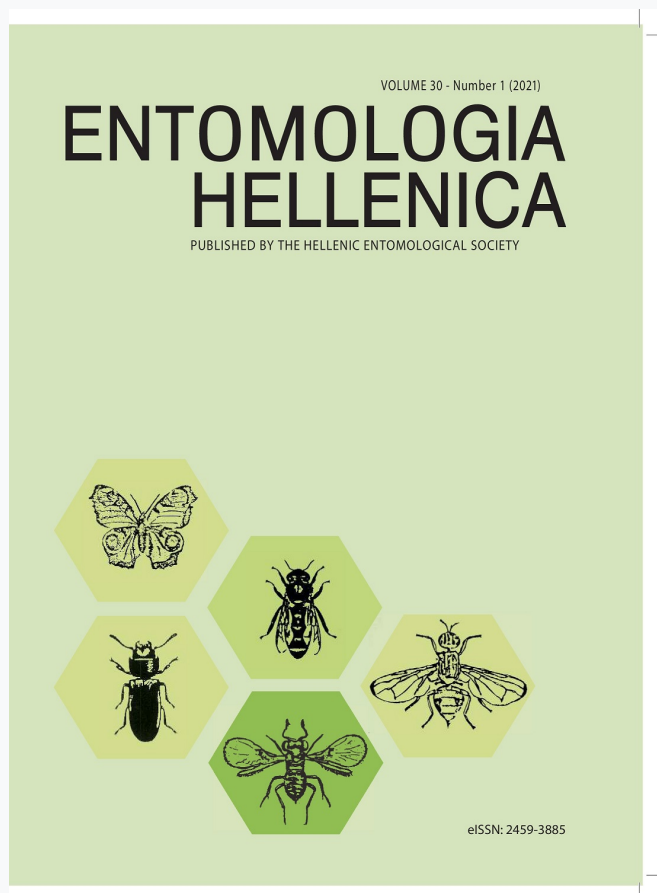


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Compatibility and combined efficacy of entomopathogenic fungi and neonicotinoid insecticides against *Myzus persicae* (Sulzer): An ecofriendly approach

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ABSTRACT

Efficacy of different entomopathogenic fungi (EPF) *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium* (= *Verticillium*) *lecanii* and neonicotinoid insecticides imidacloprid, thiamethoxam and acetamiprid were evaluated alone and their 1:1 combination at half-dose against *Myzus persicae*. Among the entomopathogenic fungi, *B. bassiana* was found to be the most effective, having the shortest median lethal time (LT₅₀) of 48.17, 48.92 and 48.87 h during the period of 2018, 2019 and 2020, respectively, followed by *L. lecanii* (49.57, 49.45 and 50.46 h) and *M. anisopliae* (51.81, 51.67 and 51.63 h). Among the three neonicotinoids, acetamiprid was found to be the most effective. Combining of *B. bassiana* and acetamiprid at half of their recommended dose led to the shortest (22.76, 23.48 and 23.06 h during 2018, 2019 and 2020, respectively) median lethal time (LT₅₀) followed by *L. lecanii* + acetamiprid (22.58, 22.68, 22.52 h) and *M. anisopliae* + acetamiprid (22.61, 23.82, 23.60 h). Combinations of entomopathogenic fungi and neonicotinoid insecticides led to co-toxicity coefficient values (CTC) > 1 and lower LT₅₀ values than each one alone, indicating their compatibility and synergistic action. Combining these EPF with sub-lethal concentration of neonicotinoids could minimize the chemical insecticides input in the environment and contribute towards a greener, ecofriendly control option of this sucking pest.

KEY WORDS: Co-toxicity coefficient, entomopathogenic fungi, *Myzus persicae*, median lethal time, neonicotinoids.

