



Interaction effects between entomopathogenic fungi and neonicotinoid insecticides against *Lipaphis erysimi* in vegetable ecosystem

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ABSTRACT

Efficacy of different entomopathogenic fungi (EPF), viz. *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium* (= *Verticillium*) *lecanii* and neonicotinoid insecticides (Imidacloprid and Thiamethoxam) were evaluated alone and their 1:1 combination against *Lipaphis erysimi* prevalent in vegetable ecosystem. Among the entomopathogenic fungi, *L. lecanii* was found most promising, registering lowest median lethal time (LT₅₀) of 41.46 h, 44.09 h and 44.83 h during the period of 2015, 2016 and 2017, respectively; followed by *B. bassiana* (44.07 h, 53.22 h and 51.60 h) and *M. anisopliae* (46.86 h, 48.83 h and 48.99 h). Amongst the two neonicotinoids, Thiamethoxam was found more efficacious than the Imidacloprid. Blending of *L. lecanii* and Thiamethoxam at half of their recommended dose took lowest (13.39 h, 15.66 h and 17.41 h during 2015, 2016 and 2017, respectively) lethal time to kill the 50% test population followed by *B. bassiana* + Thiamethoxam (15.86 h, 17.72 h, 20.86 h) and *M. anisopliae* + Thiamethoxam (18.28 h, 19.69 h, 21.86 h). Combinations of these entomopathogenic fungi and neonicotinoid insecticides had co-toxicity co-efficient values >1 and lower LT₅₀ values than each of their individual indicating the compatibility amongst them. Co-application of these EPF with sub-lethal concentration of neonicotinoids could not only be a green ecofriendly option against this sucking pest but also able to minimize the chemical insecticides load in the environment.

Key words: Co-toxicity coefficient, Entomopathogenic fungi, *Lipaphis erysimi*, Median lethal time, Neonicotinoids

Sucking insect pests are considered as one of the major biotic constraints for vegetable production in India. They are primarily phloem feeders, abstracting sap via specially adapted mouthparts and also secrete the sugar rich honey dew which is deposited on the plant surface and helps to develop the black sooty mould, thereby hindering the normal photosynthesis of the plants. Aphids are important as they attack almost all the vegetables throughout the year (Halder *et al.* 2014). Aphids alone have the potential to inflict yield losses up to 82% in case of cruciferous crops where insecticides were not applied (Razaq *et al.* 2011). Mustard aphid, *Lipaphis erysimi* Kalt. (Aphididae: Hemiptera) is an important pest of cruciferous vegetables like cabbage, cauliflower, knol khol, broccoli, mustard, etc. To control this oligophagous pest, farmers used to many synthetic insecticides including neonicotinoids. This practice has led to many fold problems like resistance to insecticides, resurgence of target insects and secondary pest outbreak, in addition to widespread killing of non-target organisms (Halder *et al.* 2012, 2018).

Considering the ill-effects of these chemical

insecticides, biological control of insect pests using different entomopathogenic microorganisms and predators and parasitoids is gaining importance due to their target specificity, self-perpetuity and obvious safety to the environment. The pest control prospects chiefly of entomopathogenic fungi, viz. of *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium* (= *Verticillium*) *lecanii* (Rai *et al.* 2014a,b; Halder *et al.* 2016) have been proved beyond doubt over the decades. Literature pertaining to compatibility of different entomopathogens and neonicotinoids against this sucking pest is very scanty. Therefore, an attempt was made to find out the median lethal time (LT₅₀) of different entomopathogenic fungi and major neonicotinoid insecticides and their combinations (1:1) for their compatibility, if any. Similarly, relative lethal toxicity in terms of median lethal time of these entomopathogens and neonicotinoids with special reference to change in susceptibility level over the years was also computed, to know the development resistance, if any.

