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Efficacy of the entomopathogenic fungus, *Beauveria bassiana* as biological control agent of black cutworm, *Agrotis ipsilon* hufnagel and compatibility with chemical insecticides

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Abstract

Beauveria bassiana and four chemical insecticides were tested for their potentiality against *Agrotis ipsilon* 3rd instar larvae under laboratory conditions. In order to derive maximum benefits from applying both of entomopathogenic fungus and chemical insecticides in IPM program for controlling *A. ipsilon* larvae, compatibility of *B. bassiana* with the tested chemical insecticides were studied. It was found that, under laboratory conditions, except buprofezin which showed slight harmful effect on the fungus mycelial growth (51.73% growth inhibition), all other insecticides showed harmless effect. Also, the virulence of *B. bassiana* spores harvested from SDYA poisoned with LC_{90} of the tested insecticides was evaluated against *A. ipsilon* 3rd instar larvae under laboratory conditions. All treatments dramatically lost their virulence except those spores harvested from thiamethoxam-poisoned SDYA which slightly affected negatively. Moreover, it was found that all tested insecticides at sub-lethal doses (LC_{50}) were compatible with *B. bassiana* under semi-field conditions. So, the combination of the tested insecticides at the sub-lethal doses with *B. bassiana* in IPM programs was recommended.

Keywords: *Agrotis ipsilon*, *Beauveria bassiana*, chemical insecticides and compatibility

1. Introduction

The black cutworm, *Agrotis ipsilon* (Hufnage), has a wide host range and can destroy more than one hundred types of crops [1]. It infects nearly all vegetables, many important grains and grasses causing huge economic losses [2, 3]. Early larval instars fed aboveground until about the fourth instar, but older larvae feed near the soil surface, cutting off young plants at ground surface and sometimes pulled them underground. When newly planted fields are infected, young plants may be disappeared entirely at night. Due to the feeding behavior and hidden life style of the larvae, besides acquiring resistance against most applied conventional chemical insecticides, application of new insecticides or new alternatives became very important. The entomopathogenic fungus, *Beauveria bassiana* is among the first entomopathogenic fungi used for microbial control of insect pests [4-8]. It naturally exists in the soil and shows good epizootic infecting the insect by adhesion to their cuticle by adhesion proteins [9, 10]. In the field, this entomopathogenic fungus may be accidentally contaminated with chemical pesticides used for controlling another pests infecting the same crop. So, the influence of chemical pesticides on it should be studied. Also, for incorporating *B. bassiana* into integrated pest management programs (IPM), it is crucial to take into account the compatibility of products applied on the crop in order to choose pesticides compatible with it and avoid the most toxic ones [11, 12]. Therefore, the present study aimed to illustrate the compatibility of *B. bassiana* as microbial control agent with some chemical insecticides belonging to different categories for controlling *A. ipsilon* 3rd instar larvae *in vitro* and under semi field conditions in order to derive maximum benefits from them.

2. Materials and Methods

2.1 Entomopathogenic Fungi

The entomopathogenic fungus, *B. bassiana* was obtained as wettable powder formulation produced by Insect Pathogen Production Unit (IPPU), Plant Protection Research Institute, Agricultural Research Center, Egypt. It was cultured on Sabouraud dextrose yeast extract agar

(SDYA) [20g/l agar, 40g/l dextrose, 10g/l peptone and 10g/l yeast extract] and incubated at 27± 2 ºC and 80 ±5% RH until full-term. The spores were harvested and different concentrations of them were prepared.

2.2 Chemical Insecticides

The synthetic chemical insecticides selected for accomplishing the current study were among those commonly applied for insect pest management. These insecticides were listed in Table 1.

Table 1: List of chemical insecticides used in the current study

S.N.	Active Ingredient	Trade name	Formulation	Source
1	Buprofezin	Ran way	25% SC	Jiangsu Seven Continent Green Chemical Co., Ltd. China.
2	Pyriproxyfen	Glister	10% EC	Jiangsu Rutam Chemistry Ltd. China
3	Emamectin benzoate	Deltalym	5% EC	Saudi Delta for chemical industries. Saudi Arabia
4	Thiamethoxam	Lex- Extra	25% WDG	Starchem Industerial Chemicals-Egypt

