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Effect of fungal isolates and imidacloprid on cabbage aphid *Brevicoryne brassicae* and its parasitoid *Diaeretiella rapae*

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Abstract

Diaeretiella rapae (McIntosh) is a primary parasite of the cabbage aphid *Brevicoryne brassicae* (L.); both species are widely distributed throughout the world. In this research, the efficacy of imidacloprid and five entomopathogenic fungi: *Acremonium sclerotigenum*, *Beauveria bassiana*, *Paecilomyces variotii*, *Simplicillium* sp. and *Lecanicillium muscarium*, against cabbage aphid and its parasitoid was evaluated. Concentration-mortality and time-mortality data were subjected to Probit analyses to estimate LC_{50} (lethal concentration) and LT_{50} (lethal time) values for each isolate and imidacloprid for adults of *B. brassicae* and *D. rapae*. The LC_{50} values for *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp., *L. muscarium* and imidacloprid were 2.7×10^3, 3.7×10^3, 8.1×10^6, 4.7×10^5 and 2.5×10^3 conidia ml^{-1} and 13.56 ppm, respectively. Cumulative mortality of *B. brassicae* and *D. rapae* 7 days after treatments was 98.56% and 99.99% by *L. muscarium* at high concentration (10^8 conidia ml^{-1}), respectively. The highest mortality of *B. brassicae* and *D. rapae* after 48 and 72 hours was observed in combinations of sublethal mixed treatments: LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *L. muscarium*), and LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *P. variotii*), LC_{25} (imidacloprid + *Simplicillium* sp.), LC_{25} (imidacloprid + *L. muscarium*), respectively. The LT_{50} values for *B. brassicae* and *D. rapae* at concentration 10^8 conidia ml^{-1} were obtained 0.26 and 2.16 days by *A. sclerotigenum*, and 7.4 and 6.22 days – by *P. variotii* isolate, respectively. This study showed that imidacloprid had harmful effect on the pest and parasitoid; however, fungal isolates were safer than imidacloprid to the parasitoid. The results of this study show that entomopathogenic fungi can be effectively used alone and mixed with sublethal concentration (LC_{25} = 9.23 ppm) of imidacloprid in integrated pest management of *B. brassicae*.

Key words: biocontrol, entomopathogenic fungi, parasitoid, pathogenicity, sublethal.

Introduction

The cabbage aphid, *Brevicoryne brassicae* L. (Hem.: Aphididae) is a serious pest of cabbage that regularly causes significant yield reductions in brassica crops (Collier, Finch, 2017). Chemical compounds have been vastly used to control *B. brassicae*, which caused development of resistance, destruction of natural enemies and environmental pollution (Pereira et al., 2018). Therefore, considerable effort has been devoted to researching environmentally safe methods for management of *B. brassicae*, particularly biological control (Brennan, 2016; Song et al., 2017). The hymenopteran parasitoid, *Diaeretiella rapae* (McIntosh) (Hym.: Braconidae, Aphidiinae) is one of the most important naturally-occurring parasitoids of *B. brassicae* (Saleh et al., 2009; Singh, Singh, 2015) and is often used in augmentative biological control programs (Neuville et al., 2015).

Among microbial control agents, entomopathogenic fungi have considerable potential to suppress various arthropod pest populations (Güçlü et al., 2010; Sandhu et al., 2012). These fungi present a potential alternative to pesticides that can provide long-lasting pest control without undesirable impacts on non-target organisms or the broader environment (Kim et al., 2006; Jones et al., 2009). However, the best candidates for use in an integrated pest management (IPM) program would be those that are virulent to the target while being innocuous to the pest's predators and parasitoids. Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces* ssp. showed a promising level of activity against aphids (Gurulingappa et al., 2011; Sandhu et al., 2012). Rashki et al. (2009) demonstrated that entomopathogenic fungi *B. bassiana* had no adverse effect on biological parameters of the parasitoid wasp, *Aphidius matricariae* (Hymenoptera: Braconidae) and can be successfully combined for biological control of *Myzus persicae* (Hemiptera:

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Aphididae). *B. bassiana* and *Paecilomyces fumosoroseus* (Wize) Brown and Smith have been reported as most important entomopathogenic fungi (Al-Olayan, 2013).

The *Lecanicillium lecanii* has been reported to cause natural epizootics in aphid and mealy bug populations in natural conditions (Shah, Pell, 2003). The *L. lecanii* isolates were more infective compared to other entomopathogenic fungi in order Hypocreales (Ascomycota) as well as *B. bassiana*, *Paecilomyces* sp. and *Metarhizium anisopliae* against aphid species (Loureiro, Moino, 2006). Additionally, imidacloprid, a neonicotinoid insecticide, is a contact and oral chemical which binds selectively to the nicotinic acetylcholine receptor to control sucking insects such as cabbage aphids (Roessink et al., 2013).

