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Evaluation the efficiency of certain entomopathogenic fungi, chemical pesticides and their residual effects on *Chrysoperla carnea* larvae and two insect pests

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Abstract
The toxicity effects of two commercial entomopathogenic fungi Biovar ((3200 viable spore/mg) and Bio-Ranza, an ((32x10^6 viable spore/mg). Also, the toxicity effect of two chemical pesticides Renocam 48%EC (Chlorpyrifos-ethyl) and Match 5% E.C (lufenuron) were studied from 2 to 4 days under semi-field conditions against nymphs of the aphid, *Aphis craccivora* (Koch), the 2nd larval instars of the green lacewing, *Chrysoperla carnea* on bean leaves and against 2nd instar larvae of cotton leaf worm, *Spodoptera littoralis* (BOISD.) on cotton leaves under semi-field conditions. Different concentrations of the two entomopathogenic fungi, Renocam 48% EC and Match 5% EC were applied, LC_{50}s and LC_{90}s values were measured and tabulated. The chemical formulated pesticides, Renocam 48% EC gave highly efficient followed by Match 5% E.C. Biovar proved moderate toxic while Bio-Ranza ranked lest. The Mortality rate increased by increasing the concentration. The persistence of tested insecticides against the 2nd larval instars of the green lacewing, *Chrysoperla carnea*, Aphis craccivora (Koch) on bean leaves and against cotton leafworm (*S.littoralis*) on cotton leaves were measured after 1,3,6,9,12,15 and 21 days after application to evaluate the residual performances during the period from 1 August to 28 December, 2018 under semi -field conditions.

Keywords: entomopathogenic fungi, *C. carnea*, *A. craccivora* and *Spodoptera littoralis*

Introduction

The green lacewing, *C. carnea* (Chrysopidae; Neuroptera) is a generalist biological agent commonly used to control insect pests [1]. Larvae feed on several harmful agricultural insect pests, however, it preys on thrips, aphids and eggs of *S. littoralis* [2] while adults feed only on nectar, pollen and honeydew. Chrysopidae show high resistance towards many widely used pesticides.
The cowpea aphid, *A. craccivora Koch* (Hemiptera: Aphididae), is one of the most damaging insect pests in worldwide on legume plants and its genetic structure is poorly understood [3]. Both nymphs and adults are caused highly damages and consequently economic losses to legume crops [4] by feeding or through transmission of at least 14 plant viral diseases, such as groundnut rosette virus, cowpea aphid-borne mosaic virus, peanut mottle virus, bean common mosaic virus, and peanut stunt virus [5]. The aphids feed on stems and terminal shoots in the seedling stage, and they move later as the plants growth to flowers and pods [6] and that caused stunted plant growth and delayed flower initiation, and around half of crop yield may be lost if the infestation is not controlled [7].
The cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a prolific and highly polyphagous insect. It is considered to be a major pest of great economic importance in many countries since it attacks a multitude of host plants (~73) [8]. The damage is mainly defoliation since the larvae (caterpillars) are principally leaf feeders and those larvae also have the habit of boring into and feeding in the interior of fruits such as tomato, young melon and pepper which near to or rest on soil [9]. Cotton worm, *S. littoralis* insect has 7 generations / year, the insect is given three generations on cotton and four generations on the rest of the crops and may affect some fruit trees and more than 150 plants.
Pesticides can help in controlling of pests in environment but they have risks to our environment, essentially when they are incorrectly applied [10]. The main pesticides classes are insecticides, bactericides, herbicides, and fungicides. Organophosphorus are insecticides that are more used in pest control than organochlorine insecticides. Organophosphorus insecticides illustrate more than 80% of total insecticides. However, fungicides illustrate 65.5% of pesticides [11]. Biological control of crop pests, with microbial pathogens (virus, bacteria, fungi and nematodes) has been known as a good tool in pest management [12].
Microbial pesticides (environment-friendly) can play an important role in crop production by providing a stable pest management program. Several asexual fungi are related with Arthropods, especially with insects Entomopathogenic fungi and that parasitize insects are useful weapons for biocontrol and promoting integrated pest management [13].
The present work aimed to study the relative toxicity and persistence of different control agents against the bean aphid, the green lacewing, *C. carnea* on bean leaves, and against cotton leafworm (*S. littoralis*) on cotton leaves plant under semi-field conditions.