2.3 The insect pest

Larvae of *A. ipsilon* were collected from insecticides-free tomato fields at the farm of Faculty of Agriculture, Mansoura Univ. They transferred to laboratory and kept at 27 ± 2 °C, 65± 5% RH, and 14hs photoperiod. To avoid cannibalism, larvae were kept individually in separate plastic cups and supplied with insecticides-free castor leaves, *Ricinus communis* for feeding. Castor leaves were washed with sterile water three times and dried with paper towel then given to the larvae. After pupation, pupae were collected and transferred to a glass jar until the emergence of adults. Cotton pads immersed in 10% sugary solution were used as nutrition source for adults. Before the fungal pathogenicity test, *A. ipsilon* 3rd instar larvae were sterilized by using 1% sodium hypochlorite for 30 seconds, then washed by sterile water.

2.4 *In-vitro* Bioassay

2.4.1 Virulence of *B. bassiana* and chemical insecticides against *A. ipsilon* 3rd instar larvae

Fresh castor leaves were sterilized according to Clair *et al*., (1997) [13] by immersing in 70% alcohol, sterile water, 5% sodium hypochlorite, respectively. Then, they washed three times with sterile water, dried and sprayed with the tested fungal or insecticidal concentrations. The sprayed castor leaves were transferred to 15 cm Petri–dishes with only one larva to avoid cannibalism and incubated at 27± 2 °C., 70 ± 5% RH, and 14hs photoperiod. Each concentration was represented by 3 replicates and each replicate contained ten larvae. Another three replicates were sprayed only with water and 0.03% aqueous Tween 80 to serve as control. Castor leaves were replaced by fresh ones after the third day of the treatment to provide nutrition. Mortality percentage was recorded daily along the seven days of the experiment.

2.4.2 Compatibility of *B. bassiana* with chemical insecticides

In vitro compatibility between *B. bassiana* and the tested insecticides was studied at the recommended concentration of application in the field (LC_{90} of each of them). The impact of the tested insecticides on germination and the radial growth of *B. bassiana* was demonstrated by poisoned food technique [14] in Sabouraud dextrose yeast extract agar (SDYA) medium. The insecticide emulsions of required concentration were mixed with autoclaved SDYA medium when still liquid, at approximately 45 ±5ºC. Under laminar flow cabinet, twenty ml of each mixture were poured into 9 cm diameter sterile petri dishes. After solidification, each amended SDYA petri dish was inoculated with agar disc with mycelium mat of *B. bassiana* cored from seven days old colony by a cork borer. Normal growth medium (SDYA) inoculated only with mycelial disc was served as control. Each treatment was replicated five times. They were incubated at 25 ±2ºC for 10 days to allow maximum growth. At 10th day after inoculation, the diameter of growing culture in petri dishes containing insecticides was measured. Conidia of *B. bassiana* grown in insecticides-poisoned SDYA were harvested and tested for their virulence compared with those grown in insecticides-free SDYA against *A. ipsilon* 3rd instar larvae.

2.5 Compatibility of *B. bassiana* with chemical insecticides under semi field conditions

Semi-field experiment was conducted to allow *A. ipsilon* larvae to exhibit its normal behavior under natural conditions. Tomato seeds were grown in plastic pots filled with autoclaved soil and kept under plastic greenhouse conditions of 27±2 ºC, 70±5 RH and 14hs photoperiod. When tomato seedlings reached 20-25 cm high, only one *A. ipsilon* 3rd instar larva was transferred to the pot at depth of 2 cm at least 6h before application. Each seedling pot was subjected to dual treatment. *B. bassiana* suspension was applied at LC_{50} by drenching and the tomato seedlings aerial parts were sprayed with LC_{50} of the tested insecticides. Each treatment was represented by three replicates in addition to another three replicates treated only with water (control). Mortality percentages were reported daily and at the final of the experiment, the cadavers were mounted with lacto phenol blue and examined microscopically to confirm the fungal infection.

