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Entomopathogenic Fungi against Cabbage Aphids, *Brevicoryne brassica* L.

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ABSTRACT

The present investigation was carried out during two successive Cabbage seasons (2016-2017 and 2017- 2018), to study the impact of entomopathogenic fungi on *Brevicoryne brassica L.* The aphid Populations were evaluated in the field early in the season in December 2016 which began to appear on cabbage plants. Thereafter number of aphids increased gradually to reach a peak of abundance during December 2016 & 2017 and January 2017 & 2018). Three concentrations were used (1 x10^5, 1 x 10^6 and 1 x 10^7 spores/ ml.). Under laboratory conditions, the results showed that *V. lecanii*, *M. anisopliae*, and *B. bassiana* have high toxicity and mortality rates occurred after 3rd day from treatment. The maximum percent of mortality (100 %) occurred after the 10th day from treatment with the 3rd concentration in *V. lecanii*. The 3rd concentration (1 x 10^7spores/ ml.) was highly toxic in *V. lecanii*, *B. bassiana*, and *M. anisopliae* to the adult of *Brevicoryne brassica L.* compared with the other two concentrations. Under field conditions, the third concentration (1 x 10^7) also, was the best concentration against *Brevicoryne brassica L.* after the third application in *V. lecanii*, *B. bassiana*, and *M. anisopliae.* The percent of reduction was ranged between 93.3 to 99.2% and 92.0 to 97.7 in the high concentrations, in the two seasons, respectively. *V. lecanii* and *B. bassiana* were highly effective than *M. anisopliae* against *Brevicoryne brassica L.* These results confirmed that *V. lecanii*, *B. bassiana*, and *M. anisopliae* isolates are promising agents for *Brevicoryne brassica L.* control in the field.

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. Fortunately, all these pests are susceptible to formulations based on entomopathogenic bacteria *Bt* of pathotype A (Cannon, 1996). 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). Modern exploitation of fungi as inundative insecticides began in the 1960s and several products based on *Beauveria bassiana* were used for control of numerous pests in the People's Republic of China (Feng *et al.*, 1994) and the Colorado potato beetle in the former USSR (Ferron *et al.*, 1991). *Metarhizium anisopliae* has potential against several pest species and is being used commercially in Brazil for control of spittlebugs in sugarcane (Wraight and Roberts, 1987). Also, uses in Egypt by (Saleh, et al., 2016, Abdel-Raheem and Lamya Ahmed Al-Keridis, 2017, Mohamed Abdel-Raheem, 2020 [a&b], Abdel-Raheem, 2019, Abdel-Raheem, *et al*., 2016_a, b& c, 2019, 2020a&b) against Some Insect Pests such as *Rhynchophorus ferrugineus*, *Bemisia tabaci*, *Aphis gossypii*, *Pegomyia mixta*, and *Pieris rapae. 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,* and *Verticillium*. 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 concentration of fungal suspension should be increased 5-10-fold (Andreeva and Shternshis, 1995). *Conidiobolus thromboides* (representative of the phylum Zygomycota), isolated from diseased pea aphids in the Novosibirsk region (Makhova and Rakshaina, 1980), was used for development of preparation designated as Pyriformin. The results of Pyriformin tests demonstrated the potential of *C. thromboides* for control of *T. vaporariorum*, *Myzus persicae*, *Aphis gossypie*, *Macrosiphum euphorbia*, *Aulacorthum solani*, *Brevicoryne brassicae* in glasshouses (Lobanova *et al*., 1989). The advantage of this fungus is a low relative humidity requirement (about 60% RH). In addition, Phytoverm® was also shown to be very effective against main pests of crops under greenhouse condition. The mortality of *A. gossypii* and *A. solani* after 3 days of application with 0.2% Phytoverm® suspension was very high under the conditions of Central Siberian Botanical Garden (Novosibirsk) (Andreeva and Shternshis, 2005).

In Egypt, many authors studied the impact of entomopathogenic fungi against some pests 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, 2015 a &b, Salem and Abdel-Raheem, 2015, and Salem *et al*., 2015.

MATERIALS AND METHODS

Fungi Culture:

Fungi: (*Verticillium lecanii*, *Metarhizium anisopliae,* and *Beauveria bassiana*) 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 days old culture (PDA) media. The suspensions were filtered through cheesecloth 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 1×10^{5}, 1×10^{6} and 1×10^{7} spores / ml.

Laboratory Inoculation:

The aphids were transferred to the Laboratory from the field and put it in Petridishes with leaf disk cabbage 25 ± 2 ˚C and 70 ±5 % RH. 5 individuals / dish, Twenty-five /concentration. The fungi were applied in a suspension containing 1 x 10 ^{5}, 1 x 10^{6,} and 1x 10 ^{7} spores/ml. in the control treatment, 1 ml. of sterilized water was added to the leaves disks. The mortality of aphids was observed daily. The mortality was corrected using Abbott's formula (Abbott, 1925).