Introduction

Sucking insects are considered as one of the major pests for vegetable production in India. They are primarily phloem feeders, removing sap via specially adapted mouthparts and also secrete sugar rich honey dew which they deposit on the plant surface and on which black sooty mould develops and thereby hindering normal photosynthesis. Amongst all sucking insects, aphids are the most important as they attack almost all vegetable crops year-round (Halder et al. 2014). Aphids alone have the potential to inflict yield losses up to 82% in case of cruciferous crops when insecticides were not applied

(Razaq et al. 2011). The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is considered an important polyphagous aphid infesting vegetable crops worldwide (Blackman & Eastop 1985). This species has more than 875 secondary host plant species (Ro et al. 1998). It reduces directly both yield and quality of the harvestable products and also acts indirectly as a vector of many plant viruses (Castle & Berger 1993; Syller 1994). To control this important pest, farmers of this region often rely on synthetic insecticides, including neonicotinoids. This practice has led to problems, such as resistance to insecticides, resurgence of target insects and secondary

pest outbreaks, in addition to widespread killing of beneficial non-target organisms (Halder et al. 2012, 2018).

Considering the adverse effects of these synthetic insecticides, presently biological control methods of insect pests using different entomopathogenic microorganisms, as well as predator and parasitoid insects is gaining importance due to their target specificity, self-perpetuity and apparent safety to the environment. 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) have been proved beyond doubt over the past decades. Literature pertaining to compatibility of different entomopathogenic fungi and neonicotinoid insecticides against this pest is scarce. 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 entomopathogenic microorganisms and neonicotinoids with special reference to change in susceptibility level over the years was also calculated, to assess the development of resistance.

Materials and Methods

Talc-based formulation of three entomopathogenic fungi (EPF), *B. bassiana* IIVR strain (1×10^{10} cfu/g), *M. anisopliae* IIVR strain (1×10^{10} cfu/g) and *L. lecanii* (2×10^9 cfu/g), each at recommended doses of 5 g/l of water were used for the experiments. Proprietary insecticidal formulations of neonicotinoids viz., Imidacloprid 17.8 SL, Thiamethoxam 25 WG and Acetamiprid 20 SP at their recommended doses, 0.35 ml/l, 0.35 g/l and 0.15 g/l, respectively, were taken

for the experimental purpose. All microbial insecticides and the neonicotinoid insecticides at their recommended as well as at half of the recommended dose at 1:1 combination with EPF were tested for their efficacy against the green peach aphid, *M. persicae*.

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 the bioassays. Twenty insects were placed in each petri dish (9 cm diameter) and were directly sprayed with 1 ml of each concentration of different entomopathogenic fungi, three neonicotinoid insecticides and their combination (1:1 ratio) under Potter's tower at 340 g/cm² pressure. The sprayed petri dishes containing the aphids were dried for five minutes under the fan. Fresh un-infested and untreated plant material was provided as food. All bioassays were performed in biocontrol laboratory under 27±1°C temperature and 70±5% relative humidity with 12:12h light:dark photoperiod. Observations were recorded every 12 hrs in each case. Moribund insects were considered as dead. All experiments were conducted during winter (December, January and February) for three consecutive years in 2018, 2019 and 2020.

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 (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) or synergistic ratio (SR) was calculated as

$$(CTC) = \frac{LT_{50} \text{ of Neonicotinoid alone}}{LT_{50} \text{ of insecticide} + EPF}$$

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 & Johnson 1960; Corbel et al. 2006; Halder et al. 2017). Similarly, relative lethal time of all these insecticides and EPF alone and their 1:1 combination were calculated using the formula

$$\text{Relative lethal time} = \frac{\text{LT}_{50} \text{ of 2019 or 2020}}{\text{LT}_{50} \text{ of 2018}}$$

Results

Marked differences were observed among these microbial insecticides alone and their combinations. The white halo fungus, *L. lecanii* was found to be the most promising entomopathogen against viviparous, apterous adults of *M. persicae*. Talc-based formulation of *L. lecanii* at its recommended dose took only 49.57 hour to kill fifty per cent of test population followed by *B. bassiana* (48.17 h) and *M. anisopliae* (51.81 h) during 2018. Amongst the three neonicotinoid insecticides, acetamiprid was found to be the best as it registered lower median lethal time (24.41 h) than the imidacloprid (27.49 h) and thiamethoxam (28.84 h). When these EPF and neonicotinoids were combined at 1:1 ratio and sprayed under a potter's tower, percentage mortality changed in time dependent manner and the combination of *L. lecanii* + acetamiprid was found to be the most effective, leading to the lowest median lethal time of 22.58 h during 2018 followed by *M. anisopliae* + acetamiprid (22.61 h). Considering individual neonicotinoids as having a base $CTC = 1$, the combinations of EPF and neonicotinoids (at 1:1 ratio) had $CTC > 1$ indicating they are compatible and synergistic in action at half of their recommended doses. From Table 1 it is also clear that maximum co-toxicity coefficient value (1.14) was recorded in the *M. anisopliae* + imidacloprid (1:1) combination.

