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Biocontrol against cabbage aphid *Brevicoryne brassicae* L.

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Abstract: *The effects of the aphid parasitoid Diaeretiella rapae (M'Intosh), the predator Coccinella undecimpunctata L. and the entomopathogenic fungus Beauveria bassiana (Bals.) on the cabbage aphid Brevicoryne brassicae L. were evaluated in this study. The effects of host densities on D. rapae parasitizing B. brassicae were studied at five densities of the parasitoid (2, 4, 8, 12 and 16 females). The highest percentages of parasitism were 92.6, 57.6 and 47% for D. rapae at 16 parasitoids/200 aphids in the field, pots and Petri-dishes in lab, respectively. The developmental time of predator C. undecimpunctata larvae instars was 14.8 days. The average number of aphids consumed during the C. undecimpunctata larva stage was 442.45 individuals of B. brassicae. The adult male consumed a total average 5073.83 of B. brassicae individuals while, the female consumed 6615 of B. brassicae individuals. Three tested concentrations (10^5, 10^6 and 10^7 spores/ml) of B. bassiana formulations were used against aphid B. brassicae. Mortality percentage after 7 days of application showed 35.55, 46.66 and 64.44%, respectively. The obtained results revealed that LC_{50} was 1.1×10^6 spores/ml and LC_{90} was 3.4×10^9 spores/ml. According to these results, D. rapae, C. undecimpunctata and B. bassiana could be used as suitable biocontrol agents against B. brassicae.*

Keywords: *Beauveria bassiana, Biocontrol, Brevicoryne brassicae, Coccinella undecimpunctata, Diaeretiella rapae*

I. Introduction

In several parts of the world and also in Egypt, the aphid *B. brassicae* is a major pest on cruciferous plants, especially on cabbage and cauliflower [1,2,3]. About 23 types of virus diseases of cruciferae and many citrus diseases are transmitted by the cabbage aphids [4]. The parasitoids and predators that found in nature are important factors in the regulation of their population densities. One of the most efficient predators of aphids is the ladybird beetle *C. undecimpunctata* [5]. The most common natural enemy of the cabbage aphid in nature is the parasitoid *D. rapae* [6,7,3].

In agriculture, entomopathogenic fungi are natural enemies that contribute in regulating their host's numbers and causing mortality in the pest's population [8]. Many fatal diseases in Egypt are caused by several species of entomopathogenic fungi as *Erynid neophidis, Onidiobolus obscures, Verticillium lecanii*, various species of *Beauveria* and *Paecilomyces farinosus* [9,10,11]. Entomopathogenic fungi can be used as insect's biocontrol agent without causing any damage to the environment or to the other non-target organisms [12]. All orders of insects are infected by entomopathogenic fungi; the most common are Coleoptera, Diptera, Hemiptera, Hymnoptera, Lepidoptera, and Orthoptera [13], but they are specifically important pathogens to the order Homoptera [14]. The present research aims to:

1- Estimate the role of the parasitoid *D. rapae* and the predator *C. undecimpunctata* against the cabbage aphid *B. brassicae* in the laboratory and in the field.

2- Evaluate the biocontrol potential of *B. bassiana* against the cabbage aphid *B. brassicae*.

II. Materials and Methods

The present study was carried out in the laboratory of the Plant Protection Research Institute, Sharkia Branch and also in the fields of Zagazig district, Sharkia governorate during winter season of 2012-2013.

The culture of *B. brassicae* was maintained on cabbage plants. The plants were maintained at temperature 25±20°C and relative humidity 65±5%. Twenty aphids were transferred to each cabbage plant for reproduction. To avoid parasitism and predation, that plants were covered with mustin.

II.1. The parasitoid *D. rapae*:

II.1.1. Bionomics of *D. rapae* parasitoid of *B. brassicae* at varying densities (2,4,8,12 and 16) under field conditions: The host *B. brassicae* and the parasitoid *D. rapae* were reared in the laboratory under 22.0±1°C and R.H. 70.0±2%. Young potted seedlings of cabbage bearing about 200 hosts were used in the experiments. The experiments were carried out in iron cages (100×100×60cm). The food source for the parasitoid was a small sponge piece soaked in 30% honey solution. Thereafter, freshly emerged mated and native female parasitoids were gently introduced into each cage having aphids on the host plant for 24h. After 24h the parasitoids were

removed and the hosts were left undisturbed until they mummified. On the day of mummification, the mummies were cut off along with a part of the leaf and gently placed into marked Petri-dishes. The base of each Petri-dish contained pieces of moist filter paper. The mummies were sexed and recorded. Five replicates of the experiment were performed for all parasitoid's densities.