2.6 Statistical analysis

Mortality percentages of the 3rd instar larvae of *A. ipsilon* were corrected by Abbott's formula [15]. The LC_{50}, LC_{90} and slope values were determined according to Finney [16]. Virulence of conidia produced from the poisoned media were evaluated and compared with the most effective one by using Sun's equation [17]. For compatibility *in vitro* of *B. bassiana* with chemical insecticides, the data were expressed as percentage of *B. bassiana* growth inhibition [18] which was calculated by equation (1).

$$X = \frac{Y-Z}{Y} \times 100 \qquad (1)$$

Where, X represents the percentage of growth inhibition, Y is the radial growth of fungus in control and Z is the radial growth of fungus in poisoned medium. According to Hassan's classification scheme [19], there were four categories of pesticides scoring index; harmless (<50% reduction in beneficial capacity), slightly harmful (50-79%), moderately harmful (80-90%) and harmful (>90%). The data were submitted to analysis of variance and means and compared by

Tukey test (p=0.05) [20].

X^2-test was used to evaluate the compatibility of *B. bassiana* with the tested chemical insecticides under semi field conditions. The type of interaction (additive, synergistic or antagonistic) was evaluated by comparing the observed and expected mortalities [21]. The expected proportional mortality M_E for the EPF/ insecticide combination was calculated by equation (2), where, M_I and M_F are the observed proportional mortalities relatively caused by chemical insecticides and EPF alone. X^2 test was then carried out using equation (3), where M_{IF} is the observed mortality for the EPF/ insecticide combination.

$$M_E = M_I + M_F\,(1-M_I) \qquad (2)$$

$$X^2 = (M_{IF} - M_E)^2 / M_E \qquad (3)$$

Additivity was indicated if $X^2 < 3.84$, Synergism was indicated if $X^2 > 3.84$ and $Mc > M_E$, where Mc is the observed mortality of the EPF/ insecticide combination and M_E is the expected mortality from the combination. Antagonism was indicated if $X^2 > 3.84$ and $Mc < M_E$.

3. Results and Discussions

3.1 Virulence of *B. bassiana* and chemical insecticides against *A. ipsilon* 3rd instar larvae under laboratory conditions

Data in Table 2. showed that both of *B. bassiana* and the tested insecticides suppressed *A. ipsilon* 3rd instar larvae with different mode of action. Mortality percentage increased with increasing concentrations and time elapsed after all treatments. In spite of the slow action of *B. bassiana*, it revealed high mortality with LC_{50}: 66.73x10^2 conidia/ml and LC_{90}: 1003.704x 10^3 conidia/ml. This slow action is due to the time dependent mechanism of fungi for tissues invasion and toxins accumulation in the victim body. The current data agreed with previous study [22] which confirmed the sensitivity of *A. ipsilon* larvae to *B. bassiana*.

Buprofezin is a potential chitin synthesis inhibitor reducing the population of the insect pest by suppressing fecundity, egg hatchability and production of malformed larvae and pupae [23]. The lipophilic properties of buprofezin can interfere with the exoskeleton chitin by contact, inhibiting chitin formation causing abnormal and deforming endocuticular deposition [24]. Also, it was found that higher concentrations have anti-feeding effect. The present data showed that buprofezin showed toxic effect against *A. ipsilon* 3rd instar with LC_{50} value: 280.508 ppm and LC_{90}: 1230.86 ppm. The mortality was clearly caused by molting failure of the larvae. The current data agreed with those obtained by Khatun *et al*., (2017) [25] when evaluated the potentiality of buprofezin against *Spodoptera littoralis*.

Pyriproxyfen is a juvenile hormone analog belongs to insect growth regulators. It mimics the action of juvenile hormones so, it inhibits the embryonic development, metamorphosis, and adult formation [26-28]. The current study showed that pyriproxyfen suppressed *A. ipsilon* 3rd instar revealing maximum mortality percentages at the 5th day of treatment. It showed LC_{50}: 278.936 ppm and LC_{90}: 1102.75 ppm. The toxicity of pyriproxyfen to *A. ipsilon* was previously emphasized [29]. It was suggested that pyriproxyfen may suppress the immune system of *A. ipsilon* where it suppressed the phagocytic plasmatocytes numbers.

Regarding to emamectin benzoate, it causes continuous flow of chlorine ions in the neurotransmitter gamma-aminobutyric acid (GABA) and H-Glutamate receptor sites, disrupting nerve impulses then the insect cadaver paralyzed and stop feeding [30]. It showed LC_{50}: 0.0146 ppm and LC_{90}: 0.048 ppm. It did not exhibit rapid knock down activity against *A. ipsilon* but paralysis was happened rapidly and feeding cessation shortly after ingestion.