The same trend was also observed during 2019. *Lecanicillium lecanii* amongst the EPF and acetamiprid amongst the neonicotinoids were found to be the most effective. Imidacloprid sprayed at the recommended dose had a median lethal time of 27.78 h but at a combination 1:1 with different EPF at half of their respective recommended doses led to lower LT_{50} values than imidacloprid and EPF individually (Table 2). Similarly, thiamethoxam alone had an LT_{50} of 29.30 h but when mixed with *L. lecanii*, *B. bassiana* and *M. anisopliae*, LT_{50} , it decreased to 27.81, 26.65 and 28.96 h, respectively. CTC values for all combinations were > 1 , indicating compatibility and synergistic action amongst them. During 2020, a consistent trend was also recorded. All entomopathogens had relatively higher median lethal time than the three neonicotinoids (Table 1). Their 1:1 mixture showed lower median lethal time than they had individually. Their combination CTC values were > 1 in all cases, indicating compatibility (Table 1).

From Table 2, a gradual change of LT_{50} over the years is evident. However, for the entomopathogens the change of median lethal time was comparatively at a slower rate than that of the tested neonicotinoids. For *L. lecanii*, the median lethal time was 49.57 h during 2018, then there was a slight increase to 50.46 h during 2020. Considering the LT_{50} value of 2018 as base (1), median lethal times were 1.02 times higher for 2020. Paradoxically, for imidacloprid LT_{50} varied from 27.49 to 28.12 h during the same period and median lethal times during 2019 and 2020 were 1.01 and 1.02 times higher from the base year, respectively. In case of thiamethoxam, the relative lethal times were 1.02 and 1.01 times higher during 2019 and 2020 than the base year. Interestingly, mixing EPF with neonicotinoids at half of their respective recommended doses (1:1) led to a shorter time of killing fifty per cent test population than their individual one.

TABLE 1. Median lethal time of neonicotinoid insecticides and EPF alone and their 1:1 combinations against *Myzus persicae*