II.1.2. The same experiment was carried out in Petri dishes and Pots in the laboratory at the same time.

II.2. The predator *C. undecimpunctata*:

Biological characters of *C. undecimpunctata* on cabbage aphid *B. brassicae*: Permanent cultures of the predator *C. undecimpunctata* and the pray *B. brassicae* were maintained in the laboratory at 21.0 ± 1^0 C and R.H. 65±5%. Twenty newly hatched first instar larvae of each predator were introduced singly into Petri-dishes of 10 cm in diameter. To facilitate the predator larva movement, the bottom of each dish was covered with a filter paper. A known number of the different stages of each aphid species were introduced daily into each dish. A small plant leaflet was introduced daily in each Petri-dish as food for the aphids. The devoured individuals were recorded. Before introducing new individuals, the rest of the aphids and their parts were removed from each Petri-dish. The number of the aphids consumed per larvae of each predator was recorded. Twenty newly adults of the predator fed on the prey as that of its larval instars, were sexed and introduced singly into the Petri-dishes. The same technique done in the larval stage was adapted during the rearing of the adult stage. Copulation took place after 5-7 days of emergence and the two sexes were immediately separated and kept singly in the dishes. The total number of eggs laid by the predator's female was recorded and the total number of the aphid's consumed by males or females were also counted.

II.3. The entomopathogenic fungi *B. bassiana*

II.3.1. Fungal inocula: Spores of fungal isolate were harvested by rinsing with sterilized water containing 0.005% Tween80 from 7 days old culture (Dox medium grown at 25±1°C for *B. bassiana* isolate). To reduce the mycelium clumping, the suspensions were filtered through cheesecloth. The spores were counted in the suspensions using a haemocytometer. The concentrations were adjusted to 10^5, 10^6 and 10^7.

II.3.2. Experimental work: Studies regarding the effect of the fungus on the infected leaves of cabbage were applied on three replicates each consists of fifteen individuals of *B. brassicae* on cabbage leaves. Two ml of spores' suspension were sprayed on the infected leaves and the control was treated with two ml of sterilized water containing 0.005 % tween80 only. The treatments and control were incubated for 7 days under laboratory conditions (25±1°C, R.H. 65±5%). Nymphs mortality was observed after 1, 3, 5and 7 days. LC_{50} and LC_{90} values were calculated after 5 and 7 days according to Finny[15]. Mortality was corrected by using Abbott's formula [16]. Final data were subjected to ANOVA and the obtained results were statistically analyzed by using Costat[17] computer program. Climatic data during the period from 2012-2013 was provided by Cairo Meteorological authority.

III. Results and discussion

III.1. The parasitoid *D. rapae*:

III.1.1. Bionomics of *D. rapae* parasitoid of *B. brassicae* at varying densities (2, 4, 8, 12 and 16) under field conditions: Data in Table (1) shows the influence of the parasitoid density on the emergence percentage of the adult parasitoids from the mummies. The maximum percentage was 80.19% at two parasitoids per cage while, the minimum percentage was 73.95% at 16 parasitoids per cage. The maximum percentage of parasitism was 92.6% was at 16 parasitoids per cage while, the minimum was 51% at two parasitoid per cage .The maximum number of parasitized aphid was 187 individuals at 16 parasitoids per cage while, the minimum was 101.4 individuals at two parasitoid per cage .There were significant differences in the total numbers of parasitized aphid and the total percentage of parasitism at all densities .In this investigation, it is clear that the total percentage of parasitism increased in the field and decreased in the laboratory, which was probably due to some weather factors.

Table (1): Effect of parasitoid number on the number of aphid parasitized, adults emerged, number of adults non emerged, percentage of adult emergence and percentage of parasitism in the field under the conditions (16.0±1°C and R.H. 75.0±2 %) in 2012-2013.