Field Application:

The application of *Verticillium lecanii*, *Metarhizium anisopliae,* and *Beauveria bassiana* In Cabbage fields was applied in Giza (Elwarraq region) Governorate during December (2016-2017) and during December (2017-2018). Cabbage plants were sprayed with the fungal suspensions to control *Brevicoryne brassica* L.

Spores of *Verticillium lecanii*, *Metarhizium anisopliae,* and *Beauveria bassiana* were applied to cabbage plants by using the concentrations of 1 x 10 ^{5}, 1x 10 ^{6,} and 1 x 10 ^{7} spores/ml.

An area was divided into three plots each plot divided into four replicates (the replicates were treated with *Verticillium lecanii*, *Metarhizium anisopliae,* and *Beauveria bassiana*) and one replicate was controlled. Agriculture practices were performed, without any pesticide treatments in the plots. The suspensions were sprayed every week for three weeks before the spray counted the live insects of *Brevicoryne brassica* L. per leaf/replicate. The suspension sprayed early in the morning.

The percent of reduction were calculated according to Handerson and Tilton formula, (1955).

Statistical Analysis:

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

RESULTS

Three concentrations of three isolates *V. lecanii*, *M. anisopliae,* and *B. bassiana* were evaluated against *Brevicoryne brassica* L. under laboratory and field conditions.

Effect of *V. lecanii*, *M. anisopliae* and *B. bassiana* on *Brevicoryne brassica* L under Laboratory Conditions:

Mortalities induced the third day. The percent of mortalities are increased gradually and reached the maximum on the tenth day after treatment (Table 1). Data also showed a positive correlation between concentrations of fungi and the percentage of aphids mortality. The percent of mortalities ranged between 81.0 to 100, 64.0 to 85.0 and 70.0 to 90.0 % with *V. lecanii, M. anisopliae,* and *B. bassiana,* respectively, in the tenth day after treatment. This means that *V. lecanii* isolation is more effective than *M. anisopliae* and *B. bassiana*.

Season (2016-2017):

During (2016-2017) season (Table 2) showed that there are significant differences between 1st and 2nd spray in all concentrations after the first application in all parts, the differences appear gradually after the second and third applications. On the other hand, the 3rd concentration (C_3) in *V. lecanii* was the best concentration against *Brevicoryne brassica* L. followed by the 3rd concentration in *Beauveria bassiana* and the third concentration in *M. anisopliae*.

Table 1: Percent mortality of *Brevicoryne brassica* L. treated with *V. lecanii*, *M. anisopliae* and *B. bassiana* at 25 ± 2 ˚C and 70 ±5 % RH.

Date	Control	*V. lecanii*			*M. anisopliae*			*B. bassiana*		
		C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	7.2	10.3	17.5	4.5	4.9	7.0	5.0	7.0	12.0
4th	0.0	15.5	21.5	28.3	7.0	13.0	18.0	14.0	19.0	23.0
5th	0.0	26.3	33.9	40.0	18.0	25.0	29.0	22.0	33.0	33.0
6th	0.0	27.4	47.8	52.8	23.0	27.0	38.0	28.0	45.0	48.0
7th	0.0	42.9	50.2	64.2	37.0	45.0	58.0	40.0	45.5	52.0
8th	0.0	52.6	71.5	79.5	43.0	62.0	67.0	49.0	63..0	69.0
9th	0.0	77.5	80.0	98.0	52.0	65.0	78.0	57.0	75.0	80.0
10th	0.0	81.0	97.2	100.0	64.0	83.0	85.0	70.0	80.0	90.0

C_1 (1 x 10^5), C_2 (1x 10^6) and C_3 (1 x 10^7) spores / ml

Table 2: Average number of *Brevicoryne brassica* L on Cabbage sprays with *Verticillium lecanii*, *Metarhizium anisopliae,* and *Beauveria bassiana* in season 2016-2017.

Before spray	Number of alive individuals										L.S.D
	Control	*Verticillium lecanii*			*Metarhizium anisopliae*			*Beauveria bassiana*			
		C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3	
1st	96.3 ± 5.2^a	97.0 ± 10.3^a	90 ±7^a	90.5 ± 6.4^a	100 ±7^a	91.0 ±3.3^a	90 ± 6^a	97 ± 5^a	95 ± 3^a	85 ± 5.3^a	9.6
2nd	104 ± 5.7^a	80 ± 9^b	71.0 ± 5.4^b	72.0 ± 2.0^b	74.0 ±11.4^b	54.5 ±10.4^b	64.7 ±18.5^b	65.5 ±10.5^b	67.5 ±10.4^b	52 ±14.3^b	12.0
3rd	114.3 ± 10.3^a	49 ± 18^b	24 ± 2^b	17.0 ± 5.2^b	28.0 ±11.0^b	29.4 ±5.5^b	25.9 ±11^b	32 ± 12^b	37.3 ±11.2^b	23.2 ±5.2^b	26.0
4th	105.7 ± 6.5^a	12.4 ± 1.3^b	3.7 ± 1.3^b	1.7 ± 1.2^b	11 ± 2.0^b	10 ±1.2^b	3.9 ± 1.3^b	6.9 ± 1.7^b	7 ± 3.3^b	6.9 ±3.9^b	8.7
Percent of reduction	-------	90.5	97.7	99.2	85.9	90.0	93.3	87.79	91.5	96.3	-------