In IPM programs, it is essential to know the influence of the compatibility between entomopathogenic fungi and pesticide used in crop protection (Sain et al., 2019). Therefore, use of entomopathogenic fungi as biopesticides in combination with sublethal dose of chemical agents should be considered. Moreover, Rashki et al. (2015) reported that sublethal dose of imidacloprid causes restlessness in aphids and enhances the likelihood of development of entomopathogenic conidia from their contaminated habitat. Thus, additional information is needed to get better understanding of the impacts of pesticides and entomopathogenic fungi on natural enemies (Kim et al., 2006; Jones et al., 2009). Entomopathogenic fungi can be used in combination with the parasitoids and predators as biological control agents for controlling the aphids. This method requires an effective time management in order to prevent the antagonistic interactions (Seiedy et al., 2015).

The aims of this study were (1) to determine susceptibility of *Brevicoryne brassicae* and its parasitoid *Diaeretiella rapae* to five entomopathogenic fungi: *Acremonium sclerotigenum*, *Beauveria bassiana*, *Paecilomyces variotii*, *Simplicillium* sp., *Lecanicillium muscarium*, and imidacloprid as insecticide with LC_{50} and LT_{50} values, and (2) estimate their combination effect for use in integrated aphid management with the least effect on parasitoid.

Materials and methods

This research was performed in a greenhouse at the Agriculture Faculty of Urmia University, Iran during 2018 under controlled conditions of 25 ± 2°C temperature, 60 ± 5% relative air humidity and 16/8 (light/dark) photoperiod.

Rearing of Brassicae oleracea. Cabbages were grown from seed in plastic flower pots (20.0 cm diameter × 23.0 cm height), filled with a potting soil mix compost and peat moss, fertilized once with $N_{15}P_{15}K_{15}$ and watered as needed.

Rearing of Brevicoryne brassicae. Cabbage aphids *B. brassicae* (were collected from Brassicaceae plants in the region of Nazlo village (37.5287° N, 45.0469° E) near Urmia city, Iran. Aphids were reared on common cabbage (*B. oleracea* var. *capitate)* at a six-leaf stage in 500 ml pots in wooden framed cages (45 × 50 × 70 cm) covered by cheesecloth and maintained in a greenhouse. Adult females from the stock colonies were used to obtain nymphs and new adults for the experiments.

Rearing of Diaeretiella rapae. A colony of *D. rapae* was reared on *B. brassicae* under the same greenhouse conditions. Aphid mummies were collected from cabbage fields by cutting infected leaves and kept until emergence in net cover cages (90 × 90 × 70 cm). All emerged parasitoides were fed a 30% honey solution and used to parasitize aphids or in bioassay experiments, 24 h later.

Parasitism test. For parasitism, a total of 250 forth nymph aphids were allowed to settle on a freshly excised leaf of cabbage (4.0 cm diameter) placed in a plastic Petri dish (9.0 cm diameter) by a fine paint brush on moistened filter paper. Therefore, fifteen mated females of *D. rapae* that emerged were gently introduced by an aspirator into Petri dishes (5 in each dish) on 250 forth nymph aphids (25 in each dish) for 24 h. After one day, the parasitoids were removed and the hosts were left undisturbed until they mummified. After mummification, they were collected and transferred to sterilized Petri dishes (9.0 cm diameter) for emergence and bioassay test. The experiment was replicated three times.

Preparation of fungal isolates and conidial suspensions. Five fungal isolates: *Acremonium sclerotigenum* FCCUU490, *Beauveria bassiana* FCCUU438, *Paecilomyces variotii*, *Simplicillium* sp. FCCUU478 and *Lecanicillium muscarium* FCCUU420, were obtained from the collection of the Plant Protection Department of Urmia University and cultured on SDA (Sabouraud dextrose agar) at 25 ± 1°C temperature for two weeks to induce sporulation. Spores were removed from the surface of media with a sterile scalpel and transferred to a test tube containing sterilized distilled water with 0.05% Tween 80 as surfactant. After the tubes were shaken, the spore suspension was filtered through three layers of muslin cloth to remove hyphal debris. Spores were counted with a Neubauer hemocytometer (LABART, Germany) and serial dilutions were made to obtain conidial concentrations of 10^4, 10^5, 10^6, 10^7 and 10^8 conidia ml^{-1}.

Insecticide. Commercial formulations of imidacloprid 350 g L^{-1} Confidor SC (Bayer CropScience, www.cropscience.bayer.com) suspension of 0.35% concentration were used in the bioassay test.

Concentration-mortality (LC) and time-mortality (LT) values of aphid and parasitoid to fungi. Bioassay test was carried out on aphid and its parasitoid. Different concentrations (10^4 to 10^8 conidia ml^{-1}) of each fungal isolate as main concentrations base of preliminary test were prepared. Aphids were treated by fungal concentrations. The sterilized cabbage leaves with 10 aphids were dipped in each concentration with 0.05% Tween 80 for 10 seconds then were placed in Petri dishes. The control treatment was dipped in water with 0.05% Tween 80. We confirmed that 0.05% Tween 80 is not harmful to aphids or conidial germination in pre-experiments. The mortality was recorded for 7 days. To estimate LT_{50} value, aphid mortality was recorded every 12 hours. Newly born nymphs were counted and removed daily from the plants. Aphid cadavers were disinfected using 2% NaClO (sodium hypochlorite) and rinsed with sterile distilled water. The cadavers were then incubated in a humidity chamber (100% relative humidity) into a Petri dish on damp filter paper to ensure that death was due to fungal treatment. Only aphids which exhibited fungal sporulation were considered to have died from the fungal treatment. The whole experiment was conducted with three replicates (Fadayivata et al., 2014).