Thiamethoxam is neonicotinoids insecticide acting on insect nicotinic acetylcholine receptors (nAChR) in the central nervous system, causing paralysis of the insect muscles [31]. The current data showed that thiamethoxam showed LC_{50} value: 82.889 ppm and LC_{90}: 320.727 ppm.

Table 2: Virulence of *B. bassiana* and chemical insecticides against *A. ipsilon* 3rd instar larvae under laboratory conditions of 27± 2 °C., 70 ± 5% RH, and 14hs photoperiod

Treatment	**Conc.**	**Mortality% at indicated day after treatment**				**LC_{50} and confidence limits at 95%**		**LC_{90} and confidence limits at 95%**		**Slope ± SE**	***X^2***
		1st	**3rd**	**5th**	**7th**						
B. bassiana	1x10^4(conidia/ml)	0	0	40.00	53.33	66.73x10^2conidia/ml		1003.704x 10^3conidia/ml		0.589 ± 0.143	0.035
	1x10^5(conidia/ml)	0	0	53.33	76.67						
	1x10^6(conidia ml)	0	3.33	66.67	90.00	3.64x10^2	24.197x10^3	303.613x10^3	1256.321x10^4		
	1x10^7(conidia ml)	0	33.33	73.33	96.67						
Buprofezin	100 ppm	0	0	13.33	23.33	280.508ppm		1230.86Ppm		1.995 ± 0.3892	1.63
	200 ppm	0	0	20.00	33.33						
	400 ppm	0	26.67	53.33	56.67	206.96	379.45	766.42	3273.4		
	800 ppm	0	33.33	76.67	86.67						
Pyriproxyfen	125 ppm	10.0	20.00	26.67	26.67	278.936ppm		1102.75Ppm		2.147 ± 0.402	1.876
	250 ppm	13.33	36.67	43.33	43.33						
	500 ppm	13.33	46.67	63.33	63.33	203.24	364.79	738.71	2405.86		
	1000 ppm	33.33	73.33	93.33	93.33						
Emamectin benzoate	0.01 ppm	0	26.67	33.33	36.67	0.0146ppm		0.048ppm		2.486 ± 0.461	0.773
	0.02 ppm	10.00	40.00	53.33	60.00						
	0.04 ppm	16.67	76.67	83.33	83.33	0.010	0.019	0.036	0.081		
	0.06 ppm	23.33	96.67	96.67	96.67						
Thiamethoxam	25 ppm	10.00	13.33	16.67	16.67	82.889ppm		320.727ppm		2.181 ± 0.402	2.642
	50 ppm	13.33	26.67	330.00	30.00						
	100 ppm	20.00	36.67	43.33	46.67	63.433	111.69	204.984	776.244		
	200 ppm	40.00	76.67	86.67	86.67						

3.2 *In vitro* compatibility of *B. bassiana* with chemical insecticides

Studying the compatibility between the bio-agent, *B. bassiana* and chemical insecticides under laboratory conditions provides an opportunity to expose the pathogen to the maximum action of the insecticides, a condition that often does not occur in the field. Growth, sporulation and pathogenicity of entomopathogenic fungi may be affected by pesticides traditionally applied [32-37]. The present data in Table 3. Showed the effect of the tested insecticides on the mycelial growth of *B. bassiana*. All treatments showed significant differences comparing with control. Except, Buprofezin, all treatments showed harmless effect to *B. bassiana* mycelial growth. Thiamethoxam recorded minimum growth inhibition (17.22%) followed by emamectin benzoate (24.37%) and pyriproxyfen (40.32%). On the other hand, Buprofezin showed slightly harmful effect recording 51.73% growth inhibition. The present data agreed with previous study[38] which recorded the compatibility of *B. bassiana* with buprofezin, pyriproxyfen, and also, agreed with Joshi *et al*., (2018) [39] who indicated the compatibility of *B. bassiana* with emamectin benzoate 5% WG. Also, our results agreed with previous studies [40, 41] which reported the compatibility of thiamethoxam with *B. bassiana*. Clearly, the impact of the tested insecticides on the fungal mycelial growth was minimum. It might be due to *B. bassiana* ability to metabolize the inactive ingredients present in the insecticide formulations or the insecticides itself using them as nutrients, enhancing the vegetative growth and conidial production of the EPF [42].

