotinoids with a focus on changes in susceptibility over time, in order to evaluate the emergence of resistance, if any.

MATERIALS AND METHODS

Talc-based formulations of the three entomopathogenic fungi (EPF), *viz. Beauveria bassiana*

(Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (1×10^{10} cfu/g), *Metarhizium anisopliae* (Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) (1×10^{10} cfu/g) and *Lecanicillium* (=*Verticillium*) *lecanii* R. Zare and W. Gams (Hypocreales: Clavicipitaceae) were utilized at their recommended doses, *i.e.* 5 g/L of water. Proprietary insecticidal formulations of three neonicotinoids *viz*., Imidacloprid 17.8% SL, Thiamethoxam 25% WG, and Acetamiprid 20% SP, were used @ 0.35 mL/L, 0.35 g/L, and 0.14 g/L, respectively. The effectiveness of each microbial pesticides and neonicotinoid insecticides against *A. craccivora* were evaluated alone at their above-mentioned doses as well as at half of their respective doses in a 1:1 ratio when used in conjunction/mixture.

Direct spray method The aphid infested leaves, twigs and tender pods of Indian bean, were brought from the experimental farm of the ICAR-IIVR, Varanasi (82°86′ E longitude and 25°18′ N latitude), and only viviparous apterous adults were used for bioassays. Twenty insects were placed in each petri-dish (9 cm in diameter), and under Potter's tower at 340 g/cm^2 pressure, they were sprayed directly with 1 mL of each concentration of various entomopathogenic fungi, three neonicotinoid pesticides, and their above-mentioned combinations. The aphids in sprayed petri-dishes were dried for five minutes under the fan. Fresh, uninfested, and untreated plant parts were offered as food. All bioassays were conducted in Insect Toxicology laboratory at 27±1°C temperature, a relative humidity of 70±5, and a photoperiod of 12:12 hours of light and dark. Every 12 hours, observations were taken for each case. Moribund insects were considered as dead. In January and February during both years (2022 and 2023), all experiments were carried out.

Data analysis Mortality data were corrected by Abbott's formula (Abbott, 1925) and analyzed by Probit analysis (Finney, 1971) with SAS program (version 9.2). Control mortality in almost all cases was below 10%. The median lethal time values (LT_{50}) were determined and any two values were considered significantly different if their respective 90% confidence limits (CL) did not overlap. Co-toxicity coefficient (CTC) or synergistic ratio (SR) was calculated with respect to entomopathogenic fungi as well as neonicotinoids and expressed by the following formulae:

$$\text{Co-toxicity co-efficient w.r.t. EPF} = \frac{LT_{50}\text{ value of entomopathogenic fungi alone}}{LT_{50}\text{ values of insecticide and EPF mixture}}$$

$$\text{Co-toxicity co-efficient w.r.t. neonicotinoids} = \frac{LT_{50}\text{ value of neonicotinoid insecticide alone}}{LT_{50}\text{ values of insecticide and EPF mixture}}$$

Values of CTC > 1 indicated they are compatible and synergistic in action with each other and when CTC < 1 showed that they are not compatible and antagonistic in action (Sun and Johnson, 1960; Corbel *et al.*, 2006, Halder *et al.*, 2021). Similarly, relative lethal time of all these insecticides and EPF alone and their 1:1 combination were calculated using the formula:

$$\text{Relative lethal time} = \frac{LT_{50}\text{ value during 2022}}{LT_{50}\text{ value during 2023}}$$

RESULTS AND DISCUSSION

Marked differences were observed among the three microbial insecticides alone and their 1:1 combinations with neonicotinoids. The white halo fungus, *L. lecanii*, has been proven to be the most promising entomopathogen against viviparous, apterous adults of *A. craccivora* during both the years. During 2022, a talc-based formulation of *L. lecanii* at its prescribed dose took 44.29 h to kill 50% of the test aphid population, followed by *B. bassiana* (45.77 h) and *M. anisopliae* (46.45 h). Acetampirid 20% SP was shown to be the most effective of the three neonicotinoid insecticides, with a lowest median lethal time (26.39 h) than Imidacloprid (27.43 h) and Thiamethoxam (26.82 h). The combination of *L. lecanii* + Acetamiprid was found to be the most effective, leading to lowest median lethal time of 21.85 h during 2022, followed by *B. bassiana* + Acetampirid (22.82 h), when these EPF and neonicotinoids were combined at half of their recommended doses and sprayed under a Potter's tower.