LC and LT values of aphid and parasitoid to imidacloprid. In order to determine LC_{50} value of imidacloprid, first in a series of preliminary tests, the maximum and minimum concentrations were determined with 80–20% mortality on the adult insect, and then between maximum and minimum, three concentrations were measured by logarithm method (Bayramzadeh et al., 2019). Thus, five concentrations (10, 13.16, 17.32, 22.80 and 30.00 ppm) with distilled water as control, each concentration in three replicates, were sprayed on cabbage leaves and 10 adult aphids were released in each replication. The mortality of different concentrations after 24, 48 and 72 hours was recorded. To estimate LT_{50} value, aphid mortality was recorded every 12 hours. Newly born nymphs were counted and removed daily from the plants.

Percentages of emergence of parasitoid from infested aphids by fungal isolates. This experiment was laid out in a completely randomized design with 12 treatments: LC_{50} *A. sclerotigenum*, LC_{50} *B. bassiana*, LC_{50} *P. variotii*, LC_{50} *Simplicillium* sp., LC_{50} *L. muscarium*, LC_{50} imidacloprid, LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *P. variotii*), LC_{25} (imidacloprid + *Simplicillium* sp.), LC_{25} (imidacloprid + *L. muscarium*) and distilled water as control sprayed in three replications on 150 mummy aphids. *B. brassica* mummies were collected from the aphid rearing cage and 50 mummy aphids were placed in each Petri dish (60 mm diameter) on leaves of cabbage in the cage. The cages were incubated in a climate chamber at 25 ± 2°C temperature, 60 ± 5% relative air humidity and 16/8 h light/dark regime until the parasitoids emerged (48 h). Then mortality of mummy was recorded.

Lethal effect of fungi and imidacloprid combination. After calculating LC_{50} and LC_{25} values for fungal isolates and imidacloprid on adult insects, combination effects of fungi and pesticide were evaluated in the laboratory. Therefore, in the preliminary test to detect pesticide side effects of five entomopathogenic fungi on conidia viability were evaluated using a method modified by Lazreg et al. (2009). A conidial suspension was adjusted to 1×10^4 conidia ml^{-1}, and 0.1 ml^{-1} was sprayed on to 6-cm diameter Petri dishes containing SDA. Petri dishes were maintained at 25 ± 2°C temperature. After 24 h of incubation, percentages of germinated conidia were calculated. Conidia were regarded as germinated when they produced a germ tube at least half of the conidial length. Germination ratios for each fungus were calculated after examining a minimum of 200 conidia from each of three replicate plates. All experiments were conducted in a completely randomized design in 12 treatments, including LC_{50} *A. sclerotigenum*, LC_{50} *B. bassiana*, LC_{50} *P. variotii*, LC_{50} *Simplicillium* sp., LC_{50} *L. muscarium*, LC_{50} imidacloprid, LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *P. variotii*), LC_{25} (imidacloprid + *Simplicillium* sp.), LC_{25} (imidacloprid + *L. muscarium*) and distilled water as control in three replications on ten aphids and its parasitoid. The mortality after 48 and 72 hours was calculated. The percentage of hatching parasitoid in all treatments was calculated as mortality at the same time.

Statistical analysis. The LC_{25}, LC_{50} and LT_{50} values (with 95% confidence limits) were calculated using the Probit analysis method after correcting according to Abbott's (1925) formula. All data were tested for normality and homogeneity of variance by the Shapiro-Wilk and Levene's test, respectively. The data collected were subjected to analysis of variance (ANOVA) and means were separated using Turkey's HSD (honestly significant difference) test at a significance level of $P \leq 0.05$ with software package SPSS, version 22.0 (IBM Inc., USA).

Results

Determination of concentration-mortality (LC_{50}) value response. Conidia viability was assessed before each bioassay. Almost 96, 97, 96, 95 and 98 % of conidia of five fungal isolates *Acremonium sclerotigenum*, *Beauveria bassiana*, *Paecilomyces variotii*, *Simplicillium* sp. and *Lecanicillium muscarium* were germinated, respectively. The LC_{50} values of fungal isolates: *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp., *L. muscarium*, and imidacloprid against the adult stage of aphids were determined. The highest mortality between the fungi isolates against the adult stage of aphid was observed in *L. muscarium* with LC_{50} value equal 2.5×10^3 conidia ml^{-1} (Table 1).

The LC_{50} values of imidacloprid and fungal isolates: *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp. and *L. muscarium*, of *Diaeretiella rapae* are shown in Table 2. Fungal isolates *L. muscarium* and *P. variotii* with LC_{50} values equal to 5.2×10^3 and 4.0×10^7 conidia ml^{-1} had the highest and the lowest effects on *D. rapae*, respectively.