MATERIALS AND METHODS

Talc based formulation of three entomopathogenic fungi (EPF), viz. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) IIVR strain (1×10^{10} cfu/g), *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) IIVR strain (1×10^{10} cfu/g)

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and *Lecanicillium* (= *Verticillium*) *lecanii* R. Zare and W. Gams (Hypocreales: Clavicipitaceae) (2×10^9 cfu/g) commercial formulation each at recommended doses of 5 g/l of water were taken for the experiments. Proprietary insecticidal formulations of duo neonicotinoids, viz. Imidacloprid 17.8 SL and Thiamethoxam 25 WG at their recommended doses, 0.35 ml/l and 0.35 g/l, respectively, were taken for the experimental purpose. All the microbial insecticides and neonicotinoid insecticides alone at their recommended doses as well as half of their recommended doses at 1:1 combinations with EPF were tested for their efficacy against the mustard aphid, *Lipaphis erysimi* (Aphididae: Hemiptera) and green lace wing, *Chrysoperla zastrowi sillemi* (Chrysopidae: Hemiptera), a polyphagous predator of *L. erysimi*.

Direct spray method: The aphid infested leaves of cabbage were brought from the experimental farm of the ICAR-Indian Institute of Vegetable Research, Varanasi and only viviparous apterous adults were used for bioassay tests. Insects (20) were placed in each petridish (9 cm diameter) which was directly sprayed with 1 ml of each concentration of different entomopathogenic fungi and duo neonicotinoid insecticides and their combination (1:1 ratio) under Potter's tower at 340 g/cm² pressure. The sprayed petridishes containing the aphids were dried for 5 min. under the fan. Fresh un-infested and untreated plant materials were given as food. All the bioassays were done in biocontrol laboratory under 27±1°C temperature and 70±5% relative humidity with 12:12 h light: dark photoperiod. Observations were recorded at every 12 h interval in each case. Moribund insects were considered as dead. All the experiments were conducted during the *rabi* seasons for three consecutive years, viz. 2015, 2016 and 2017. Similarly, entomopathogens and duo neonicotinoids alone and their 1:1 mixture were also tested by direct spray method on second instar grubs of *C. z. sillemi* reared on *Corcyra* eggs under laboratory conditions and mortality was recorded after 24 and 48 h after the treatment (HAT). These pesticides were classified for recognizing adverse effects (percent reduction of mortality over control) on beneficial and non-target arthropods as given by IOBC where: class I - harmless (<30%), class II - slightly harmful (30-79%), class III - moderately harmful (80-99%) and class IV - harmful (>99%) (Hassan 1992).

Data analysis: The mortality data were corrected by Abbott's formula (Abbott 1925) and analyzed by probit analysis (Finney 1971) with SAS program (version 9.2). The control mortality in almost all cases was below 10%. The median lethal times (LT₅₀) were determined and any two values were considered significantly different if their respective 90% confidence limits (CL) did not overlap.

Co-toxicity coefficient (CTC) was calculated by the following formula –

$$\text{Co-toxicity coefficient} = \frac{\text{LT}_{50} \text{ value of Imidacloprid or Thiamethoxam alone}}{\text{LT}_{50} \text{ values of insecticide and EPF mixtures}}$$

When the value of co-toxicity coefficient was >1

indicating they are compatible with each other and when it was < 1 showed that they are not compatible (Halder *et al.* 2017).

Similarly, relative lethal time of all these insecticides and EPF alone and their 1:1 combinations were calculated using the following formula – Relative lethal time = LT₅₀ worked out during 2016 or 2017/LT₅₀ calculated during 2015

RESULTS AND DISCUSSION

Marked differences were observed among these microbial insecticides alone and their combinations. The white halo fungus, *L. lecanii* was found most promising entomopathogen against viviparous, apterous adults of *L. erysimi*. Talc based formulation of *L. lecanii* at its recommended dose took only 41.46 h to kill the fifty per cent of test population followed by *B. bassiana* (44.07 h) and *M. anisopliae* (46.86 h) during 2015. Potential of these entomopathogenic fungi against *L. erysimi* was also confirmed by several workers (Parmar and Kapadia 2007, Halder *et al.* 2013). Amongst the duo neonicotinoid insecticides, Thiamethoxam was found better as it registered lower median lethal time (21.63 h) than the Imidacloprid (30.15 h). When these EPF and neonicotinoids were blended at 1:1 ratio and sprayed under potter's tower, the per cent mortality were changed in time dependent manner and *L. lecanii* + Thiamethoxam was found most promising registering lowest median lethal time of 13.39 h during 2015 followed by *B. bassiana* + Thiamethoxam (15.86 h). Co-toxicity coefficient (CTC) values when calculated, considering Imidacloprid/Thiamethoxam as base (1), all these EPF and two neonicotinoids (at 1:1 ratio) had CTC values >1 indicating they are compatible at half of their recommended doses. From the Table 1 it is also clear that maximum co-toxicity coefficient value (1.62) was recorded in *L. lecanii* + Thiamethoxam combination.