Materials and Methods

1. Growing of tested insects

Rearing of *C. carnea*: The green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) is an aphid's predator very common in nature. Only the larval stages can feed on aphids or other species of insects, while the adult usually feeds on nectar, honeydew and other sugar sources. The adult female lay eggs right in the middle of an aphid colony, the larvae are pale brown or grey and start preying after emergence. Lacewing adults live longer and lays more eggs when provided nectar, pollen and insect honeydews. *C.*

carnea adults were collected from the field by insect sweeping net and brought to the laboratory. The collected adults put in chimney glass (17 cm height, 7 cm top diameter and 8.5 cm bottom diameter). The bottom of each chimney cage was placed on a Petri-dish and its top was covered with black muslin for laying their eggs on it and kept in position by rubber bands [14]. The artificial diet for adults was prepared by adding yeast oxide: factors sugar: water as a ratio (5: 6: 10) and put together in a beaker, which mixed with a mixer. The diet should be a viscous pulp, which is easy to spread using a brush or spatula. A piece of cotton with the mixture (artificial diet) was offered to adults. Adults laying their eggs on the muslin cloth on their stalks glued. Eggs were collected daily by scissors and newly black muslin cloth was replaced. The larvae are fed preferably with fresh eggs of Icerya aegyptiaca or P. gossypiella, which supplied until pupate.

Rearing of Aphis craccivora

Aphis craccivora is considered the most preferable prey for different predators; a strong culture of this aphid should be available during the rearing time to maintain the predator rearing process. The bean seeds were planted in Plastic bags (25× 40×15 cm) contained soil with peatmus. The seeds were planted at 1-2 cm deep and followed with irrigation and fertilizers as required. When the first leaflet appeared after about one week from cultivation. cowpea leaves were infested with *A. craccivora* which distributed over the new foliage of cultivated trays. *A. craccivora* colonies were cultured under laboratory conditions (30±2ºC and 65±5 %R.H.) on broad beans (Vicia faba). Such bean leaves were infested by different stages of aphids and kept under a glass chimney which its upper opening was covered with white muslin. The potted plants were irrigated and fertilized whenever necessary and kept in wooden cages (100×135×135 cm) with nylon gauze sides. *A. craccivora* instars were originally collected from infested beans, cultivated in Agriculture faculty farm.

Rearing of cotton leafworm (*S. littoralis*) culture

Insect larvae used were obtained from laboratory strains of *S. littoralis* reared on castor oil leaves for several generations under controlled conditions of 25± 2 ℃ and 65 ± 5 % R.H. Four replicates were prepared for each concentrate [15].

2. The tested pesticides were

Biovar, entomopathogenic fungi (3200 viable spore/mg), containing the fungus Beauveria bassiana. Applied at a field rate of (2 gm/liter) of water [16].

Bio-Ranza, entomopathogenic fungi ($32x10^6$ viable spore/gm), containing the fungus Metarrihizium anisoplae. Applied at a field rate of (2 gm/liter) of water.

Renocam 48% EC was applied at a field rate of (5ml/liter) of water.

Match 5% EC was applied at a field rate of (0.8 ml/liter) of water.

3. The applied method

The experiments were carried out in the laboratory-field of Plant Protection Research Institute, ARC, Dakahlia, Egypt. The method of indirect exposure was used throughout the present investigation. Five concentrations of each natural control agent (0.1, 0.5,1.0, 1.5, 2.0 g/L), Renocam 48% EC (0.1, 0.5,1.0, 2.0 and 3.0 ml/L) and Match 5% EC (0.05, 0.1, 0.5,1.0 and 2.0 ml/L) were used; leaves of bean plants were divided into five replicates. Every five Plastic bags contained leaves of bean plants were sprayed in each concentration of each tested pesticide in clean water as untreated check (control). Each 20 of nymphs of *A. craccivora* and *C. carnea* were transferred to the treated Plastic bags contained leaves of bean plants, which then covered with muslin cloth held in position by rubber bands. After 24,48,72 hours the alive aphid or predator was counted. As the same method leaves of cotton plants were treated in each concentration of the tested pesticides. Each 10 of 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.) were transferred to the treated Plastic bags contained leaves of cotton plants, which then covered with muslin cloth. After from 48 to 72 hours the alive larvae were counted.