Year 2018						
Treatments	Heterogeneity		Regression equation (Y=)	Median lethal time (LT ₅₀) (h)	Fiducial limit	Co-toxicity coefficient
	df	χ^2				
<i>Beauveria bassiana</i>	5	6.876	3.135X - 0.275	48.17	55.83 - 41.57	--
<i>Metarhizium anisopliae</i>	5	5.604	4.782X - 3.199	51.81	57.69 - 46.53	--
<i>Lecanicillium lecanii</i>	5	6.315	3.754X - 1.364	49.57	56.04 - 43.85	--
Imidacloprid 17.8 SL	5	0.641	4.539X - 1.532	27.49	30.97 - 24.37	--
<i>Beauveria bassiana</i> + Imidacloprid (1:1)	4	0.925	4.186X - 0.878	25.35	28.99 - 22.18	1.09
<i>Metarhizium anisopliae</i> + Imidacloprid (1:1)	4	0.572	4.470X - 1.175	24.07	27.48 - 21.08	1.14
<i>Lecanicillium lecanii</i> + Imidacloprid (1:1)	4	0.089	4.700X - 1.761	25.29	28.71 - 22.25	1.09
Thiamethoxam 25 WG	5	0.139	3.773X - 0.508	28.84	33.02 - 25.19	--
<i>Beauveria bassiana</i> + Thiamethoxam (1:1)	5	4.809	5.794X - 3.169	25.71	28.45 - 23.23	1.12
<i>Metarhizium anisopliae</i> + Thiamethoxam (1:1)	4	0.147	3.993X - 0.340	24.75	28.49 - 21.55	1.17
<i>Lecanicillium lecanii</i> + Thiamethoxam (1:1)	5	3.801	3.798X - 0.545	27.48	32.72 - 25.43	1.05
Acetamiprid 20% SP	5	0.348	3.989X - 0.535	24.41	28.10 - 21.18	--
<i>Beauveria bassiana</i> + Acetamiprid (1:1)	5	1.388	5.061X - 1.869	22.76	25.79 - 20.09	1.07
<i>Metarhizium anisopliae</i> + Acetamiprid (1:1)	4	0.385	3.387X - 0.413	22.61	27.11 - 18.85	1.08
<i>Lecanicillium lecanii</i> + Acetamiprid (1:1)	5	1.826	3.667X - 5.038	22.58	27.93 - 20.37	1.08
Year 2019						
<i>Beauveria bassiana</i>	5	2.921	4.116X - 1.955	48.92	54.38 - 44.02	--
<i>Metarhizium anisopliae</i>	5	2.766	2.862X + 0.097	51.67	62.25 - 42.88	--
<i>Lecanicillium lecanii</i>	5	2.228	3.328X - 0.638	49.45	57.74 - 42.34	--
Imidacloprid 17.8 SL	5	1.987	1.814X + 2.381	27.78	36.13 - 21.36	--
<i>Beauveria bassiana</i> + Imidacloprid (1:1)	4	2.556	1.829X - 2.396	26.54	34.41 - 20.47	1.05
<i>Metarhizium anisopliae</i> + Imidacloprid (1:1)	4	3.716	1.979X - 2.159	27.24	33.39 - 22.23	1.02
<i>Lecanicillium lecanii</i> + Imidacloprid (1:1)	4	3.939	2.011X - 2.156	25.97	31.70 - 21.28	1.00
Thiamethoxam 25 WG	5	1.332	1.623X - 2.618	29.30	39.67 - 21.77	--
<i>Beauveria bassiana</i> + Thiamethoxam (1:1)	5	2.274	1.897X - 2.295	26.65	34.07 - 20.85	1.10
<i>Metarhizium anisopliae</i> + Thiamethoxam (1:1)	5	2.342	1.980X - 2.106	28.96	35.73 - 23.48	1.01
<i>Lecanicillium lecanii</i> + Thiamethoxam (1:1)	4	2.544	2.040X - 2.054	27.81	34.02 - 22.72	1.05
Acetamiprid 20% SP	5	1.684	1.791X - 2.511	24.49	31.25 - 19.20	--
<i>Beauveria bassiana</i> + Acetamiprid (1:1)	5	3.633	1.972X - 2.296	23.48	29.26 - 18.85	1.04
<i>Metarhizium anisopliae</i> + Acetamiprid (1:1)	5	3.348	1.944X - 2.322	23.82	29.20 - 19.03	1.03
<i>Lecanicillium lecanii</i> + Acetamiprid (1:1)	4	3.119	1.796X - 2.565	22.68	28.69 - 17.93	1.08

(Table 1. continues to next page)

(Table 1. continued from previous page)

