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Nano Entomopathogenic Fungi As biological Control Agents on Cabbage Worm, *Pieris rapae* L. (Lepidoptera: Pieridae)

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ABSTRACT

The present investigation was carried out during two successive Cabbage seasons (2014-2015 and 2015- 2016), to study the impact of entomopathogenic fungi on Cabbage worm, Pieris rapae L. The Pieris rapae Populations were evaluated in the field early in the season in October 2014.which began to appear on cabbage plants. Thereafter number of Pieris rapae increased gradually to reach a peak of abundance during November 2014 and November 2015.). Three concentrations were used (1 x10^3, 1 x 10^4 and 1 x 10^5 spores/ ml.). Under laboratory conditions the results showed that B. bassiana, M. anisopliae and V. lecanii have a latent toxicity because mortalities were occurred after the third day from treatment. The maximum percent of mortality (100 %) was occurred after the tenth day from treatment with the third concentration in V. lecanii. The Second concentration (1 x 10^4spores/ ml.) was the highly toxic in B. bassiana, M. anisopliae and V. lecanii to the larvae of Pieris rapae compared with the other two concentrations. Under field conditions the third concentration (1 x 10^5) also, was the best concentration against Pieris rapae L. after the third application in B. bassiana, M. anisopliae and V. lecanii. The percent of reduction was ranged between 60 and 70% in all concentrations. B. bassiana and M. anisopliae were high effective than V. lecanii against Pieris rapae L. These results confirmed that B. Bassiana, M. anisopliae and V. lecanii isolates are promising agents for Pieris rapae L. control in the field.

Key words: Nano Entomopathogenic fungi, Cabbage worm, *Pieris rapae*.

INTRODUCTION

Cabbage is the most common vegetable crop grown in Egypt. Lepidopteran pest insects, such as beet webworm, *Pyrausta sticticalis*, cabbage moth, *Mamestra brassicae*, diamondback moth, *Plutella xyllostella* and the large white butterfly, *Pieris brassicae, Brevicoryne brassica L.* are all able to completely eliminate yield. According to this susceptibility (Shternshis, 2005). Naturally occurring entomopathogens are important regulatory factors in insect populations. Many species are employed as biological control agents of insect pests Lacey *et al* 2001. Fungi are important in the natural regulation of many insect pests and pest populations are often decimated in widespread epizootics (McCoy *et al.*, 1988). Several products based on *Beauveria bassiana* were used for control of numerous pests in the People's Republic of China Feng *et al.*, 1994). *Metarhizium anisopliae* has potential against several pest species and is being used commercially in Brazil for control of spittle bugs in sugarcane (Wraight and Roberts, 1987). *Paecilomyces fumosoroseus* and *Verticillium lecanii* are commercially produced and used for control of whiteflies and aphids in greenhouses in Europe and the USA (Copping, 2001). The most common fungi used for insect control belong to the genera *Beauveria, Metarhizium, Paecilomyces, Verticillium, Aschersonia, and Conidiobolus*. Treatment with suspensions of *Verticillium (Lecanicillium) lecanii* (Verticillin®), and *Beauveria*

bassiana (Boverin®) has resulted in efficient control of aphids and whitefly (Ogarkov and Ogarkova, 2000, and Abdel-Raheem, *et al*.2009). The advantage of this fungus is a low relative humidity requirement (about 60% RH).
In Egypt many authors studied the impact of entomopathogenic fungi against some insect such as *Spodoptera littoralis,* Rabie, 2002), Aphids, (Abdel-Rahman, *et al.* 2004), *Cassida vittata* Vill. and *Scrobipalpa ocellatella* Boh. (Abdel-Raheem, 2005 and Rabie, *et al.* 2005), and *Spodoptera littoralis*, *S. exigua*, *Aphis craccivora* and *Bemisia tabaci* (Genn.) (Zaki, 1998, Zaki and Abdel-Raheem 2010 , Abdel-Raheem *et al*. 2009, Salem, *et al*., 2015 and Abdel-Raheem *et al*. , 2009, 2011, 2015[a]&[b], 2016).