4. Statistical analysis

The mortality percentages were calculated and plotted by LDP line program. The tested compounds were compared for their efficiency on the nymphs of *A. craccivora* and *C. carnea* and 2nd instar larvae of cotton leaf worm according to their LC_{50} and LC_{90} of the toxicity lines.

5. Measurment the persistance of tested insecticides against the 2nd larval instars of the green lacewing, *Chrysoperla carnea, aphis craccivora* (koch) and against cotton leafwor (*s.littoralis*):

The field experiments were conducted according to the ministry of agriculture protocol (2010) field-lab experiment at small scale, Dakahlia governorate during the season (2018). A hand sprayer equipped with one nozzle was used for spraying. With the purpose of evaluating the initial as well as the residual effect of the insecticides with a complete recommended rate against the tested aphesis. Around 160 plant bags were treated with tested pesticides and 40 untreated plant bags(control) in Plant protection Research Institute, Mansoura branch and introduced to the starved 2nd instars larvae and each four bags (one concentration) covered with muslin cloth, every plant bag contained ten larvae for each plant bag treatment. Percentage of mortality were calculated after 1,3,6,9,12,15 and 21 days from application to evaluate the residual performances in bean and cotton field during the period from 1 August to 23 December, 2018. The obtained data were corrected by Abbott's formula [17].

Results and Discussion

1. Measurement of insecticidal efficincy (relative toxicity) under semi- field conditions

On the 2nd larval instars of the green lacewing, *C. carnea* on bean plants

Data presented in Table (1) show the potency of two natural compounds namely (Biovar and Bio-Ranza) compared with two chemical pesticides, Renocam 48% EC and Match 5% EC against the 2nd larval instars of ***C. carnea*** using indirect exposure technique**.** Tabulated data indicated that, the potency of the tested compounds was varied tremendously due to the nature of the tested compounds, the used concentration and the tested stage. As a general trend, data proved that at any of the tested compounds the higher the concentration, the higher was the rate of mortality and vice versa.

Data in Table (1) show that, Biovar and Bio-Ranza proved less toxic to the 2nd larval instars of *C. carnea* (LC_{50} 1.89 g/L (6.8×10^6 viable spore) and (LC_{50} 2.63 g/L (8.6×10^7 viable spore) sequentially. On the other hand, Renocam 48% EC and gave highly efficient against nymphs (LC_{50} 0.80 ml/L) after 48h while Match 5 % EC ranked next showing (LC_{50} 1.10 ml/L) after 72h. On base of the LC_{90} values, the tested compounds showed the same trend, Biovar proved the interior against the 2nd larval instars of ***C. carnea*** (LC_{90} 4.01 gm/L (1.1×10^7 viable spore)), while Bio-Ranza ranked next showing (LC_{90} 4.98 gm/L (1.5×10^8 viable spore)). Renocam 48% EC gave highly efficient against nymphs (LC_{90} 1.54 ml/L) after 48h while Match 5 % EC ranked next showing (LC_{90} 1.87 ml/L) after 72h. According to the LC_{50} and LC_{90}, Renocam 48% EC proved to be the most toxic compound, followed by Match 5 % EC and Biovar, while Bio-Ranza came in the last category.