Year 2020						
<i>Beauveria bassiana</i>	5	3.595	4.210X – 2.111	48.87	55.17 – 43.29	--
<i>Metarhizium anisopliae</i>	4	2.412	3.785X – 1.483	51.63	59.01 – 45.17	--
<i>Lecanicillium lecanii</i>	5	2.674	3.527X – 1.006	50.46	58.14 – 43.79	--
Imidacloprid 17.8 SL	5	3.749	2.408X + 1.511	28.12	34.50 – 22.91	--
<i>Beauveria bassiana</i> + Imidacloprid (1:1)	5	0.357	4.247X – 1.032	26.32	29.46 – 23.51	1.07
<i>Metarhizium anisopliae</i> + Imidacloprid (1:1)	4	0.364	4.230X – 1.059	27.07	30.29 – 24.19	1.04
<i>Lecanicillium lecanii</i> + Imidacloprid (1:1)	5	0.996	3.474X + 0.084	25.99	29.80 – 22.69	1.08
Thiamethoxam 25 WG	5	1.556	2.805X + 0.899	28.98	34.37 – 24.43	--
<i>Beauveria bassiana</i> + Thiamethoxam (1:1)	5	0.203	3.486X + 0.053	26.24	30.42 – 22.63	1.11
<i>Metarhizium anisopliae</i> + Thiamethoxam (1:1)	5	1.354	3.334X + 0.133	28.51	32.59 – 24.94	1.00
<i>Lecanicillium lecanii</i> + Thiamethoxam (1:1)	5	1.456	4.201X – 1.031	27.27	30.52 – 24.35	1.06
Acetamiprid 20% SP	5	5.398	4.228X – 0.880	24.59	28.55 – 21.19	--
<i>Beauveria bassiana</i> + Acetamiprid (1:1)	5	0.516	3.516X + 0.208	23.06	27.24 – 19.51	1.07
<i>Metarhizium anisopliae</i> + Acetamiprid (1:1)	4	0.308	3.887X – 0.337	23.60	27.31 – 20.39	1.04
<i>Lecanicillium lecanii</i> + Acetamiprid (1:1)	6	0.679	3.674X + 0.051	22.52	26.27 – 18.84	1.09

TABLE 2. Relative lethal time of entomopathogens and neonicotinoids to *Myzus persicae* by direct spray method

Pesticides	Median lethal time (LT50) (in hour)			
	2018	2019	2020	LSD at 5% (pesticide wise)
<i>Beauveria bassiana</i>	48.17 (1)	48.92 (1.02)	48.87 (1.00)	0.14 (0.01)
<i>Metarhizium anisopliae</i>	51.81 (1)	51.67 (1.00)	51.63 (1.00)	0.03 (0)
<i>Lecanicillium lecanii</i>	49.57 (1)	49.45 (1.00)	50.46 (1.02)	0.18 (0.03)
Imidacloprid 17.8 SL	27.49 (1)	27.78 (1.01)	28.12 (1.02)	0.14 (0.02)
<i>B. bassiana</i> + Imidacloprid (1:1)	25.35 (1)	26.54 (1.05)	26.32 (1.04)	0.29 (0.06)
<i>Metarhizium anisopliae</i> + Imidacloprid (1:1)	24.07 (1)	27.24 (1.13)	27.07 (1.13)	0.80 (0.16)
<i>L. lecanii</i> + Imidacloprid (1:1)	25.29 (1)	25.97 (1.03)	25.99 (1.03)	0.18 (0.04)
Thiamethoxam 25 WG	28.84 (1)	29.30 (1.02)	28.98 (1.01)	0.10 (0.02)
<i>B. bassiana</i> + Thiamethoxam (1:1)	25.71 (1)	26.65 (1.04)	26.24 (1.02)	0.21 (0.05)
<i>M. anisopliae</i> + Thiamethoxam (1:1)	24.75 (1)	28.96 (1.17)	28.51 (1.00)	1.02 (0.21)
<i>L. lecanii</i> + Thiamethoxam (1:1)	27.48 (1)	27.81 (1.01)	27.27 (1.00)	0.12 (0.01)
Acetamiprid 20 SP	24.41 (1)	24.49 (1.00)	24.59 (1.01)	0.04 (0.01)
<i>B. bassiana</i> + Acetamiprid SP (1:1)	22.76 (1)	23.48 (1.03)	23.06 (1.01)	0.17 (0.03)
<i>M. anisopliae</i> + Acetamiprid SP (1:1)	22.61 (1)	23.82 (1.05)	23.60 (1.04)	0.31 (0.06)
<i>Lecanicillium lecanii</i> + Acetamiprid SP (1:1)	22.58 (1)	22.68 (1.00)	22.52 (1.00)	0.04 (0)
LSD at 5% (year wise)	4.37 (0)	4.16 (0.12)	4.26 (0.07)	--

Discussion

Combined application of *B. bassiana* and imidacloprid resulted in higher percentage mortality of *Thrips tabaci* infesting onion than either *B. bassiana* or imidacloprid alone (Mazraáwi 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* (James & 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 combined with *M. anisopliae* to get enhanced effect (Rachappa et al. 2007). Imidacloprid showed synergism when combined with *M. anisopliae* for control of the burrower bug, *Cyrtomenus bergi* and white grub (*Popillia japonica*) larvae in the laboratory and greenhouse (Jaramillo et al. 2005).