Parasitoid densities	Mean ± SD No. of aphid parasitized	Mean ± SD No. of adults emerged	Mean ± SD No. of adults non emerged	Mean ± SD Percentage of adult emergence	Mean ± SD Percentage of parasitism
2 ♀	101.4 e ± 4.32	81.4 c ± 3.35	19.8 c ± 1.3	80.19 a ± 0.94	51 e ± 2.09
4 ♀	114.6 d ± 3.96	91 c ± 3.38	25.6 c ± 0.92	77.74 ab ± 0.79	61.9 d ± 2.14
8 ♀	142.6 c ± 5.61	108.4 b ± 3.1	37.6 b ± 2.15	75.72 ab ± 1.51	72.8 c ± 2.71
12♀	168 b ± 3.51	124.8 a ± 3.34	44.2 ab ± 1.82	74.79 b ± 4.5	84.5 b ± 1.67

16♀	187 a ± 2.09	133.4 a ± 4.91	51.2 a ± 4.61	73.95 b ± 1.54	92.6 a ± 1.2
F value	76.79	35.40	26.25	3.61	44.78

III.1.2. Bionomics of *D. rapae* parasitoid of *B. brassicae* at varying densities (2, 4, 8, 12 and 16) in pots: Table (2) shows the influence of the parasitoid density on the emergence percentage of the adult parasitoids from the mummies. The maximum percentage was 79.0% for *D. rapae* at two parasitoids per cage and a minimum percentage of 73.9% at 16 parasitoids per cage. With the increase of parasitoid density the rate of parasitism and the number of parasitized aphids increased. The maximum percentage of parasitism was 57.6% at 16 parasitoids per cage and a minimum of 28% was recorded at two parasitoids per cage. The maximum number of aphid parasitized for *D. rapae* 114.4 was recorded at 16 parasitoids per cage and a minimum of 54.4 was recorded at two parasitoids per cage.

Table (2): Effect of parasitoid densities on the number of aphid parasitized, adults emerged, number of adults non emerged, percentage of adult emergence and percentage of parasitism in the pots under the laboratory conditions (18.0±1°C and R.H. 73.0±2%) in 2012-2013.

Parasitoid densities	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	No. of aphid parasitized	No. of adults emerged	No. of adults non emerged	Percentage of adult emergence	Percentage of parasitism
2 ♀	54.4 e ± 3.25	41.8 d ± 2	11.8 c ± 1.74	79 a ± 2.75	28 d ± 1.9
4 ♀	71.6 d ± 3.43	55.4 c ± 3.74	16.2 c ± 1.35	77.9 a ± 2.05	35 c ± 2.63
8 ♀	87.2 c ± 3.03	67 b ± 3.9	21 b ± 1.44	76.35 a ± 1.74	43.9 b ± 1.31
12♀	103.6 b ± 2.83	77.8 a ± 3.59	25.6 b ± 1.2	73.45 a ± 1.83	52.1 a ± 1.36
16♀	114.4 a ± 4.08	83.8 a ± 1.82	32.2 a ± 2.12	73.9 a ± 1.33	57.6 a ± 2.28
F value	51.32	28.97	24.42	1.46	37.7

III.1.3. Bionomics of *D. rapae* parasitoid of *B. brassicae* at varying densities (2, 4, 8, 12 and 16) in Petri-dishes: Table (3) shows that the maximum percentage of adult emergence of the parasitoids from mummies was 80.65% at two parasitoids per cage, while the minimum percentage was 73.29% which recorded at 16 parasitoids per cage. With the increase of parasitoid density, the percentage of parasitism increased to 47% for *D. rapae* at 16 parasitoids per cage while, it was a minimum of 20.6% at two parasitoids per cage. There were significant differences in the percentage of parasitism and number of parasitized aphid among two, four, eight, 12 and 16 parasitoid per cage. The maximum number of aphid parasitized was 94.8 for *D. rapae* at 16 parasitoids per cage with a minimum of 41.8 was at one parasitoid per cage.

Table (3): Effect of parasitoid density on the number of aphid parasitized, adults emerged, number of adults non emerged, percentage of adult emergence and percentage of parasitism in the laboratory in Petri-dishes under laboratory conditions (16.0±1°C and R.H. 75.0±2%) in 2012-2013.