On nymphs of A. craccivora on bean plant

Data in Table (2) show that, Toxicity lines of Biovar and Bio-Ranza compared with Renocam 48% EC and Match 5 % EC against the nymphs aphid on bean plant using indirect exposure technique. Biovar proved the interior against nymphs of *A. craccivora* (LC_{50} 0.99 mg/L, (3.4×10^6 viable spore)), while Bio-Ranza showed (LC_{50} 1.40 mg/L, 4.2×10^7 viable spore)). Renocam 48% EC gave highly efficient against nymphs (LC_{50} 0.54 ml/L) while Match 5 % EC ranked next showing (LC_{50} 1.37 ml/L). On the base of the LC_{90} values, the tested compounds showed the same trend, Biovar proved the interior against nymphs of *A. craccivora* (LC_{90} 2.66 (8.1 $\times10^6$ viable spore)), while Bio-Ranza ranked next showing (LC_{90} 2.98 (9.8 $\times10^7$ viable spore)). Renocam 48% EC gave highly efficient against nymphs (LC_{90} 1.14 ml/L) while Match 5 % EC ranked next showing (LC_{90} 2.50 ml/L). According to the LC_{50}, Renocam 48% EC and Biovar proved to be more effective compounds than Match 5 % EC, while Bio-Ranza came in the last category. while, LC_{90s}values showed that Renocam 48% EC and Match 5 % EC proved to be more effective compounds than Biovar and Bio-Ranza came in the last category.

On 2nd instar larvae of cotton leaf worm, S. littoralis (BOISD.) on cotton plant

Data in Table (3) show that, Biovar proved moderate toxic to the 2nd instar larvae of *S. littoralis* (LC_{50} 1.75g/L, 5.6×10^6 viable spore), while Bio-Ranza ranked next showing (LC_{50} 1.99 g/L, 6.5×10^7 viable spore). On the other hand, Match 5% E.C (LC_{50} 0.48 ml/L) gave highly efficient against 2nd instar larvae of *S. littoralis* after 72h followed by Renocam 48% EC (LC_{50} 1.29 ml/L) after 48h from treatment. The LC_{90} values of the tested compounds showed the same trend, Biovar proved the interior against the 2nd instar larvae of *S. littoralis* (LC_{90} 4.8 g/L, 1.8×10^7 viable spore), while Bio-Ranza ranked next showing (LC_{90} 5.75 g/L, 2.3×10^8 viable spore). While Match 5% EC (LC_{50} 0.90 ml/L) gave highly efficient against 2nd instar larvae of *S. littoralis*, then Renocam 48% EC (LC_{90} 2.40 ml/L). According to the LC_{50} and LC_{90}, Match 5% E.C (IGR) proved to be the most toxic compound followed by Renocam 48% EC while Biovar and Bio-Ranza came in the last category. These results agree with those obtained by Sahab and Sabbour [18] who reported that under field conditions, the tested fungi showed significant infestations' decrease in the plots treated with B. bassiana, followed by M. anisopliae.

Table 1: The efficiency of Biovar, Bio-Ranza, Renocam 48% EC and Match 5% EC against the 2nd larval instars of the green lacewing, *Chrysoperla carnea* under semi field conditions.

Conc. (g/L)	% Mortality		Conc. (ml/L)	% Mortality of Renocam 48% EC	Conc. (ml/L)	% Mortality of Match 5% EC
	Biovara	Bioranza				
Control	0.0	0.0	Control	0.0	Control	0.0
0.1	28.8	19.1	0.1	22.5	0.05	2.3
0.5	33.4	27.8	0.5	40.2	0.1	20.2
1.0	39.5	35.6	1.0	58.3	0.5	48.3
1.5	45.6	44.3	2.0	96.9	1.0	49.7
2.0	50.4	55.1	3.0	100	2.0	97.8
LC_{50}	1.89 (6.8×10^6 viable spore)	2.63 (8.6×10^7 viable spore)	LC_{50}	0.8	LC_{50}	1.10
LC_{90}	4.01 (1.1×10^7viable spore)	4.98 (1.5×10^8 viable spore)	LC_{90}	1.54	LC_{90}	1.87

Table 2: The efficiency of Biovar, Bio-Ranza, Renocam 48% EC and Match 5% EC against nymphs of the bean aphid, *Aphis craccivora* (Koch) on bean leaves under semi field conditions.