Table 1. The LC_{25}, LC_{50} and LC_{95} values of fungal isolates (conidia ml^{-1}) and imidacloprid (ppm) against the adult stage of *Brevicoryne brassicae*

Isolate	Slope ± SE	Intercept + 5	χ2 (df)	*P*	LC_{25} (CLs)	LC_{50} (CLs)	LC_{90} (CLs)
Acremonium sclerotigenum	0.59 ± 0.14	2.973	1.053(3)	0.788	1.9×10^2 ($0.5–1.8 \times 10^3$)	2.7×10^3 ($7.5 \times 10^1–1.2 \times 10^4$)	1.6×10^6 ($4.3 \times 10^5–3.4 \times 10^7$)
Beauveria bassiana	0.70 ± 0.17	2.496	1.909(3)	0.592	4.1×10^2 ($2.4–2.6 \times 10^3$)	3.7×10^3 ($1.7 \times 10^2–1.3 \times 10^4$)	8.4×10^5 ($2.5 \times 10^5–1.5 \times 10^7$)
Paecilomyces variotii	0.35 ± 0.08	2.525	0.630(3)	0.889	1.1×10^5 ($7.02 \times 10^3–4.6 \times 10^5$)	8.1×10^6 ($2.0 \times 10^6–7.3 \times 10^7$)	3.2×10^{11} ($6.3 \times 10^9–5.8 \times 10^{15}$)
Simplicillium sp.	0.21 ± 0.07	3.781	0.049(3)	0.997	3.4×10^2 ($1.4 \times 10^2–1.3 \times 10^4$)	4.7×10^5 ($1.1 \times 10^4–5.8 \times 10^6$)	2.1×10^{13} ($1.8 \times 10^{10}–1.0 \times 10^{29}$)
Lecanicillium muscarium	0.64 ± 0.16	2.808	0.682(3)	0.877	2.2×10^2 ($0.5–1.9 \times 10^3$)	2.5×10^3 ($5.9 \times 10^1–1.1 \times 10^4$)	9.3×10^5 ($2.5 \times 10^5–1.9 \times 10^7$)
Imidacloprid	4.04 ± 0.73	0.422	2.561(3)	0.464	9.23 (6.57–11.07)	13.56 (11.39–15.40)	34.62 (27.45–54.72)

SE – standard error, df – degree of freedom, CLs – confidence limits

Table 2. The LC_{25}, LC_{50} and LC_{95} values of fungal isolates (conidia ml^{-1}) and imidacloprid (ppm) against the adult stage of *Diaeretiella rapae*

Isolate	Slope ± SE	Intercept + 5	χ^2 (df)	*P*	LC_{25} (CLs)	LC_{50} (CLs)	LC_{90} (CLs)
Acremonium sclerotigenum	0.41 ± 0.09	3.282	0.436(3)	0.933	3.2×10^2 ($1.1–3.4 \times 10^3$)	1.3×10^4 ($6.5 \times 10^2–5.9 \times 10^4$)	1.1×10^8 ($1.4 \times 10^7–1.3 \times 10^{10}$)
Beauveria bassiana	0.54 ± 0.09	2.385	2.562(3)	0.464	3.5×10^3 ($2.3 \times 10^2–1.5 \times 10^5$)	6.0×10^4 ($1.3 \times 10^4–1.6 \times 10^5$)	6.2×10^7 ($1.2 \times 10^7–1.3 \times 10^9$)
Paecilomyces variotii	0.29 ± 0.08	2.792	0.576(3)	0.902	1.9×10^5 ($6.3 \times 10^3–1.06 \times 10^6$)	4.0×10^7 ($7.2 \times 10^6–3.7 \times 10^9$)	1.8×10^{13} ($5.3 \times 10^{10}–3.9 \times 10^{20}$)
Simplicillium sp.	0.26 ± 0.07	3.601	0.266(3)	0.902	5.2×10^2 ($0.2–1.7 \times 10^4$)	1.7×10^5 ($7.2 \times 10^3–1.0 \times 10^6$)	2.6×10^{11} ($2.2 \times 10^9–3.8 \times 10^{18}$)
Lecanicillium muscarium	0.40 ± 0.10	3.365	1.705(3)	0.636	1.5×10^2 ($0.24–1.9 \times 10^3$)	5.2×10^3 ($1.3 \times 10^2–2.7 \times 10^4$)	2.8×10^7 ($4.5 \times 10^6–2.1 \times 10^9$)
Imidacloprid	3.23 ± 0.58	1.431	3.230(3)	0.702	7.82 (3.22–12.5)	12.64 (0.15–21.41)	31.44 (19.48–606.32)

Explanation under Table 1

Determination of time-mortality (LT_{50}) value response. The LT_{50} values of imidacloprid and fungal isolates *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp. and *L. muscarium* at 10^8 and 10^7 conidia ml^{-1} against *B. brassicae* and *D. rapae* are shown in Tables 3 and 4.

The LT_{50} values of imidacloprid and fungal isolates at two (10^8 and 10^7 conidia ml^{-1}) concentrations showed that *A. sclerotigenum* was the most effective entomopathogenic fungi in control of *B. brassicae* after imidacloprid (Table 3). The LT_{50} values of imidacloprid and fungal isolates at two (10^8 and 10^7 conidia ml^{-1}) concentrations showed that *L. muscarium* was the most effective entomopathogenic fungi against *D. rapae* after imidacloprid (Table 4).