The combination of EPF and neonicotinoids (in 1:1 ratio) had a CTC > 1 value, indicating that they are compatible and work synergistically at half of their recommended doses when compared with respect to neonicotinoids or entomopathogenic fungi alone. The combinations of *M. anisopliae* + Thiamethoxam and *L. lecanii* + Acetamiprid (1:1) recorded the highest co-toxicity coefficient value (1.19) when neonicotinoid insecticide was considered as base. In enigma, combination of *L. lecanii* + Acetamiprid and *B. bassiana* + Thiamethoxam had the highest CTC (2.03) with respect to white halo fungus *L. lecanii* and *B. bassiana*, respectively, during 2022 (Table 1).

During 2023 also, the same pattern was recorded. *Lecanicillium lecanii* amongst the EPF and acetamiprid amongst the neonicotinoids were found to be the most effective. Imidacloprid had a median lethal time of 28.85 h when sprayed at the recommended dose; however a 1:1 combination with various EPF at half of each of their respective recommended doses resulted in lower LT_{50} values than Imidacloprid and EPF alone (Table 2). Similar to this, when combined with *L. lecanii*, *B. bassiana*, and *M. anisopliae*, Thiamethoxam's median lethal time values were dropped to 22.93, 23.80 and 26.11 h, respectively. In all combinations, CTC values were more than 1, showing their compatibility and potential for synergy. A consistent trend was also observed during 2023. In comparison to the three neonicotinoids, all entomopathogens exhibited a median fatal time that were considerably higher (Tables 1 and 2). Their 1:1 combo had a shorter median lethal time than either of them alone. In every instance, their combined CTC values were greater than 1, showing compatibility. A gradual change of LT_{50} over the years is evident (Table 2). The median lethal duration for *L. lecanii* was 44.29 hours in 2022, and it increased slightly to 46.60 hours in 2023.

With 2022's LT_{50} value as a base (1), 2023's median lethal durations for *L. lecanii* was 1.05 times longer. Similar to Imidacloprid, the median lethal times in 2023 was 1.05 times longer than in the base year, ranging from 27.43 to 28.85 hours within the same period. The relative lethal times for thiamethoxam were 1.09 times greater in 2023 than in the preceding year (Table 3). It's interesting to note that killing 50% of the test population required less time when EPF and neonicotinoids were combined at half of their respective recommended doses.

Table 1: Median lethal time of EPF and neonicotinoid insecticides alone and their combinations at half of the recommended doses against A. craccivora during 2022

Insecticide	Heterogenity		Regression Equation (Y=)	LT_{50} (hr)	Fiducial limit	Co-toxicity coefficient w.r.t.	
	df	χ^2				EPF	Neonicotinoid
Beauveria bassiana	3	0.153	5.798X – 4.629	45.77	50.31 – 41.64	--	--
Metarhizium anisopliae	3	1.598	5.676X – 4.462	46.45	51.14 – 42.19	--	--
Lecanicillium lecanii	3	2.714	5.204X – 3.567	44.29	49.13 – 39.92	--	--
Imidacloprid 17.8% SL	3	0.105	3.971X – 0.711	27.43	33.22 – 22.64	--	--
Thiamethoxam 25% WG	3	0.276	4.023X – 0.747	26.82	32.61 – 22.06	--	--
Acetamiprid 20% SP	3	1.004	5.012X – 2.125	26.39	30.09 – 22.41	--	--
B. bassiana + Imidacloprid	4	2.837	1.908X + 2.335	24.92	32.40 – 19.16	1.84	1.10
M. anisopliae + Imidacloprid	4	1.085	1.991X + 2.191	25.76	33.06 – 20.07	1.80	1.07
L. lecanii + Imidacloprid	4	1.039	2.449X + 1.595	24.54	29.97 – 20.10	1.81	1.12
B. bassiana + Thiamethoxam	4	5.057	1.871X + 2.462	22.59	29.35 – 17.38	2.03	1.19
M. anisopliae + Thiamethoxam	4	5.379	1.697X + 2.600	25.94	34.99 – 19.23	1.79	1.03
L. lecanii + Thiamethoxam	4	2.986	2.401X + 1.741	22.55	27.74 – 18.32	1.96	1.19
B. bassiana + Acetamiprid	4	3.312	1.907X + 2.409	22.82	32.04 – 16.26	2.01	1.16
M. anisopliae + Acetamiprid	4	6.209	1.999X + 2.265	23.32	32.34 – 16.82	1.99	1.13
L. lecanii + Acetamiprid	4	6.456	2.486X +1.669	21.85	28.14 – 16.97	2.03	1.21