Table 3: Showed the effect of tested chemical insecticides on the mycelial growth of *B. bassiana*

Treatment	Mean diameter of mycelial growth (mm)	Percent reduction over control	The Effect
Buprofezin	41.8^b	51.73	slightly harmful
Pyriproxyfen	51.8^c	40.32	Harmless
Emamectin benzoate	66.4^d	24.37	Harmless
Thiamethoxam	72.2^e	17.22	Harmless
Control	8.72^a	------	------

3.3 Virulence of *B. bassiana* grown in insecticide-poisoned SDYA against *A. ipsilon* 3rd instar larvae under laboratory conditions

The virulence of *B. bassiana* spores harvested from SDYA (control) and SADYA poisoned with LC_{90} of the tested chemical insecticides was evaluated against *A. ipsilon* 3rd instar larvae under laboratory conditions. Data in Table 4 showed that except spores from SDYA poisoned with thiamethoxam, all other spores harvested from insecticides-poisoned SDYA were sapped of their strength compared with those grown in normal SDYA. Virulence loss of *B. bassiana* grown on SDYA poisoned with chemical insecticides were parallel with the reduction of fungal mycelia growth resulted by these insecticides. *B. bassiana* grown in SDYA poisoned with thiamethoxam showed slight loss of virulence showing LC_{50} value: 68.31x10^2 conidia/ml and toxicity index of 97.69%. *B. bassiana* grown in emamectin benzoate- poisoned SDYA showed a moderate loss of virulence showing LC_{50} value: 114.18x10^2 conidia/ml and toxicity index of 58.44%, followed by the fungal spores grown in pyriproxyfen-poisoned SDYA (LC_{50}: 152.37x10^2 conidia/ml and toxicity index: 43.8%). *B. bassiana* grown in buprofezin-poisoned SDYA media showed the greatest loss of virulence against *A. ipsilon* 3rd instar larvae (LC_{50}: 875.36x10^2 conidia/ml). Previous data illustrated that although the most tested insecticides were harmless to the fungal mycelial growth, most of them negatively affected the virulence of *B. bassiana* spores later produced. This means, in case of exposing the entomopathogenic fungus to high concentrations of chemical insecticides at the long term, it may eliminate the efficacy of it. So, when *B. bassiana* incorporated into IPM programs, application of chemical insecticides and applicable concentrations must be carefully considered to preserve the bio control agent.

Table 4: Virulence of *B. bassiana* grown in SDYA containing insecticides against *A. ipsilon* 3rd instar larvae under laboratory conditions of 27± 2 °C., 70 ± 5% RH, and 14hs photoperiod

Treatments	Conc. (conidia /ml)	Mortality% at indicated day after treatment	LC_{50} and confidence limits at 95% (Conidia/ml)		LC_{90} and confidence limits at 95% (Conidia/ml)		Slope ± SE	X^2	Toxicity index
B. bassiana grown in untreated sadya	1x10^4	53.33	66.73x10^2 conidia/ml		1003.704x 10^3 conidia/ml		0.589± 0.143	0.035	100
	1x10^5	76.67							
	1x10^6	90.00	3.64x10^2	24.197x10^3	303.613x10^3	1256.321x10^4			
	1x10^7	96.67							
B. bassiana grown in Buprofezin treated sadya media	1x10^4	30.00	875.36x10^2		4729.822x 10^4		0.469± 0.113	0.674	7.62
	1x10^5	53.33							
	1x10^6	73.33	150.17x10^2	3282.111x10^3	6750.519x10^3	6623.14x10^6			
	1x10^7	80.00							
B. bassiana grown in Pyriproxyfen treated sadya media	1x10^4	46.67	152.37x10^2		2530.62x10^4		0.398± 0.115	0.008	43.8
	1x10^5	63.33							
	1x10^6	76.67	2.86x10^2	704.81x10^2	3149.6x10^3	1841.26x10^7			
	1x10^7	86.67							
B. bassiana grown in Emamectin benzoate treated sadya media	1x10^4	50.00	114.18x10^2		1272.673x10^3		0.626± 0.141	1.682	58.44
	1x10^5	73.33							
	1x10^6	83.33	12.46x10^2	349.99x10^2	401.46x10^3	1325.7249x10^4			
	1x10^7	100.00							
B. bassiana grown in Thiamethoxam treated sadya media	1x10^4	56.67	68.31x10^2		460.174x10^3		0.701± 0.157	0282	97.69
	1x10^5	76.67							
	1x10^6	96.67	6.71x10^2	210.08x10^2	169.439x10^3	284.746x10^4			
	1x10^7	100							