Parasitoid densities	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	No. of aphid parasitized	No. of adults emerged	No. of adults non emerged	Percentage of adult emergence	Percentage of parasitism
2♀	41.8 d ± 2.74	33.2 d ± 2.21	7.8 d ± 0.85	80.65 a ± 1.65	20.6 e ± 1.15
4♀	59.4 c ± 4.53	49.2 c ± 2.88	13.4 c ± 1.38	77.67 ab ± 2	30.1 d ± 2.14
8♀	73 b ± 4.23	55 bc ± 2.73	17.2 bc ± 0.79	76.79 ab ± 1.42	35.9 c ± 1.61
12♀	79.8 b ± 1.93	60 b ± 1.84	19 b ± 1.3	74.88 ab ± 1.31	41.1 b ± 1.38
16♀	94.8 a ± 3.24	71.4 a ± 2.01	25 a ± 1.92	73.28 b ± 1.44	47 a ± 1.81
F value	33.63	35.19	23.76	3.11	39.32

However, Herakly and Abou El-Ezz[18] reported that *D. rapae* is one of the major natural factors in suppressing *B. brassicae* population. As the density of the parasitoid increase, the number of mummies, the emerged adults and the fecundity of the parasitoid *D. rapae* increased. The previous results indicated that, by increasing the parasitoid densities the percentages of parasitism were increased at lower host densities as the parasitoid had the ability to attack higher number of its host [19]. These findings agree with those of El-Naggar *et al.*[20] who reported that the density of the parasitoid had an influence on the percentage of parasitism. The highest percentage reached was 91.40% at 16 *D. rapae* female parasitoids per cage, while the minimum value was 55.6% at one female per cage. By increasing the number of parasitoids, the percentage of the parasitism increased. However, Zahra *et al.*[21] mentioned the rate of parasitism and functional response by *D. rapae* on different host densities of *Diuraphis noxia* (Mordvilho). There was a decrease in the number of hosts parasitized by the parasitoid with the increasing in host density as there was intra-specific competition among the female parasitoids at high density.

Meanwhile, Abdulrehman and Powell[22] and saleh[3] reported that aphid parasitoids are highly potential biocontrol agents but their efficiency is depend on the presence in the right place at the right time and right host. By identifying the physical and chemical signals regulating the parasitoid behavior, this will provide exciting

opportunities to manipulate the parasitoids in the field. The parasitoids will selectively bred to attack specific hosts and then primed to an appropriate plant volatiles as foraging cues before release and this could be used in inundative releases.

III.2. The predator *C. undecimpunctata*:

Biological characters of *C. undecimpunctata* on cabbage aphid *B. brassicae*: Tables (4 & 5) show the results of certain studied biological characters of the predator *C. undecimpunctata* on *B. brassicae*. The average duration of the predator larval instars was 14.8 days when fed on this aphid species. During the four larval instars of the predator, the averages of the total consumption were 46.2, 74.45, 117.1 and 204.7 aphid individuals, respectively, with a general average of 442.45 individuals during the larval stage. It is clear that the third and fourth larva instars consumed 26.47% and 46.26%, respectively of the total consumption. Mean while, the first and second instars consumptions were 10.44% and 16.83%. The male predator in the adult stage consumed a total average of 5073.83 aphid individuals, with a daily rate of 71.13 individuals during its longevity period which lasted an average of 71.33 days. The female predator consumed a total average of 6615 of *B. brassicae* individuals, with a daily rate of 76.25 during its longevity which lasted 86.75 days. The number of the deposited eggs by the female predator averaged 211.25 during the oviposition period which reached an average of 45.5 days.

Table (4): Duration of larval instars of *C. undecimpuncatata* and their feeding capacity when fed on *B. brassicae* under laboratory conditions (21.0±1^0 C and R.H. 65±5%) during winter season of 2012-2013.

Larval instar	Duration in days ± SD	Daily average consumption	Total consumption per instar	%
1st	3.4±0.11	13.58	46.2±2.03	10.44
2nd	3.55±0.114	11.52	74.45±3.01	16.83
3rd	3.5±0.115	11.66	117.1±3.44	26.47
4th	4.35±0.109	16.64	204.7±3.41	46.26
Total	14.8±0.186	50.22	442.45±7.15	100

Table (5): Longevity (in days) and fecundity of predator *C. undecimpuncatata* reared on *B. brassicae* under laboratory conditions (21.0±1^0 C and R.H. 65±5%) during winter season of 2012-2013.