Comparison of different concentrations of fungal isolates on B. brassicae and D. rapae. The bioassay results showed that fungal concentrations (10^8, 10^7, 10^6, 10^5 and 10^4 conidia ml^{-1}) against adult *B. brassicae* showed significant differences with ($F_{5, 12} = 45.78$, $P = 0.001$), ($F_{5, 12} = 56.98$, $P = 0.001$), ($F_{5, 12} = 39.69$, $P = 0.001$), ($F_{5, 12} = 44.64$, $P = 0.001$) and ($F_{5, 12} = 21.56$, $P = 0.001$) and on *D. rapae* ($F_{5, 12} = 112.80$, $P = 0.001$), ($F_{5, 12} = 36.21$, $P = 0.001$), ($F_{5, 12} = 23.27$,

Table 3. The LT_{50} values of fungal isolates at 10^8 and 10^7 conidia ml^{-1} and imidacloprid at LC_{50} = 13.56 ppm against *Brevicoryne brassicae* after 7 days

Fungal conidia ml^{-1}	Isolate	Intercept(a) + 5	χ^2	*P*	Slope ± SE	LT_{50} (day) (CLs)
10^8	*A. sclerotigenum*	5.76	1.52	0.91	6.32 ± 0.44	0.26 (0.03–0.70)
	B. bassiana	3.48	5.70	0.33	5.34 ± 0.62	1.92 (1.66–2.16)
	P. variotii	3.41	5.31	0.37	1.81 ± 0.40	7.40 (5.62–13.38)
	Simplicillium sp.	3.96	1.54	0.90	2.23 ± 0.36	2.92 (2.33–3.53)
	L. muscarium	4.41	0.91	0.96	4.96 ± 0.75	1.32 (1.09–1.52)
10^7	*A. sclerotigenum*	4.84	2.18	0.82	3.09 ± 0.37	1.18 (0.69–1.59)
	B. bassiana	2.88	5.24	0.38	4.21 ± 0.52	3.19 (2.80–3.57)
	P. variotii	2.50	6.87	0.23	2.80 ± 0.56	7.78 (6.28–11.76)
	Simplicillium sp.	3.29	1.23	0.94	2.73 ± 0.42	4.27 (3.58–5.00)
	L. muscarium	3.27	0.744	0.98	2.81 ± 0.43	4.14 (3.54–4.89)
13.56 ppm	Imidacloprid	4.32	0.47	0.52	2.34 ± 0.71	1.93 (1.42–2.77)

Explanation under Table 1

Table 4. The LT_{50} values of fungal isolates at 10^8 and 10^7 conidia ml^{-1} and imidacloprid at LC_{50} = 12.64 ppm against *Diaeretiella rapae* after 7 days

Fungal conidia ml^{-1}	Isolate	Intercept(a) + 5	χ^2	*P*	Slope ± SE	LT_{50} (day) (CLs)
10^8	*A. sclerotigenum*	3.46	2.06	0.84	3.15 ± 0.40	2.16 (1.77–2.52)
	B. bassiana	3.16	11.15	0.04	3.65 ± 0.45	3.16 (2.29–4.08)
	P. variotii	3.68	1.92	0.86	1.65 ± 0.37	6.22 (4.74–10.41)
	Simplicillium sp.	3.46	4.14	0.52	2.41 ± 0. 39	4.38 (3.67–5.40)
	L. muscarium	4.02	3.05	0.69	4.17 ± 0. 52	1.71 (1.42–1.97)
10^7	*A. sclerotigenum*	3.72	2.46	0.75	2.46 ± 0.37	3.32 (2.76–3.96)
	B. bassiana	2.84	9.44	0.093	3.31 ± 0.47	4.46 (3.45–6.15)
	P. variotii	2.38	0.61	0.98	2.80 ± 0.63	8.61 (6.76–14.85)
	Simplicillium sp.	2.79	4.62	0.46	2.69 ± 0.50	6.59 (5.45–9.07)
	L. muscarium	3.54	4.06	0.54	3.03 ± 0.40	3.04 (2.57–3.51)
12.64 ppm	Imidacloprid	4.34	0.20	0.65	3.00 ± 0.73	1.65 (1.25–2.06)

Explanation under Table 1

$P = 0.001$), ($F_{5, 12} = 28.02$, $P = 0.001$) and ($F_{5, 12} = 51.25$, $P = 0.001$) conditions, respectively. The cumulative mortality of aphid and parasitoid caused by *L. muscarium*, *B. bassiana* and *A. sclerotigenum* was 98.6, 97.2, 96.4 % and 99.9, 96.5 and 98.6 %, respectively (Table 5).