3.4 Compatibility of *B. bassiana* with chemical insecticides under semi field conditions

Compatibility of *B. bassiana* with chemical insecticides was conducted under semi-field conditions to have more realistic indications to determine to what extent the entomopathogenic fungus, *B. bassiana* inoculum was influenced by chemical insecticides. Data in Table 5. revealed that *B. bassiana* were compatible with all tested chemical insecticides even though they differ in the degree of compatibility. Thiamethoxam was the most compatible with *B. bassiana* showed synergistic relation achieving maximum mortality (100%) in less time than it takes for *B. bassiana* or thiamethoxam alone. Also, emamectin benzoate showed synergism with *B. bassiana* achieving maximum mortality (100%) at the 7th day after treatment. Additivity was dominant relation in combination between *B. bassiana* and pyriproxyfen or buprofezin.

Although all insecticides inhibited both the mycelia growth and virulence of *B. bassiana* when applied at LC_{90} in the poisoned media, the combined use of the fungus and insecticides on the ground was very different. Under semi-field conditions, when insecticides combined at sub-lethal doses (LC_{50}) with *B. bassiana*, there was an enhancement of mortality percentages of *A. ipsilon* larvae. Mortality percentage was increased due to mycosis of *B. bassiana* in addition to toxicity by insecticides with no harmful effects on the fungus. The combined factors weaken the insect physiology to critical degree making it more susceptible to the pathogen infection [43] and also delay appearance of resistance to new insecticides [44, 45]. These results agreed with previous study [46] which illustrated positive results on combination of *B. bassiana* and chemical insecticides at reduced doses for Coleoptera control.

Table 5: Compatibility of *B. bassiana* with chemical insecticides under semi field conditions

Treatment	Days after treatment	Observed mortality%	Expected mortality%	*X2*	Relation (Type of interaction)
B. bassiana	1	0	-------		
	3	13.33	-------		
	5	43.33	-------		
	7	53.33	-------		
Buprofezin	1	0	-------		
	3	13.33	-------		
	5	40.00	-------		
	7	53.33	-------		
Pyriproxyfen	1	0	-------		
	3	0	-------		
	5	36.67	-------		
	7	56.67	-------		
Emamectin benzoate	1	20.00	-------		
	3	43.33	-------		
	5	53.33	-------		
	7	53.33	-------		
Thiamethoxam	1	36.67	-------		
	3	43.33	-------		
	5	53.33	-------		
	7	53.33	-------		
B. bassiana + Buprofezin	1	0	0	0	Additive
	3	26.67	24.88	0.13	Additive
	5	66.67	66.00	0.006	Additive
	7	80.00	78.22	0.04	Additive
B. bassiana + Pyriproxyfen	1	0	0	0	Additive
	3	26.67	13.33	13.35	Synergistic
	5	66.67	64.11	0.10	Additive
	7	86.67	79.78	0.6	Additive
B. bassiana + Emamectin benzoate	1	23.33	20.00	0.55	Additive
	3	60.00	50.88	1.63	Additive
	5	96.67	73.55	7.27	Synergistic
	7	100	78.22	6.06	Synergistic
B. bassiana + Thiamethoxam	1	43.33	36.67	1.21	Additive
	3	73.33	50.88	9.91	Synergistic
	5	100.00	73.55	9.51	Synergistic
	7	100.00	78.22	6.06	Synergistic

4. Conclusion

B. bassiana proved itself worthy as microbial control agents for controlling many insect pests in general and for controlling *A. ipsilon* in the present study. To preserve the biocontrol agent, *B. bassiana*, compatibility of it with chemical insecticides *in vitro* and under semi field conditions were studied. It was found that *in vitro*, almost all tested insecticides showed no harmful effect to *B. bassiana* mycelial growth. But, most of them negatively affected the virulence of the fungal conidia produced on insecticides poisoned media. Also, it was found that all tested insecticides at sub-lethal doses (LC_{50}) were compatible with *B. bassiana* under semi-field conditions. So, the combination of them in IPM programs was recommended. Further studies in possibility of applying these insecticides and *B. bassiana* as mixtures can provide additional information on the mechanism of the insect toxicity by these combinations.

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