Conc. (g/L)	% Mortality		Conc. (ml/L)	% Mortality of Renocam 48% EC	Conc. (ml/L)	% Mortality of Match 5% EC
	Biovara	Bioranza				
Control	0.0	0.0	Control	0.0	Control	0.0
0.1	30.5%	22.4	0.1	35.6	0.05	5.0
0.5	38.5	32.9	0.5	44.5	0.1	20.9
1.0	55.1	47.9	1.0	88.4	0.5	33.7
1.5	66.7	60.7	2.0	100	1.0	41.6
2.0	79.9	72.1	3.0	100	2.0	82.1
LC_{50}	0.99 (3.4×10^6 viable spore)	1.40 (4.2×10^7 viable spore)	LC_{50}	0.54	LC_{50}	1.37
LC_{90}	2.66 (8.1 $\times10^6$ viable spore)	2.98 (9.8×10^7viable spore)	LC_{90}	1.14	LC_{90}	2.50

Table 3: The efficiency of Biovar, Bio-Ranza, Renocam 48% EC and Match 5% EC against the 2nd instar larvae of cotton leaf worm *Spodoptera littoralis* (BOISD.) under semi field conditions.

Conc (g/L)	% Mortality		Conc (ml/L)	% Mortality of Renocam 48% EC	Conc (ml/L)	% Mortality of Match 5% EC
	Biovara	Bioranza				
Control	0.0	0.0	Control	0.0	Control	0.0
0.1	17.5%	13.9%	0.1	21.5	0.05	9.90
0.5	22.9	19.5	0.5	33.9	0.1	31.5
1.0	27.3	22.9	1.0	45.7	0.5	63.1
1.5	32.4	26.4	2.0	70.0	1.0	100
2.0	57.9	41.2	3.0	100	2.0	100
LC_{50}	1.75 (5.6×10^6 viable spore)	1.99 (6.5×10^7 viable spore)	LC_{50}	1.29	LC_{50}	0.48
LC_{90}	4.80 (1.9×10^7viable spore)	5.75 (2.3×10^8 viable spore)	LC_{90}	2.40	LC_{90}	0.90

2. Measurement of the persistance of tested insecticides under semi -field conditions

On the 2nd larval instars of the green lacewing, *C. carnea* on bean leaves

Data presented in table (4) showed that; all the tested chemical insecticides improved the insecticidal action of the complete recommended rate. Using Renocam 48% EC gave the highest average residual effect (47.8 %) followed by Biovar (25.6%) in complete recommended rate. These results depend on the increasing acidity of these chemical Organophosphorous insecticide (Renocam 48% EC) [19]. The decrease in pH value indicates an increase in positive charge of spray solution leading to increasing attraction between spray solution and treated plants leaves surface, which has a negative charges, then will increase its persistence on the plant surface. on the other hand, Using Bio-Ranza gives less average residual effect (13.6 %).

On nymphs of A. craccivora on bean leaves under semi -field conditions

The field efficiency of Biovar, Bio-Ranza, Renocam 48% EC and Match 5% E.C was applied at field rate against nymphs of the bean aphid, *Aphis craccivora* (Koch) on bean leaves. Data concerning in table (5) showed the effect of different pesticides types against bean aphid, *Aphis craccivora* (Koch) on bean leaves during 2018. It is clear that the recommended doses of Match 5% E.C (35.2 %) has less resistance percentage on been plant against nymphs of the bean followed by Bio-Ranza (39.5%) then Biovar (48.9%) while Renocam 48% EC has the most residual percentage (74.4%) under semi-field conditions.

On the 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.) on cotton leaves under semi -field conditions:

The field efficiency of Biovar, Bio-Ranza, Renocam 48% EC and Match 5% E.C was applied at field rate against the 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.) on cotton leaves. Data concerning the effect of different pesticides types against *S. littoralis* (BOISD.) during 2018. From table (6), It is clear that the recommended doses of Bio-Ranza have a less residual effect (29.9%) on *S. littoralis* (BOISD.) followed by Biovar (37.8 %) while Match 5% EC and Renocam 48% EC has the most residual percentage (44.9%, 57.2 %) under semi-field conditions. These findings agree with Gomaa and Ibrahim [20] who reported that Biovar formulation was effective on the 2nd instar larvae of *S. littoralis* followed by Bioranza.

Table 4: Residual effect of tested insecticides against the 2nd larval instars of the green lacewing, *C. carnea* under semi field conditions.