Table 2: Median lethal time of EPF and neonicotinoid insecticides alone and their combinations at half of the recommended doses against *A. craccivora* during 2023

Insecticide	Heterogenity		Regression Equation (Y=)	LT_{50} (hr)	Fiducial Limit	Co-toxicity coefficient w.r.t.	
	df	χ^2				EPF	Neonicotinoid
Beauveria bassiana	3	4.479	4.641X – 2.798	47.89	52.94 – 43.34	--	--
Metarhizium anisopliae	3	0.226	5.389X – 4.074	48.27	52.68 – 44.23	--	--
Lecanicillium lecanii	3	1.569	5.123X – 3.547	46.60	51.13 – 42.47	--	--
Imidacloprid 17.8% SL	4	1.381	4.399X – 1.424	28.85	33.89 – 24.56	--	--
Thiamethoxam 25% WG	3	0.476	5.066X – 2.424	29.21	33.79 – 25.25	--	--
Acetamiprid 20% SP	4	0.182	3.526X – 0.146	28.78	35.28 – 23.48	--	--
B. bassiana + Imidacloprid	5	5.291	2.646X + 1.277	25.51	30.85 – 21.09	1.88	1.13
M. anisopliae + Imidacloprid	4	0.183	4.341X – 1.159	26.25	31.63 – 21.78	1.82	1.10
L. lecanii + Imidacloprid	4	5.729	2.418X + 1.628	24.81	30.42 – 20.23	1.93	1.16
B. bassiana + Thiamethoxam	4	1.829	2.597X + 1.424	23.80	28.62 – 19.80	2.03	1.23
M. anisopliae + Thiamethoxam	4	4.475	2.549X + 1.379	26.11	32.05 – 21.60	1.85	1.12
L. lecanii + Thiamethoxam	4	6.332	2.575X + 1.497	22.93	27.67 – 19.01	2.11	1.27
B. bassiana + Acetamiprid	4	6.043	2.546X + 1.518	23.29	28.17 – 19.26	1.99	1.24
M. anisopliae + Acetamiprid	4	6.005	2.576X + 1.445	24.01	29.14 – 19.79	1.94	1.20
L. lecanii + Acetamiprid	4	1.265	3.003X + 0.898	22.67	26.60 – 19.33	2.06	1.27

Table 3: Relative lethal time of entomopathogens and neonicotinoid insecticides alone and their combination at half of their recommended doses to *A. craccivora* by direct spray method

Pesticide	Median lethal time (LT_{50}) in hour	
	2022	2023
Beauveria bassiana	45.77 (1)[#]	47.89 (1.05)
Metarhizium anisopliae	46.45 (1)	48.27 (1.04)
Lecanicillium lecanii	44.29 (1)	46.60 (1.05)
Imidacloprid 17.8% SL	27.43 (1)	28.85 (1.05)
Thiamethoxam 25% WG	26.82 (1)	29.21 (1.09)
Acetamiprid 20% SP	26.39 (1)	28.78 (1.09)
B. bassiana + Imidacloprid	24.92 (1)	25.51 (1.02)
M. anisopliae + Imidacloprid	25.76 (1)	26.25 (1.02)
L. lecanii + Imidacloprid	24.54 (1)	24.81 (1.01)
B. bassiana + Thiamethoxam	22.59 (1)	23.80 (1.05)
M. anisopliae + Thiamethoxam	25.94 (1)	26.11 (1.01)
L. lecanii + Thiamethoxam	22.55 (1)	22.93 (1.02)
B. bassiana + Acetamiprid	22.82 (1)	23.29 (1.02)
M. anisopliae + Acetamiprid	23.32 (1)	24.01 (1.03)
L. lecanii + Acetamiprid	21.85 (1)	22.67 (1.04)