MATERIALS AND METHODS

Fungi culture:
Fungi: (*Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii*) were grown on Potato dextrose agar (PDA) (1 Kg potatoes, 80 gr. Agar, 100 gr. Dextrose and 4 lit. Distilled water. The media was autoclaved at 120 ºC for 20 minutes, and poured in Petri- dishes (10 cm diameter x 1.5 cm). Then incubated the fungi and kept at 25 ±1 ˚C and 92± 5 % RH. The fungal isolates were re-cultured every 14 – 30 days and kept at 4 ˚C.

Preparing of the concentrations:
Spores of fungal isolates harvested by rising with sterilized 0.5 % Tween 80 from 14 day old culture (PDA) media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm2).The concentrations were used 1 x 10^{3}, 1x 10^{4} and 1 x 10^{5} spores / ml.

Laboratory inoculation:
The cabbage worm, *Pieris rapae* L. were transferred to the Lab. from the field and put it in Petri-dishes with leaf disk cabbage 25 ± 2 C and 85 ±5 % RH. Five individuals / dish, Twenty-five /concentration. The fungi were applied in a suspension containing 1x 10^{3}, 1 x 10^{4} and 1x 10^{5} spores / ml. in the control treatment 1 ml. of sterilized water was added to the leaves disks. The mortality of cabbage worm was observed daily.

Field application:
The application of *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* In Cabbage fields was applied in Giza, Governorate during October (2014-2015) and during October (2015-2016). Cabbage plants were sprayed with the fungal suspensions to control cabbage worm, *Pieris rapae* L. Conidio spores of *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* were applied to cabbage plants by using the concentrations of 1x 10^{3}, 1x 10^{4} and 1 x 10^{5} spores / ml.

An area was divided into four plots each plot divided into five replicates (two replicates were treated with *Beauveria bassiana*, *Metarhizium anisopliae* and the others were treated with *Verticillium lecanii*) and one replicate was control. Agriculture practices were performed, without any pesticide treatments in the plots. The suspensions were sprayed three times every week before the spray counted the live insects of cabbage worm, *Pieris rapae* L. Per plant / replicate. The suspension sprayed early in the morning.

- The percent of reduction were calculated according to Handerson and Tilton formula.
- Statistical analysis

Data were analyzed by analysis of variance (one ways classification ANOVA) and followed by a least significant difference (L.S.D at 5%) (SAS Institute Inc., 2003).

RESULTS

Three concentrations of three isolates *B. bassiana*, *M. anisopliae* and *V. lecanii* were evaluated against cabbage worm, *Pieris rapae* L. under laboratory and field conditions.

Effect of *B. bassiana*, *M. anisopliae* and *V. lecanii* on cabbage worm, *Pieris rapae* L. under laboratory conditions:
The data in Table (1) indicated that *B. bassiana*, *M. anisopliae* and *V. lecanii* have not effect on cabbage worm, *Pieris rapae* L. after two days from treatment.

Table (1): Effect of *Beauveria bassiana, M. anisopliae* and *Verticillium lecanii* on Cabbage worm, *Pieris rapae* L. under laboratory conditions:

Table (1): % Mortality of *Pieris rapae* L. larvae infection with *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* at 25 ± 2°C and 85 ±5 % RH

After treatment	Cont.	*B. bassiana*			*M. anisopliae*			*V. lecanii*		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	2.2	7.2	10.7	1.7	3.0	4.2	1.5	1.5	2.3
4th	0.0	4.3	18.3	22.2	4.0	10.2	15.0	3.3	6.0	10.0
5th	0.0	23	24.0	30.3	14.2	21.0	23.3	10.0	11.3	19.0
6th	0.0	24.2	35.0	38.0	19.0	22.0	30.0	15.0	16.0	23.2
7th	0.0	41.0	44.3	46.0	32.0	35.0	43.0	27.2	27.0	33.0
8th	0.0	44.7	45.9	53.2	40.0	40.0	45.2	30.0	33.0	37.2
9th	0.0	65.4	70.0	75.0	45.0	55.7	67.0	33.4	40.0	43.0
10th	5.0	70	72.4	80	60.0	66.2	75.7	40.0	45.2	55.2