Combination effects of imidacloprid and fungus on B. brassicae and D. rapae. The effect of different treatments on adult of *B. brassicae* and its parasitoid *D. rapae* after 48 and 72 hours showed that there were significant differences between the treatments

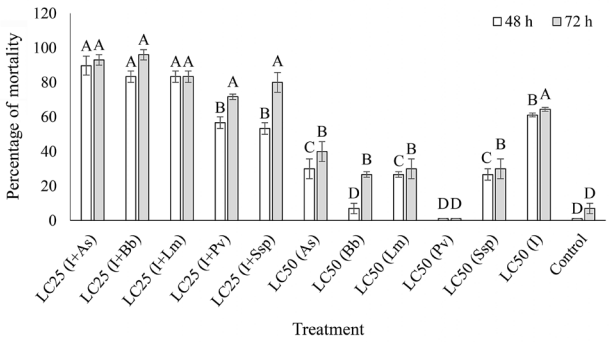
Table 5. Effect of conidial concentration of fungal isolates against adult of *B. brassicae* and *D. rapae*

Isolate	Concentration (conidia ml^{-1})	Mortality (mean ± SE)					
		Lecanicillium muscarium	*Acremonium sclerotigenum*	*Beauveria bassiana*	*Simplicillium* sp.	*Paecilomyces variotii*	Control
Brevicoryne brassicae	10^8	98.6 ± 0.01 a	97.2 ± 0.01 a	96.4 ± 0.01 a	71.2 ± 3.32 b	63.3 ± 0.33 b	7.3 ± 0.33 c
	10^7	95.7 ± 0.33 a	95.3 ± 0.33 a	94.7 ± 0.33 a	61.0 ± 0.33 b	46.7 ± 0.88 b	12.2 ± 0.03 c
	10^6	93.3 ± 1.15 a	86.7 ± 1.15 a	94.3 ± 0.57 a	63.2 ± 1.01 b	43.3 ± 0.57 b	9.7 ± 0.03 c
	10^5	83.3 ± 0.33 a	76.7 ± 0.57 a	80.5 ± 1.03 a	53.3 ± 0.57 b	31.4 ± 1.01 c	20.0 ± 0.03 c
	10^4	73.3 ± 1.15 a	60.0 ± 0.01 b	66.7 ± 1.15 a	36.7 ± 0.57 c	20.0 ± 0.03 c	13.3 ± 1.01 c
Diaeretiella rapae	10^8	99.9 ± 0.01 a	96.5 ± 0.57 a	98.6 ± 0.33 a	76.7 ± 0.57 b	56.6 ± 0.66 c	7.2 ± 0.01 d
	10^7	91.2 ± 1.73 a	86.4 ± 0.66 a	90.2 ± 0.57 a	60.0 ± 0.74 b	41.6 ± 0.08 b	12.3 ± 0.03 c
	10^6	80.3 ± 0.57 a	76.7 ± 0.33a	73.3 ± 1.30 a	43.8 ± 0.01 b	33.1 ± 0.07 b	6.7 ± 0.33 c
	10^5	63.3 ± 0.66 a	56.7 ± 0.33 a	53.3 ± 0.23 a	46.7 ± 0.33 b	26.7 ± 0.33 c	20.1 ± 0.03 c
	10^4	56.7 ± 0.33 a	51.0 ± 0.57 a	44.1 ± 0.36 a	26.7 ± 0.23 b	10.6 ± 0.03 bc	6.7 ± 1.01 c

Note. Means followed by the same superscript letter(s), within the same rows are insignificantly different ($P \leq 0.05$) according to Tukey HSD test; SE – standard error.

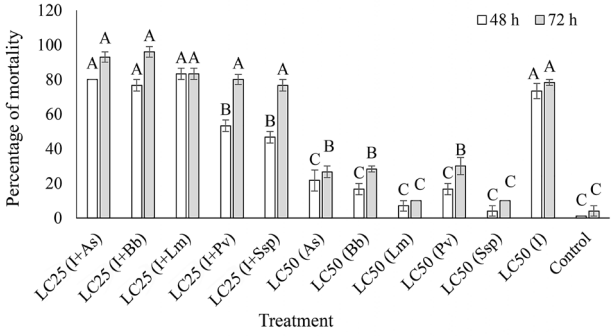
with ($F_{11, 24} = 95.68$, $P = 0.001$), ($F_{11, 24} = 75.29$, $P = 0.001$) and ($F_{11, 24} = 89.36$, $P = 0.001$), ($F_{11, 24} = 156.09$, $P = 0.001$), respectively. According to the results, the highest mortality of *B. brassicae* after 48 hours was by LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *L. muscarium*) and imidacloprid, and after 72 hours by LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *P. variotii*), LC_{25} (imidacloprid + *Simplicillium* sp.), LC_{25} (imidacloprid + *L. muscarium*) and imidacloprid (Fig. 1). The highest mortality of *D. rapae* after 48 hours was by LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *L. muscarium*) and imidacloprid, and after 72 hours by LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*), LC_{25} (imidacloprid + *L. muscarium*) and imidacloprid (Fig. 2).

The percentage of D. rapae emergence. The effect of different treatments on the percentages of *D. rapae* emergence showed that there were significant differences between the treatment with ($F_{11, 24} = 65.129$, $P = 0.001$). The result showed that the percentage of parasitoid emergence in *P. variotii* and *Simplicillium* sp. treatments was higher than in the other treatments (Fig. 3).