Treatment	% of corrected mortality after treatment (days) Residual effect								% mean residual effect
	1	3	6	9	12	15	18	21	
Biovar	0.0	5.2	42.7	55.0	20.0	30.0	37.0	15.0	25.6 %
Bio-Ranza	0.0	0.0	22.6	37.2	10.0	5.4	18.0	5.7	13.6 %
Renocam 48% EC	100	85.1	62.3	50.4	39.1	27.2	13.1	5.0	47.8 %
Match 5% EC	30.4	25.9	28.9	20.2	15.6	8.4	0.0	0.0	16.42%

Table 5: Residual effect of tested insecticides against against nymphs of the bean aphid, *A. craccivora* (Koch) on bean leaves under semi field conditions.

Treatment	% of corrected mortality after treatment (days) (Residual effect)								% mean residual effect
	1	3	6	9	12	15	18	21	
Biovar	0.0	46.2	60.1	79.7	53.0	37.0	35.0	20.0	48.9 %
Bio-Ranza	0.0	25.0	57.6	67.2	48.0	35.0	22.0	10.9	39.5 %
Renocam 48% EC	100	100	100	90.4	83.5	61.2	45.1	15.0	74.4 %
Match 5 % EC	81.5	68.7	58.9	44.2	23.3	5.4	0.0	0.0	35.2 %

Table 6: Residual effect of tested insecticides against against the 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.) on cotton leaves under semi field conditions.

Treatment	% of corrected mortality after treatment (days) Residual effect								% mean residual effect
	1	3	6	9	12	15	18	21	
Biovar	0.0	3.4	30.6	60.0	50.3	45.0	30.5	12.9	37.8 %
Bio-Ranza	0.0	1.3	27.1	53.0	33.0	40.0	15.0	9.5	29.9 %
Renocam 48% EC	100	89.5	75.3	71.7	50.3	31.7	24.2	15.3	57.2 %
Match 5 % EC.	88.2	78.6	64.8	57.6	44.6	20.4	5.0	0.0	44.9 %

Conclustion

It is concluded that traditionally the impact of pesticides on natural enemies has been based on acute mortality from topical exposure or accumulation from residues on the foliage, and expressed as an LC_{50} referring to the concentration of the product that causes 47.8 % mortality of the natural enemy species. The residual toxicity of larval instar of *C. carnea* and nymphs of *A. craccivora* on treated been leaves in the semi-field condition by airblast sprayer. Impact declined from 100 to 13.1% and from 100 to 45.1% mortality after 18 d in case of using of Renocam 48% EC pesticide. While, if we used Match 5 % EC, the percentage of the mortality decreased from 30.4 to 8.4 and from 81.5 to 8.4 % after 15 d from treatment. The application of entomopathogenic fungi against 2nd larval instars of the green lacewing, *C.carnea*, Biovar showed increase percentage of mortality to 55.0% after 9 d of treatment and then decreased to 15% after 21 d from treatment. Also using Bio-Ranza increase percentage of mortality to 37.2% after 9 d of treatment and then decreased to 5.7% after 21 d from treatment. The application of entomopathogenic fungi against nymphs of the bean aphid, Biovar showed increase percentage of mortality to 79.7% after 9 d of treatment and then decreased to 20.0% after 21 d from treatment. Also using Bio-Ranza increase percentage of mortality to 67.2% after 9 d of treatment and then decreased to 10.9% after 21 d from treatment.

Levels of toxicity were determined on some cotton insect pests by many investigators [21]. The residual toxicity of larval instar of 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.) on treated cotton leaves in the semi-field condition by air blast sprayer. Renocam 48% EC and Match 5 % EC pesticides caused about 100 and 88.2% mortality. Impact declined to 100 to 15.3%, 88.2 to 5.0 % mortality after 18 d in case of using of Renocam 48% EC and Match 5 % EC respectively. The application of entomopathogenic fungi against 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD.), Biovar showed increase percentage of mortality to 60.0% after 9 d of treatment and then decreased to 12.9 % after 21 d from treatment. also using Bio-Ranza increase percentage of mortality to 53.0 % after 9 d of treatment and then decreased to 9.5 % after 21 d from treatment. A significant decrease in the population of *S. littoralis* larvae was recorded after one week from application of Biovar and Bio-Ranza on cotton. The first instar was zero in all treatments compared with control [22]. The increase and decrease in percentage of mortality of both entomopathogenic fungi are due to environmental conditions and ground cover affect the efficiency of the fungus under field conditions [23].

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