Same trend was also observed during the year 2016. *L. lecanii* amongst the EPF and Thiamethoxam amongst the neonicotinoids were found most promising against this oligophagous sap sucker. The descending order of EPF based on their toxicity was *L. lecanii* > *B. bassiana* > *M. anisopliae*. Imidacloprid when sprayed at recommended dose had median lethal time of 33.69 h but combination (1:1) with different EPF at half of their respective recommended doses recorded lower LT₅₀ values than any of their individual (Table 2). Similarly, Thiamethoxam alone took 24.93 hour but when mixed with *L. lecanii*, *B. bassiana* and *M. anisopliae* took 15.66 h, 17.72 h and 19.69 h, respectively, to kill the 50% test population. Co-toxicity coefficient (CTC) values for all these combinations were >1 indicating compatibility amongst them. During the third year, consistent trend was observed. All the entomopathogens had relatively higher median lethal time than the duo neonicotinoids. Mixture of them at 1:1 ratio showed lower median lethal time than any of their individual. CTC values for their combination was also recorded >1 in all the cases depicting compatibility (Table 1).

Combined application of *B. bassiana* and Imidacloprid

Table 1 Median lethal time of neonicotinoid insecticides and EPF alone and their 1:1 combinations against adults of *Lipaphis erysimi*