[#]Figures in parenthesis are relative lethal time

Black bean aphid, *Aphis craccivora* Koch is a highly polyphagous pest that attacks a series of agri-horticultural crops belonging to eight plant families. As part of its feeding damage, it sucks and drains the plant sap, which ultimately lowers the availability of nutrients and water to the plants and spreads plant viruses. When infestations are severe, especially during the seedling stage, aphid feeding can cause symptoms such as chlorosis, stunting that delays the commencement of blooming, and even plant death in cowpea (Blackman and Eastop, 2006; Keating *et al.,* 2015). Aphids secrete sugar-rich honey dews that, in addition to acting as a vector and draining plant sap, serve as an ideal medium for the growth of fungi that causes sooty-mould, which ultimately impedes photosynthesis. Numerous plant viruses, including rosette, mottle, stunt, and stripe, are spread by it (Porter *et al.,* 1984). The bean aphid is viviparous, polymorphic (having both apterous and alate forms), and undergoes year-round parthenogenetic reproduction in tropical regions (Namitha *et al.,* 2021).

The insectopathogenic fungi are possible options for the management of vegetable insect pests (Halder *et al.,* 2016 and 2018a). Al Mazraáwi (2007) tested several combinations of *B. bassiana* and Imidacloprid for controlling onion thrips (*Thrips tabaci*) in both field and greenhouse settings. When *B. bassiana* (at field rates) and Imidacloprid (at 1/10 of field rates) were applied together in laboratory bioassay, the highest mortality (97%) was achieved compared to 88%, 94%, and 21% for *B. bassiana* alone, Imidacloprid alone, and the control, respectively. In greenhouse bioassay, significantly highest mortality (80%) was achieved in combination of *B. bassiana* and Imidacloprid at field rates. Additionally, they hypothesized that combining *B. bassiana* and Imidacloprid might result in a decrease in the rate of insecticide application while increasing the effectiveness of the biological control agent.

Furlong and Groden, (2001) reported that Imidacloprid interacted with *B. bassiana* to produce a synergistic response in larval mortality of Colorado potato beetle, *Leptinotarsa decemlineata* Say. They also suggested that possible role of starvation-induced stress factors underlying the observed synergistic interactions. Rajanikanth *et al.* (2010) further proved the effectiveness and compatibility of Imidacloprid and *B. bassiana* against the polypaghous insect pest *Spodoptera litura* (Fab.).

Paula *et al.* (2011) documented that the combining *M. anisopliae* with a very low concentration of the chemical insecticide Imidacloprid improved its effectiveness against *Aedes aegypti* (Linn.) and resulted in higher mortality following relatively short exposure times. According to Reddy *et al.* (2016), Imidacloprid combined with entomopathogenic fungi (*B. bassiana, M. anisopliae,* and *L. lecanii*) at recommended concentrations showed increased mortality compared to Imidacloprid alone against brown plant hopper (*Nilaparvata lugens* (Stål)) infesting paddy under glasshouse conditions. The neonicotinoids insecticide, Imidacloprid (at half the mean concentration) was found by Alizadeh *et al.* (2007) to be compatible with formulations of *V. lecanii* and they concluded that this could be used simultaneously with this entomopathogenic in integrated pest management.

Conclusion

Thus it is concluded that neonicotinoids insecticides combined with insectopathogenic fungi each at half their recommended doses were not only compatible but also synergistic in action. Without compromising the mortality of sucking pest, their mixtures would help lessen the load of toxic pesticides in environment and can be considered as an ecofriendly technology for sucking pest management like *A. craccivora*.

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