C_1 (1 x 10^5), C_2 (1x 10^6) and C_3 (1 x 10^7) spores / ml

Season (2017-2018):

During (2017-2018) season (Table 3) showed that there are significant differences also between 1st and 2rd spray in all concentrations after the first application in all parts, the differences appear gradually after the second and third applications. On the other hand, the third concentration (C_3) in *V. lecanii* was the best concentration against *Brevicoryne brassica* L. followed by the third concentration in *Beauveria Bassiana* and the third concentration in *M. anisopliae.* The Percent of reduction in all treatment was ranged between 90.0 to 97.7 % in all concentrations.

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

Table 3: Average number of *Brevicoryne brassica* L. on Cabbage sprays with *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* in season 2017-2018.

Before spray	Number of alive individuals										L.S. D
	Control	*Verticillium lecanii*			*Metarhizium anisopliae*			*Beauveria bassiana*			
		C1	C2	C3	C1	C2	C3	C1	C2	C3	
1st	114.5 ±6.6^{a}	130.2± 2.2 ^{a}	129.2 ±6.2 ^{a}	104.0 ±13.5 ^{a}	134 ±5^{a}	133.2± 15.3 ^{a}	125.5± 13.2 ^{a}	117.5± 15.5 ^{a}	116.8± 15.2 ^{a}	112.2 ±12.2 ^{a}	18.5
2nd	115 ± 10^{a}	83.3 ±11.0^{b}	62.8 ±9.9 ^{b}	34 ± 8^{b}	90 ±1.2^{b}	72 ±12^{b}	70.2 ±2.7^{b}	65.5 ±11.9^{b}	57.5 ±15.2^{b}	38.6 ±5.5^{b}	30.4
3rd	114.4 ± 3.4^{a}	47.7 ± 4.7^{b}	27.7 ±6.2^{b}	20.9 ±2.8^{b}	42 ±10^{b}	40 ±11^{b}	29.5 ±7.5^{b}	35.5 ±4.5^{b}	30.6 ±5.8^{b}	24.6 ±2.3^{b}	16.5
4th	113.5 ± 10.7^{a}	9 ± 5 ^{b}	7.0 ±1.2 ^{b}	2.7 ± 2.3 ^{b}	9.9 ±0.3 ^{b}	5.9 ±2.9 ^{b}	4.5 ±0.5 ^{b}	8.9 ±1.2 ^{b}	6.9 ±1.3 ^{b}	4.0 ±5.0 ^{b}	14.6
Percent of reduction	----	93.0	94.5	97.7	90.0	91.0	92.0	91.0	93.0	95.0	------

C_1 (1 x 10^{5}), C_2 (1x 10^{6}) and C_3 (1 x 10^{7}) spores / ml

DISCUSSION

This study obtained the percent of mortalities with all concentrations (C_1, C_2, and C_3) of *V. lecanii* isolation which was 81.0, 97.2 and100 %, respectively. The corresponding results with *B. bassiana* isolation were 70.0, 80.0, and 90.0 %, respectively. This result compatible with (Ogarkov and Ogarkova, 2000, Ismail, et al., 2016 and Abdel-Raheem, et al., 2009, 2015 a &b, 2016 a, b, & c, 2019) they found that The most common fungi used for insect and mite control belong to the genera *Beauveria, Metarhizium, Paecilomyces, Verticillium, Aschersonia, and Conidiobolus*. Treatment with suspensions of *Verticillium (Lecanicillium) lecanii* (Verticillin®) and *Beauveria bassiana* (Boverin®) caused management of *Brevicoryne brassica L.* and *B. tabaci*. (Maniania, 1991 and Mohamed Abdel-Raheem 2015, 2020[b]) who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. Zaki (1998) reported that *B. bassiana* as entomopathogenic fungi showed high effects on the aphid *Aphis craccivora* and the whitefly *B. tabaci* infesting cucumber. (Gindin *et al.*, 2000 and Ismail and Abdel-Raheem, 2010), reported that *V. lecanii* caused higher virulence in the early stages of whitefly and reduced with older instars. Abdel-Baky *et al.* (2005) mentioned that entomopathogenic fungi caused good mortality to whitefly.

The third concentration (C_3) in *V. lecanii* was the best concentration against *Brevicoryne brassica* L. followed by the third concentration in *Beauveria Bassiana* and the third concentration in *M. anisopliae*. These results are in agreement with those found by (Pineda *et al*., 2007 and Sabry, et al., 2011) who stated that survival of Aphids nymphs decreased by 22 and 34% after the first and second fungal applications, respectively, in one trial, and by 72 and 81% in the other trial. This means that the third concentration in *V. lecanii* was the best concentration against *Brevicoryne brassica*.

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