Treatment	Heterogeneity		Regression equation (Y=)	Median lethal time (LT50) (h)	Fiducial limit	Co-toxicity coefficient
	df	χ^2				
Year 2015						
<i>B. bassiana</i>	5	2.902	3.236X – 0.324	44.07	50.35 – 38.58	-
<i>M. anisopliae</i>	5	8.102	3.152X – 0.267	46.86	53.99 – 40.67	-
<i>L. lecanii</i>	5	3.175	2.605X + 0.759	41.46	50.82 – 37.18	-
Imidacloprid 17. 8 SL	4	3.673	4.059X – 1.004	30.15	33.90 – 26.82	-
<i>B. bassiana</i> + Imidacloprid (1:1)	4	0.939	4.383X – 1.181	25.72	29.05 – 22.77	1.17
<i>M. anisopliae</i> + Imidacloprid (1:1)	6	5.012	4.147X – 0.899	26.45	29.96 – 23.36	1.14
<i>L. lecanii</i> + Imidacloprid (1:1)	6	6.599	5.713X – 2.562	21.22	23.62 – 19.07	1.42
Thiamethoxam 25 WG	5	2.306	3.884X – 0.185	21.63	25.27 – 18.52	-
<i>B. bassiana</i> + Thiamethoxam (1:1)	4	3.084	3.241X + 1.109	15.86	20.58 – 12.22	1.36
<i>M. anisopliae</i> + Thiamethoxam (1:1)	5	0.619	3.3321X+ 0.808	18.28	22.85 – 14.63	1.18
<i>L. lecanii</i> + Thiamethoxam (1:1)	5	0.575	2.795X + 2.047	13.39	19.96 – 9.22	1.62
Year 2016						
<i>B. bassiana</i>	5	0.329	2.230X + 1.150	53.22	64.39 – 43.99	-
<i>M. anisopliae</i>	5	3.191	2.367X + 0.649	68.83	85.86 – 55.18	-
<i>L. lecanii</i>	5	1.469	1.851X + 1.956	44.09	54.85 – 35.44	-
Imidacloprid 17. 8 SL	4	2.089	2.088X + 1.810	33.69	42.91 – 26.46	-
<i>B. bassiana</i> + Imidacloprid (1:1)	4	1.362	3.498X – 0.084	28.41	33.34 – 24.21	1.19
<i>M. anisopliae</i> + Imidacloprid (1:1)	6	0.249	3.637X + 0.319	28.99	33.90 – 24.80	1.16
<i>L. lecanii</i> + Imidacloprid (1:1)	6	0.084	3.523X + 0.118	24.31	28.32 – 20.51	1.39
Thiamethoxam 25 WG	5	1.104	3.134X + 0.582	24.93	29.45 – 21.10	-
<i>B. bassiana</i> + Thiamethoxam (1:1)	4	0.634	1.796X + 2.758	17.72	23.24 – 13.51	1.12
<i>M. anisopliae</i> + Thiamethoxam (1:1)	5	0.798	1.890X + 2.553	19.69	25.18 – 15.40	1.01
<i>L. lecanii</i> + Thiamethoxam (1:1)	5	1.221	1.948X + 2.673	15.66	20.56 – 11.92	1.27
Year 2017						
<i>B. bassiana</i>	5	0.446	3.802X – 1.426	48.99	57.16 – 42.01	-
<i>M. anisopliae</i>	5	0.507	3.911X – 1.698	51.60	60.63 – 43.91	-
<i>L. lecanii</i>	5	0.337	3.909X – 1.456	44.83	51.27 – 39.20	-
Imidacloprid 17. 8 SL	4	0.251	3.296X – 0.284	40.10	46.40 – 34.65	-
<i>B. bassiana</i> + Imidacloprid (1:1)	4	1.533	2.308X + 1.533	31.79	39.22 – 25.77	1.26
<i>M. anisopliae</i> + Imidacloprid (1:1)	6	0.266	2.376X + 1.426	31.91	40.18 – 25.34	1.26
<i>L. lecanii</i> + Imidacloprid (1:1)	6	0.408	2.809X + 1.009	26.35	31.45 – 22.07	1.52
Thiamethoxam 25 WG	5	0.131	2.902X +0.738	29.43	34.53 – 25.08	-
<i>B. bassiana</i> + Thiamethoxam (1:1)	4	0.932	2.104X + 2.225	20.86	26.31 – 16.54	1.41
<i>M. anisopliae</i> + Thiamethoxam (1:1)	5	1.703	2.984X + 1.002	21.86	26.69 – 17.90	1.35
<i>L. lecanii</i> + Thiamethoxam (1:1)	5	2.390	1.733X + 2.850	17.41	23.31– 13.04	1.69

resulted in higher percentage mortalities of *Thrips tabaci* infesting onion than either *B. bassiana* or Imidacloprid alone (Mazra'awi 2007). It was also reported that highest mortality of 80% resulted from their combined application at field rates compared with 55%, 75% and 22% for *B. bassiana* alone, Imidacloprid alone and the control, respectively. Adding Imidacloprid to *B. bassiana* always increased mortality of

whitefly (*Bemisia argentifolii*), however, the increase was less than additive (James and Elzen 2001). Compatibility of Thiamethoxam and *B. bassiana* was also confirmed against coffee berry borer, *Hypothenemus hampei* (Oliveira *et al.* 2003). It was also reported that Imidacloprid could safely be used combining with *M. anisopliae* to get enhanced effect (Rachappa *et al.* 2007). Imidacloprid was shown to

Table 2 Relative lethal time of entomopathogens and neonicotinoids to *Lipaphis erysimi* by direct spray method