Adults stages	Periods in days±SD	Daily average consumption	Total consumption per adult stage±SD	Average No. of eggs	
				Daily	Total
A: Female preoviposition	10.88±0.69	75.6	822.6±43.58	4.64	211.25±11.69
oviposition	45.5±1.48	81.39	3703.9±151.1		
postoviposition	61.37±4.91	91.06	3588.5±374.42		
Longevity	86.75±3.68	66.89	8115±399.77		
B: Male longevity	71.33±5.136	82.86	6573.83±472.64	-	-

These findings agree with the results of Abou Zeid *et al*.[23] who reported that at 26-28°C, the incubation period of *C. undecimpuncatata* was 2.7 days. Eraky and Nasser[24] mentioned that the incubation period for the same predator was 2.0 days at 30°C. While, this period was 3.5 days at 25.0±2.0°C [25]. Ghanim and El-Adl[26] found that when the larvae of *C. undecimpuncatata* and *C. vicina isis* reared on both aphids, *Rhopalosiphum maidis* (Fitch) and *S. avenae* they took 10.0 and 9.0 days. The larvae, pupae and complete immature stages averaged 7.0, 2.5 and 12.0 days at 30°C when *C. undecimpuncatata* reared on *R. padi* L. [24]. On the other hand, Mohammed[27] found that the larvae and pupae averaged 9.2 and 2.9 days at 26°C when *C. undecimpuncatata* reared on *Macrosiphum pisi* (Harris). Meanwhile, Barakat[28] reported that when the predator *C. undecimpuncatata* reared on *H. pruni*, the incubation period, larva, pupa and total developmental period lasted 2.7, 11.0, 7.6 and 24.3 days at 26.8°C and the four larval instars consumed 15.7, 50.4, 48.2 and 157.9 individuals, respectively.

On the other hand, Mohammed[27] found that when the predator *C. undecimpuncatata* reared on *M. pisi*, the average of the total consumption during the four larval stages of was 27.7, 68.4, 123.9 and 144.0 individuals, respectively.

Whereas, El-Hag and Zaitoon[25] mentioned that for *C. undecimpuncatata* females, the oviposition and the longevity periods were 29.8 and 70.0 days. They also found that the number of eggs laid per female and daily mean of eggs was 370.5 and 142 eggs when reared on *B. brassicae* and *R. padi*, while Mohammed[27] reported that ovipostion and longevity period for the same predator females was 56.6 days with (481 eggs) and an average of 4599.2 *M. pisi* individuals were consumed by the female during this period. On the other hand, Barakat[28] found that oviposition and longevity period for the female predator was 43.5 days with (343.5 eggs) during this period. The female consumed an average of 2531.7 *H. pruni* individuals, while the male consumed an average of 2017.6 *H. pruni* individuals.

III.3. The entomopathogenic fungi *B. bassiana*:

Laboratory evaluation: Data given in Table (6) shows the efficiency of *B. bassiana* spore suspension on nymph instars of cabbage aphid *B. brassicae* after application with different concentrations of *B. bassiana* spores under laboratory conditions of (25±1°C, R.H. 65±5%). The concentrations were adjusted to 10^5,10^6 and 10^7 spores/ml. Mortality percentages after 5 days of application showed 22.22%, 28.88% and 42.22% and after 7 days showed 35.55%, 46.66% and 64.44%, respectively.

Table (6): Mortality percentages of nymph instars of *B. brassicae* after application with different concentrations of *B. bassiana* spores' suspension under laboratory conditions (25±1°C, R.H. 65±5%) during winter season of 2012-2013.

Concentration	After 1 day			After 3 days			After 5 days			After 7 days		
	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %
1×10^5 spores/ml	44	1	2.22	39	6	13.33	35	10	22.22	29	16	35.55
1×10^6 spores/ml	42	3	6.66	36	9	20	32	13	28.88	24	21	46.66
1×10^7 spores/ml	41	4	8.88	34	11	24.44	26	19	42.22	16	29	64.44

Tables (7 & 8) clarified the LC_{50} and LC_{90} values of *B. bassiana* spores/ml after 5 and 7 days of application on the nymph instars of cabbage aphid, *B. brassicae*. The obtained results revealed that, after 5 days, the LC_{50} and LC_{90} were 5.8×10^7 and 1.7×10^{12} spores/ml (Fig. 1). While, after 7 days, the LC_{50} and LC_{90} were 1.1×10^6 and 3.4×10^9 spores/ml (Fig. 2)

Experiments were conducted as a measure of mortality of different fungal isolates against aphids according to time-dose dependent mortality response. After the treatment, the mortality observed was low on day 1 and 2, then it increased from day 7 to 9. With the increase in the spores concentration of the conidial suspensions and the exposure time, the mortality in the infected aphids with the fungal isolates increased [29,30].