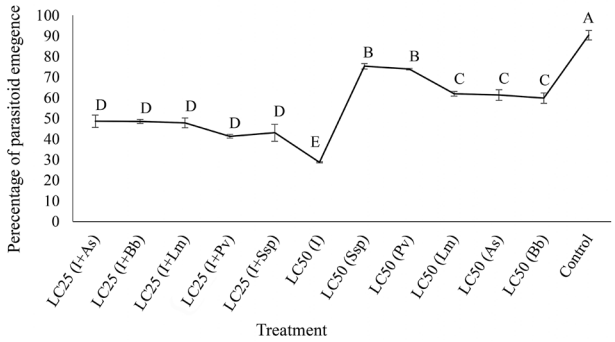
Note. The columns with the same letters indicate non-significant differences between the treatments; I – imidacloprid, *As – Acremonium sclerotigenum*, *Bb – Beauveria bassiana*, *Lm – Lecanicillium muscarium*, *Pv – Paecilomyces variotii*, *Ssp – Simplicillium* sp.

Figure 1. Mean (±SE) efficacy (%) of treatments against adult of *Brevicoryne brassicae* after 48 and 72 hours in laboratory conditions



Note. The columns with the same letters indicate non-significant differences between treatments; explanation under Figure 1.

Figure 2. Mean (±SE) efficacy (%) of treatments against adult of *Diaeretiella rapae* after 48 and 72 hours in laboratory conditions



Note. The same letters indicate non-significant differences between the treatments; explanation under Figure 1.

Figure 3. Mean (±SE) emergence (%) of *Diaeretiella rapae* on treated *Brevicoryne brassicae*

Discussion

The role of entomopathogenic fungi as prominent biological control agents has shown that the fungal isolates affected the aphids and parasitoids (Kim, 2007; Roditakis et al., 2008). The results of our experiment showed that, based on LC_{50} and LT_{50} values, *L. muscarium* was an effective isolate against aphid and parasitoid. Combinations of fungal isolates with imidacloprid, LC_{25} (imidacloprid + *A. sclerotigenum*), LC_{25} (imidacloprid + *B. bassiana*) and LC_{25} (imidacloprid + *L. muscarium*) were more effective against aphid. All fungal isolates showed virulence against *B. brassicae* after 3–7 days of treatments. The fungal isolate *Lecanicillium lecanii* was the most virulent among the fungal isolates tested, followed by *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *M. anisopliae* against the adult aphids (Asi et al., 2009), and in our study, *A. sclerotigenum*, *B. bassiana* and *P. variotii* were virulent among isolates. The results indicated that *M. anisopliae* had larvicidal effect on *Aedes albopictus* with 1.09×10^5 conidia ml^{-1}, while it took 45.41 h to kill 50% of the tested population; in our research 50% mortality by *A. sclerotigenum* at 10^8 concentration was observed after 0.28 days (6.72 h).

In current study, LC_{50} and LT_{50} values of *B. bassiana* were 3.7×10^3 conidia ml^{-1} and 1.92 day at 10^8 concentration – similar to Saruhan et al. (2015), who tested *B. bassiana* on different aphids such as *B. brassicae* and *Aphis fabae*. It was found to be effective at all concentrations, i.e. 10^6, 10^7 and 10^8 conidia ml^{-1}, against all aphid species, but the uppermost (10^8 conidia ml^{-1}) concentration provided maximum control within a short period of time. Taking long time to kill 50% population by fungal isolates compared to synthetic insecticides is the only drawback for the application of entomopathogenic fungi, but these biopesticides are safe for use (Bilal et al., 2012). The fitness of the parasitoids that emerge from insecticide treated hosts is very important (Bayram et al., 2010; Saber, 2011).

The parasitoid *Aphidius colemani* developed normally (approximately 90% adult emergence) when its cotton aphid (*Aphis gossypii*) host was treated with *V. lecanii* conidia 5 or 7 days after parasitization. Fungus exposure 1 day before or up to 3 days after parasitization reduced *A. colemani* emergence from 0% to 10% (Kim et al., 2005). In our experiment, 7 days after application of fungal isolates, the emergence percentage of *D. rapae* decreased from 30% to 70% (Fig. 3). The LT_{50} values showed *L. muscarium* to be the most effective entomopathogenic fungus against *Aphis fabae* at both 20°C and 25°C temperatures (1.77 and 1.93 day), followed by *Simplicillium lamellicola* (2.12 and 1.96 day) and *V. lecanii* (2.33 and 2.03 day). Also in this research, LT_{50} values of *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp. and *L. muscarium* on adult of *B. brassicae* was 0.26, 1.92, 7.40, 2.92 and 1.32 days, respectively.

Various authors have reported different results in the combined action of fungal strains and chemical insecticides. For example, the combination on *B. bassiana*-diflubenzuron was more effective than the pairing of *B. bassiana* with other chemical agents in field applications against grasshoppers in the United States (Foster et al., 1996) and in populations of grasshoppers in Mali treated with a mixture of *B. bassiana* and diflubenzuron (Delgado et al., 1999). It was observed that the decrease in the number of insects continued until the end of the monitoring (Bisadze et al., 2013).