Mortalities are occurred in the third day. Thus, it is clear that the entomopathogenic fungi weather *B. bassiana*, *M. anisopliae* or *V. lecanii* have a latent effect not acute. The percent of mortalities are increased gradually and reached to the maximum in the tenth day from treatment. Data also showed a positive correlation between concentrations of fungi and the percentage of *Pieris rapae* L. larvae mortality. The percent of mortalities ranged between 70 to 80, 60 to 75.7 and 40.0 to 55.2 % with *B. bassiana, M. anisopliae* and *V. lecanii*, respectively, in the tenth day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae* and *V. lecanii*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation were 70, 72.4 and80 %, respectively. The corresponding results with *V. lecanii* isolation were 40.0, 45.2 and 55.2 %, respectively. This result compatible with (Ogarkov and Ogarkova, 2000)

Table (2): % Mortality of *Pieris rapae* L. Pupae infection with *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* at 25 ± 2 °C and 85 ±5 % RH

After treatment	Cont.	*B. bassiana*			*M. anisopliae*			*V. lecanii*		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	2.0	5.5	10.7	1.2	3.1	4.5	1.3	1.6	2.0
4th	0.0	3.3	16.0	22.2	3.0	10.7	15.5	3.3	5.2	9.0
5th	0.0	20	22.0	30.3	14.5	20.0	23.7	10.0	12.0	18.0
6th	0.0	22.0	30.0	38.0	18.0	23.0	32.5	16.0	16.0	24.0
7th	0.0	40.0	41.7	46.0	32.3	36.0	44.4	27.7	28.0	34.0
8th	0.0	42.3	45.0	53.2	41.0	43.0	47.3	32.0	34.0	38.2
9th	0.0	62.0	65..0	72.0	46.0	56.5	68.0	34.4	43.0	47.0
10th	0.0	65.0	70.0	76.0	58.0	68.5	70.1	42.0	46.0	60.0

Also, mortalities are occurred in the third day. The percent of mortalities are increased gradually and reached to the maximum in the tenth day from treatment. Data also showed a positive correlation between concentrations of fungi and the percentage of *Pieris rapae* L. Pupae mortality. The percent of mortalities ranged between 65 to 76, 58 to 70.1 and 42.0 to 60 % with *B. bassiana, M. anisopliae* and *V. lecanii*, respectively, in the tenth day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae* and *V. lecanii*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation were 65, 70 and76 %, respectively. The corresponding results with *V. lecanii* isolation were 42.0, 46.0 and 60.0 %, respectively

Table (3): Average number of *Pieris rapae* L. on Cabbage sprays with *B. bassiana, M. anisopliae* and *V. lecanii* in season 2014-2015

Before spray	Number of alive individuals										L.S.D
	Cont.	*B. bassiana*			*M. anisopliae*			*V. lecanii*			
		C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	
1st	94.2± 5.0^a	85± 5 ^{a}	90± 5 ^{a}	95± 5 ^{a}	90± 10 ^{a}	90.3± 10 ^{a}	90± 10 ^{a}	93.3± 101 ^{a}	90± 10 ^{a}	92.0± 7 ^{a}	10.8
2nd	100± 5.0^a	68.3± 3.5^b	66.7± 3.5^b	65.7± 3.5^b	73.3± 3	70.7± 2 ^{b}	66.7± 3 ^{b}	75± 5^b	73.3± 5^b	70.3± 2^b	15.3
3rd	102± 6.5^a	35± 5^b	37.0± 5. ^{b}	38.0± 5. ^{b}	40.3± 3.6^b	36.0± 5.0^b	37.7± 5.5^b	50± 20^b	48± 5^b	40± 5.3^b	25. 4
4th	103± 5.0^a	7.7± 1^b	6± 3.5^b	6± 3.9^b	6± 1^b	7.0 ± 1.7^b	7.0± 1.5^b	8.0± 1.5^b	7.0± 1.2^b	6.5± 1.5^b	8.63
Percent of reduction	-------	65.9	69.4	70.9	50.3	55.4	66.0	48.2	50.2	55.5	-------