Pesticide	Median lethal time (LT ₅₀) in hour			
	2015	2016	2017	LSD at 5% (pesticide wise)
<i>B. bassiana</i>	44.07 (1) ^a	53.22 (1.21)	51.60 (1.17)	1.479 (0.28)
<i>M. anisopliae</i>	46.86 (1)	48.83 (1.04)	48.99 (1.05)	0.360 (0.07)
<i>L. lecanii</i>	41.46 (1)	44.09 (1.06)	44.83 (1.08)	0.884 (0.11)
Imidacloprid 17.8 SL	30.15 (1)	33.69 (1.12)	40.10 (1.33)	2.288 (0.42)
<i>B. bassiana</i> + Imidacloprid (1:1)	25.72 (1)	28.41 (1.11)	31.79 (1.24)	1.518 (0.31)
<i>M. anisopliae</i> + Imidacloprid (1:1)	26.45 (1)	28.99 (1.10)	31.91 (1.21)	1.129 (0.27)
<i>L. lecanii</i> + Imidacloprid (1:1)	21.22 (1)	24.31 (1.15)	26.35 (1.24)	1.740 (0.47)
Thiamethoxam 25 WG	21.63 (1)	24.93 (1.15)	29.43 (1.36)	1.489 (0.39)
<i>B. bassiana</i> + Thiamethoxam (1:1)	15.86 (1)	17.72 (1.12)	20.86 (1.32)	1.584 (0.40)
<i>M. anisopliae</i> + Thiamethoxam (1:1)	18.28 (1)	19.69 (1.08)	21.86 (1.20)	2.555 (0.66)
<i>L. lecanii</i> + Thiamethoxam (1:1)	13.39 (1)	15.66 (1.17)	17.41 (1.30)	2.148 (0.64)
LSD (P=0.05) (year wise)	3.29 (0)	2.81 (0.26)	2.83 (0.30)	--

^aFigures in the parenthesis are the relative lethal time

be synergist for *M. anisopliae* against the burrower bug, *Cyrtomenus bergi* and white grub (*Popillia japonica*) larvae in the laboratory and greenhouse (Jaramillo *et al.* 2005).

From the Table 2, it is evident that there is gradual change of median lethal time over the years. However, for the entomopathogens the change of median lethal time was comparatively slower as compared to Imidacloprid and Thiamethoxam. For the white halo fungus, *L. lecanii* the median lethal time was 41.46 h during 2015 and there were slight increases of 44.09 h and 44.83 h during the year 2016 and 2017, respectively. Considering the LT₅₀ value of the year 2015 as base (1), median lethal times were 1.06 and 1.08 times higher for the year 2016 and 2017. Paradoxically, for Imidacloprid the median lethal times varied from 30.15–40.10 hour during the same period and the median lethal times were 1.12 and 1.33 times higher during the year 2016 and 2017 from the base year. In case of Thiamethoxam, the relative lethal times were 1.15 and 1.36 times higher

during 2016 and 2017 than the base year. From Table 2 it is also clear that changes of median lethal times were higher in neonicotinoids like Imidacloprid and Thiamethoxam implicating possibly development of resistance of the duo insecticides to the mustard aphid compared to any of the entomopathogenic fungi. Interestingly, mixing of EPF with neonicotinoids at half of their respective recommended doses at 1:1 ratio not only took lower time to kill the 50% test population than their individual one but also had the lower relative lethal time than corresponding neonicotinoids.

Co-application of fungi like *B. bassiana*, *M. anisopliae*, and *L. lecanii* with suitable sub-lethal concentration of neonicotinoids as two-in-one tank mix have been successfully employed against various insect pests to reduce the selection pressure insecticides and to avoid concurrent resistance risks in target pests (Senthilkumar *et al.* 2007). Majority of mechanisms of resistance occur through induction of enzymes, especially mono-oxygenases and to some extent