Meanwhile, Akmal *et al.*[31] showed that at the 7th day after the treatment at a concentration of 1×10^8 spores/ml, the maximum mortality 100% of *B. bassiana* on *B. brassicae* was obtained, while the minimum mortality of 99.2% obtained by the treatment of 1×10^6 spores/ml. In contrast to this no mortality was recorded in control. The value of LC_{50} 6.28×10^5 showed that at the 3rd day of treatment, the 50% mortality was obtained

On the other hand, Akbari *et al.*[32] in Iran showed that the adult aphids of *B. brassicae* were treated with fungal concentrations 1×10^3 to 1×10^7 spores/ml. The lowest LT_{50} was obtained at 7.67 days for Iran 429C (*B. bassiana*) isolate at concentration 1×10^7 spores/ml.

Table (7): Lethal concentration ($LC_{25\text{-}99}$) of *B. bassiana* spores/ml after 5 days of application against nymph instar of *B. brassicae* under laboratory conditions (25 ± 1°C, R.H. 65± 5%) in 2012-2013.

Lethal concentration	Concentration of *B. bassiana* spores/ml			Slope
	Concentration	Lower limit	Upper limit	
LC_{25}	258760	8837	874830	0.287
LC_{50}	5.8×10^7	9.5×10^6	9.1×10^{10}	
LC_{75}	1.3×10^{10}	2.9×10^8	3.2×10^{17}	
LC_{90}	1.7×10^{12}	6×10^9	2.8×10^{23}	
LC_{95}	3.1×10^{13}	3.6×10^{10}	1×10^{27}	
LC_{99}	7.4×10^{19}	1×10^{12}	5.1×10^{33}	

Table (8): Lethal concentration ($LC_{25\text{-}99}$) of *B. bassiana* spores/ml after 7 days of application against nymph instar of *B. brassicae* under laboratory conditions (25 ± 1°C, R.H. 65± 5%) in 2012-2013.

Lethal concentration	Concentration of *B. bassiana* spores/ml			Slope
	Concentration	Lower limit	Upper limit	
LC_{25}	18062.3	353.66	81558.6	0.37
LC_{50}	1.1×10^6	4.5×10^5	3.5×10^6	
LC_{75}	7.9×10^7	1.5×10^7	5.4×10^9	
LC_{90}	4.3×10^9	2.2×10^8	7.2×10^{12}	
LC_{95}	3.3×10^{10}	1×10^9	5.5×10^{14}	
LC_{99}	2.2×10^{12}	1.8×10^{10}	1.9×10^{18}	

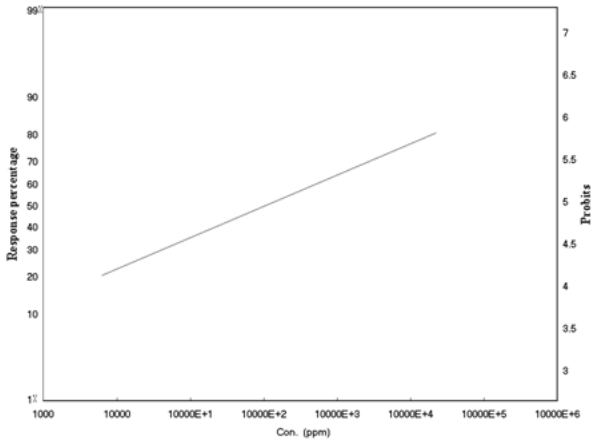

Fig. (1): Concentration mortality probit line of *B. bassiana* spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after 5 days.

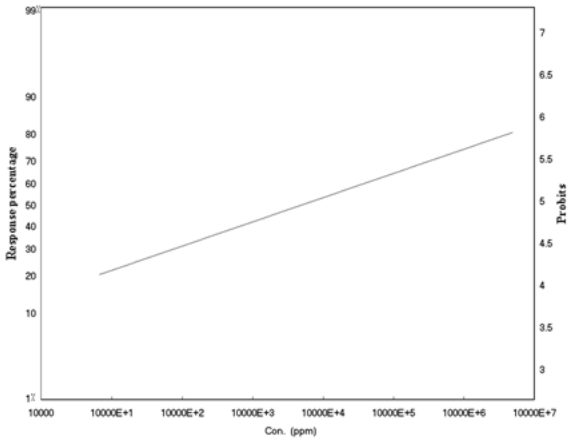

Fig. (2): Concentration mortality probit line of *B. bassiana* spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after 7 days.

IV. Conclusion

The obtained results revealed that the parasitoid *D. rapae*, the predator *C. undecimpunctata* and the entomopathogenic fungus *B. bassiana* could be recommended as biocontrol agents against the cabbage aphid *B. brassicae* under Egyptian conditions.

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