Field application:

Season (2014-2015):

During (2014-2015) season (Table 3) showed that there are significant difference between 1st (c1) and 2nd (c2) spores concentrations and control after the first application in all parts, the differences appear gradually after the second and third application. On the other hand the third concentration (C3) in *Beauveria Bassiana* was the best concentration against *Pieris rapae* L. followed by the third concentration in *M. anisopliae* and the third concentration in *V. lecanii*. This means that the third concentration in *Beauveria Bassiana* was the best concentrations against *Pieris rapae*.

Season (2015-2016):

During (2015-2016) season (Table 4) showed that there are significant difference also between 1^{st} (c1) and 3^{rd} (c2) spores concentrations and control after the first application in all parts, the differences appear gradually after the second and third application. On the other hand the third concentration (C3) in *B. Bassiana* was the best concentration against *Pieris rapae* followed by the third concentration in *M. anisopliae* and the third concentration in *V. lecanii*. The Percent of reduction in all treatment was ranged between 48.5 to 75.8% in all concentrations.

Table (4): Average number of *Pieris rapae* L. on Cabbage sprays with *B. bassiana*, *M. anisopliae* and *V. lecanii* in season 2015-2016

Before spray	Number of alive individuals										L.S.D
	Cont.	*B. bassiana*			*M. anisopliae*			*V. lecanii*			
		C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	
1^{st}	97.2± 5.0^{a}	87± 5^{a}	93± 5^{a}	90± 5^{a}	92± 10^{a}	95.3± 10^{a}	95± 10^{a}	90.3± 101^{a}	92± 10^{a}	95.0± 7^{a}	11.5
2^{nd}	99± 5.0^{a}	67.3± 3^{b}	66.0± 3.5^{b}	65.0± 3^{b}	69.0± 2^{b}	70.0± 2^{b}	66.0± 3^{b}	76± 3^{b}	74.0± 5^{b}	69.3± 2^{b}	14.5
3^{rd}	100± 6^{a}	38± 5^{b}	35.0± $5.^{b}$	32.0± $5.^{b}$	40.0 ± 3.6^{b}	36.0± 5.0^{b}	37.0± 5.5^{b}	52± 1^{b}	50± 3^{b}	39± 5^{b}	24. 2
4^{th}	101± 5.0^{a}	7.5± 2^{b}	7± 3^{b}	6± 2^{b}	8.0± 1^{b}	7.0 ± 2^{b}	7.0± 2^{b}	8.2± 3^{b}	7.0± 3^{b}	6.7± 2^{b}	8.7
Percent of reduction	-------	66.0	70.0	75.0	52.0	54.5	66.0	48.0	50.0	53.0	-------

These results confirmed that *B. Bassiana*, *M. anisopliae* and *V. lecanii* isolates are promising agents for Cabbage worm control in the field.

DISCUSSION

Beauveria bassiana, Metarhizium anisopliae and *Verticilliun lecanii* are naturally fungi that are found in the soil of most fields, Mohamed Abdel-Raheem,2015, Mohamed Abdel-Raheem, *et al.*, 2016, Magda and Abdel-Raheem, 2015, Sabry, *et al*., 2011and Saleh, *et al.*, 2016. These fungi are entomopathogenic which causing disease to insects. Fungal infection begins when conidia (asexual spores, the seeds of a fungus) attach to insect's cuticle, the spores germinate and penetrate the insect's skin and enter the host. *B. bassiana* was the most effects on the larvae and Pupae of *Pieris rapae* than *M. anisopliae* and *V. lecanii*. Sabbour &Abdel-Raheem, 2016.

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