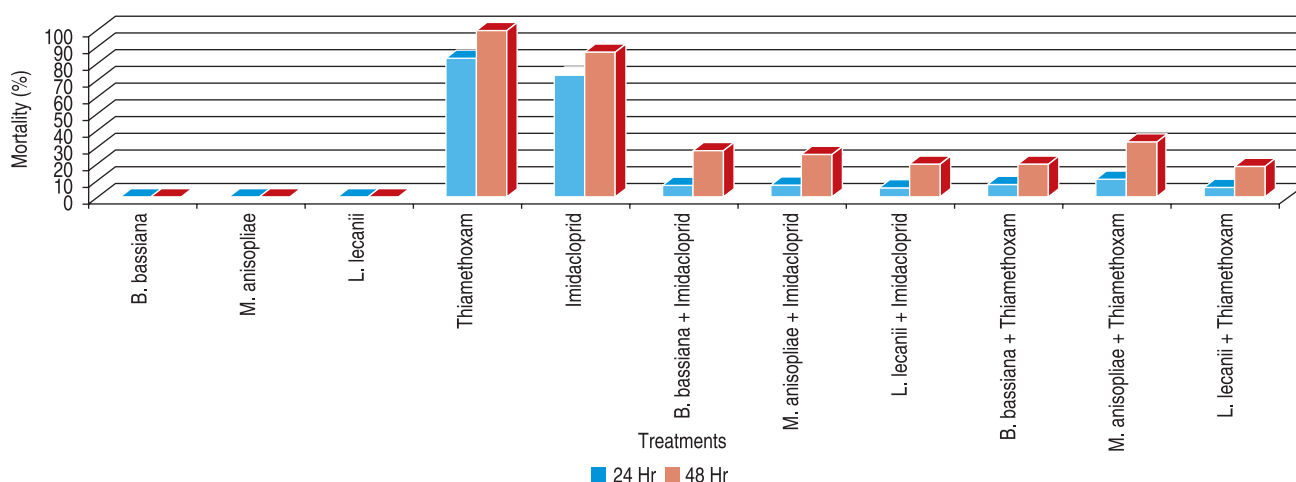


Fig 1 Toxicity of different entomopathogens and neonicotinoids alone and their combination (1:1) against second instar grubs of *C. z. sillemi*.

the esterases. Entomopathogenic fungi have an ability to induce a high degree of susceptibility to insecticides in target pests by suppressing enzyme activities and predispose them for fungal infection (Ambethgar 2009). *Cephalosporium lecanii* was inhibited by several test insecticides, viz. Dichlorvos, Carbaryl, Monocrotophos, Malathion and Endrin (Easwaramoorthy and Jayaraj 1977), Fenthion and Phosphamidon (Easwaramoorthy *et al.* 1978) at higher concentrations under *in vitro* conditions. But the efficacy of the fungus was enhanced when applied in combination with sub-lethal concentrations of the same insecticides to control coffee green scale. A significant increase in the mortality of coffee green bug *Coccus viridis* was achieved by the combination of phosphamidon and fenthion with *V. lecanii* (Easwaramoorthy *et al.* 1978). In our present study also half of the recommended dose of *L. lecanii* in combination with sub-lethal dose of neonicotinoids found effective in controlling *L. erysimi*. Recently, Rajanikanth *et al.* (2010) also observed the compatibility between Imidacloprid and *B. bassiana*.

Toxicity against second instar grubs of *C. z. sillemi* indicated that these entomopathogenic fungi did not cause any mortality both 24 and 48 h after the treatment (HAT). In contrast, duo neonicotinoids caused significantly higher mortality. Thiamethoxam proved most toxic towards this polyphagous predator as 83.33 and 100% mortalities were recorded after 24 and 48 HAT, respectively, followed by Imidacloprid (73.25 and 86.78% mortalities at 24 and 48 HAT). Interestingly, when these EPF and neonicotinoids were mixed together they were found relatively safer. Amongst the mixtures, lowest mortality (5.56%) was recorded on *L. lecanii* + Imidacloprid at 24 HAT followed by *L. lecanii* + Thiamethoxam (18.75%) at 24 HAT. As per IOBC classification on biosafety of pesticides to the beneficial and non-target arthropods, all the EPF alone and their 1:1 combination with neonicotinoids were practically harmless (<30% mortality) to the second instar grubs of *C. z. sillemi* except *M. anisopliae* + Thiamethoxam which had mortality 33.33% after 48 HAT recorded as slightly harmful. Interestingly duo neonicotinoids at their recommended doses were found moderately toxic (mortality ranged between 80-98%), whereas Thiamethoxam was found harmful by registering 100% mortality at 48 HAT. So, from the present study, it is evident that mixing of EPF with chemical insecticides like neonicotinoids not only enhanced their individual efficacy, but also reduces the synthetic insecticides load to the environment and also found to be safe to the grubs of *C. z. sillemi*, a potential polyphagous predator in vegetable ecosystem.

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