The present study showed that LC_{50} concentration of imidacloprid severely decreased the emergence of parasitoids, but when LC_{25} of imidacloprid plus LC_{25} of each fungal isolate had been used, parasitoid emergence decreased (Fig. 3). Similarly, reduction in emergence rate was reported in *Encarsia inaron* after treatment with imidacloprid (Sohrabi et al., 2012). Imidacloprid also led to significant mortality of *Eretmocerus eremicus*, *E. mundus* and *Encarsia formosa* (Sugiyama et al., 2011) and *E. inaron* adult (Sohrabi et al., 2012).

According to the above results of current research, imidacloprid was highly toxic to adult *D. rapae* with an LC_{50} value of 12.64 ppm. In our experiment, imidacloprid had harmful effects on both cabbage aphid and its parasitoid with 64.42% and 78.33% mortality, respectively. Similar result was observed in research of Towfiq et al. (2010), who reported that pirimiphos-methyl, thiamethoxam, malathion and thiacloprid were highly toxic to parasitoids and cabbage aphids. According to the manufacturer's label, a single application of *B. bassiana* (insecticide Vertalec) can effectively control *A. gossypii* for up to 5 days in greenhouse environments (Kim, 2007).

In our experiment, *B. brassicae* was controlled by *B. bassiana* after 7 days. Among the entomopathogenic fungi, *L. muscarium*, *A. sclerotigenum* and *B. bassiana* had higher mortality rate of *B. brassicae* and *D. rapae* in combination with imidacloprid, but fungal isolates had the lower effect on *D. rapae* in alone form. Results of current experiment showed that fungal isolates can be used for control of *B. brassicae* in IPM program.

Conclusion

There is a general misperception that entomopathogenic fungi are not harmful to natural enemies. However, based on the information in this article, it is necessary to exercise caution in applying entomopathogenic fungi alone and in combination with a pesticide for conservation and augmentation of natural enemies.

Hence, further studies should be conducted under more realistic environments (e.g., greenhouse and field) on simultaneous application of pesticide, entomopathogenic fungi and parasitoids in management this pest.

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Grybinių izoliatų ir imidakloprido poveikis kopūstiniam amarui *Brevicoryne brassicae* ir jo parazitoidui *Diaeretiella rapae*

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Santrauka

Diaeretiella rapae (M'Intosh) yra pirminis kopūstinio amaro *Brevicoryne brassicae* (L.) parazitas; abi rūšys yra plačiai paplitusios visame pasaulyje. Tyrimo metu buvo įvertintas imidakloprido ir penkių entomopatogeninių grybų: *Acremonium sclerotigenum*, *Beauveria bassiana*, *Paecilomyces variotii*, *Simplicillium* sp. ir *Lecanicillium muscarium*, efektyvumas kopūstiniam amarui ir jo parazitoidui. Siekiant įvertinti visų izoliatų ir imidakloprido LC_{50} (mirtinos koncentracijos) ir LT_{50} (mirtinos laiko trukmės) vertes *B. brassicae* ir *D. rapae* suaugėliams, šie mirtingumo duomenys analizuoti taikant Probit analizę. *A. sclerotigenum*, *B. bassiana*, *P. variotii*, *Simplicillium* sp., *L. muscarium* ir imidakloprido LC_{50} vertės buvo atitinkamai $2,7 \times 10^3$, $3,7 \times 10^3$, $8,1 \times 10^6$, $4,7 \times 10^5$ bei $2,5 \times 10^3$ konidijų ml^{-1} ir 13,56 ppm. Po apdorojimo praėjus 7 dienoms bendras *B. brassicae* ir *D. rapae* mirtingumas buvo atitinkamai 98,56 ir 99,99 % panaudojus didelę *L. muscarium* koncentraciją (10^8 konidijų ml^{-1}). Didžiausias *B. brassicae* ir *D. rapae* mirtingumas po 48 ir 72 valandų buvo nustatytas paveikus šių derinių subletalinėmis kombinacijomis: LC_{25} (imidaklopridas + *A. sclerotigenum*), LC_{25} (imidaklopridas + *B. bassiana*) bei LC_{25} (imidaklopridas + *L. muscarium*) ir LC_{25} (imidaklopridas + *A. sclerotigenum*), LC_{25} (imidaklopridas + *B. bassiana*), LC_{25} (imidaklopridas + *P. variotii*), LC_{25} (imidaklopridas + *Simplicillium* sp.) bei LC_{25} (imidaklopridas + *L. muscarium*). *B. brassicae* ir *D. rapae* paveikus 10^8 konidijų ml^{-1} koncentracija, LT50 vertės buvo 0,26 bei 2,16 dienos, panaudojus *A. sclerotigenum* izoliatą, ir 7,4 bei 6,22 dienos, panaudojus *P. variotii* izoliatą. Nustatyta, kad imidaklopridas turėjo žalingą poveikį ir kenkėjui, ir parazitoidui; grybiniai izoliatai parazitoidui buvo saugesni už imidaklopridą.
Tyrimo rezultatai parodė, kad taikant integruotą *B. brassicae* kontrolę, entomopatogeniniai grybai gali būti efektyviai naudojami atskirai ir mišinyje su subletaline (LC_{25} = 9,23 ppm) imidakloprido koncentracija.

Reikšminiai žodžiai: biologinė kontrolė, entomopatogeniniai grybai, parazitoidas, patogeniškumas, subletalinis.