Co-application of fungi like *B. bassiana*, *M. anisopliae*, *L. lecanii* with suitable sub-lethal concentration of neonicotinoids as two-in-one tank mixture have been successfully employed against various insect pests in order to reduce the selection pressure of insecticides and to avoid concurrent resistance risks in target pests (Senthilkumar & Raghupathy 2007). The majority of mechanisms of resistance occur through induction of enzymes, especially mono-oxygenases, glutathione-S-transferases and esterases (Martin et al. 2002; Liu 2015). 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 &

Jayaraj 1977), fenthion and phosphamidon (Easwaramoorthy et al. 1978) at higher concentrations *in-vitro*. Nevertheless, the efficacy of this fungus was improved when applied in combination with sub-lethal concentrations of the same insecticides to control coffee green scale, *Coccus viridis* (Easwaramoorthy et al. 1978). Also Rajanikanth et al. (2010) observed the compatibility between imidacloprid and *B. bassiana*.

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**Συμβατότητα και συνδυαστική αποτελεσματικότητα
εντομοπαθογόνων μυκήτων και νεονικοτινοειδών εντομοκτόνων
κατά του *Myzus persicae* (Sulzer): μια φιλική προς το
περιβάλλον προσέγγιση.**

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ΠΕΡΙΛΗΨΗ

Η αποτελεσματικότητα των διαφόρων εντομοπαθογόνων μυκήτων *Beauveria bassiana*, *Metarhizium anisopliae* και *Lecanicillium* (= *Verticillium*) *lecanii* και νεονικοτινοειδών εντομοκτόνων imidacloprid, thiamethoxam και acetamiprid αξιολογήθηκε όταν εφαρμόστηκαν μόνα τους και σε συνδυασμό τους 1:1 στη μισή δόση κατά του *Myzus persicae*. Μεταξύ των εντομοπαθογόνων μυκήτων, ο *B. bassiana* βρέθηκε να είναι ο πιο αποτελεσματικός, έχοντας τον μικρότερο μέσο θανατηφόρο χρόνο (LT₅₀) 48.17, 48.92 και 48.87 ώρες κατά την περίοδο 2018, 2019 και 2020, αντίστοιχα, ακολουθούμενο από τον *L. lecanii* (49.57, 49.45 και 50.46 ώρες) και *M. anisopliae* (51.81, 51.67 και 51.63 ώρες). Μεταξύ των τριών νεονικοτινοειδών, το acetamiprid βρέθηκε να είναι το πιο αποτελεσματικό. Ο συνδυασμός *B. bassiana* και acetamiprid στο ήμισυ της συνιστώμενης δόσης τους, οδήγησε στο συντομότερο (22.76, 23.48 και 23.06 ώρες κατά τη διάρκεια του 2018, 2019 και 2020, αντίστοιχα) μέσο θανατηφόρο χρόνος (LT₅₀) ακολουθούμενος από το συνδυασμό *L. lecanii* + acetamiprid (22.58, 22.68 , 22.52 ώρες) και *M. Anisopliae* + acetamiprid (22.61, 23.82, 23.60 ώρες). Οι συνδυασμοί εντομοπαθογόνων μυκήτων και νεονικοτινοειδών εντομοκτόνων οδήγησαν σε τιμές συντελεστή συνδυαστικής τοξικότητας (CTC) > 1 και χαμηλότερες τιμές LT₅₀ από το κάθε ένα ξεχωριστά, υποδεικνύοντας τη συμβατότητά και τη συνεργιστική τους δράση. Ο συνδυασμός των εντομοπαθογόνων μυκήτων με τη θανατηφόρα συγκέντρωση νεονικοτινοειδών εντομοκτόνων θα μπορούσε να ελαχιστοποιήσει την εισροή χημικών εντομοκτόνων στο περιβάλλον και να συμβάλει σε μια πιο πράσινη, φιλική επιλογή καταπολέμησης αυτού του εχθρού των καλλιεργειών.