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Virulence of Some Entomopathogenic Fungi on Cabbage Aphids, *Brevicoryne brassica* L.

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Abstract: The present investigation was carried out during two successive Cabbage seasons (2014-2015 and 2015 - 2016), to study the virulence of entomopathogenic fungi on *Brevicoryne brassica L.* The aphid populations were evaluated in the field early in the season in December 2014 which began to appear on cabbage plants. Thereafter the number of aphids increased gradually to reach a peak of abundance during December 2014 and January 2015. Three concentrations were used (1 x10^2, 1 x 10^3 and 1 x 10^4 spores/ ml.). Under laboratory conditions the results showed that *V. lecanii, M. anisopliae* and *B. bassiana* have a latent toxicity because mortalities were occurred after the third day from treatment. The maximum percent of mortality (100 %) occurred after the 10^{th} day from treatment with the 3^{rd} concentration in *V. lecanii*. The 3^{rd} concentration (1 x 10^4spores/ ml.) was highly toxic in *V. lecanii, M. anisopliae* and *B. bassiana* to the adult of *Brevicoryne brassica L.* compared with the other two concentrations. Under field conditions the third concentration (1 x 10^4) also, was the best concentration against *Brevicoryne brassica L.* after the third application in *V. lecanii, M. anisopliae* and *B. bassiana.* The percent of reduction ranged between 89.4 and 96.3% in all concentrations. *V. lecanii* and *M. anisopliae* were highly effective than *B. bassiana* against *Brevicoryne brassica* L. These results confirmed that *V. lecanii, M. anisopliae* and *B. Bassiana* isolates are promising agents for *Brevicoryne brassica* L. control in the field.
Key words: Entomopathogenic fungi, *Brevicoryne brassica* L..

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 [1] according to this susceptibility [2]. Naturally occurring entomopathogens are important regulatory factors in insect populations. Many species are employed as biological control agents of insect pests [3]. Fungi are important in the natural regulation of many insect pests and pest populations are often decimated in widespread epizootics [4]. They normally invade via the external cuticle and need not be ingested to initiate disease as is the case with most other insect pathogens. This makes them prime candidates for use against plant sucking insects such as aphids. Although fungi have great potential for development as microbial control agents, only a few have been used on an operational scale. 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 [5] and the Colorado potato beetle in the former USSR [6]. *Metarhizium anisopliae* has

potential against several pest species and is used commercially in Brazil for control of spittle bugs in sugarcane [7]. *Paecilomyces fumosoroseus* and *Verticillium lecanii* are commercially produced and used for control of whiteflies and aphids in greenhouses in Europe and the USA[8]. *Metarhizium anisopliae* has recently received registration in the United States for control of various ticks, beetles, flies, gnats, thrips and termites. Unfortunately, to date there are no fungi registered for insect control in Canada although there may well be a market for such products particularly for control of insect pests in greenhouses where environmental variability is not a significant issue. 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[9, 10]. The concentration of fungal suspension should be increased 5-10 fold[11]. *Conidiobolus thromboides* (representative of the phylum Zygomycota), isolated from diseased pea aphids in the Novosibirsk region[12], 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[13]. 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) [14].

In Egypt many authors studied the impact of entomopathogenic fungi against some insect such as *Spodoptera littoralis*[15], Aphids[16], *Cassida vittata* Vill. and *Scrobipalpa ocellatella* Boh[17, 18], and *Spodoptera littoralis, S. exigua, Aphis craccivora* and *Bemisia tabaci* (Genn**.)**[19-27].

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 fungi were incubated 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 were 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 10^2, 1x 10^3 and 1 x 10^4 spores / ml.

Laboratory inoculation:

The aphids were transferred to the Lab. from the field and placed in Petri-dishes with leaf disk cabbage 20 ± 2 ˚C and 65 ±5 % RH. Five individuals/dish, Twenty-five /concentration. The fungi were applied in a suspension containing 1 x 10^2, 1 x 10^3 and 1x 10^4 spores / ml. in the control treatment 1 ml. of sterilized water was added to the leaves disks. The mortality of aphids was observed daily.

Field application:

The application of *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* In Cabbage fields was applied in El-Behira Governorate during December (2014 - 2015) and during December (2015 - 2016). Cabbage plants were sprayed with the fungal suspensions to control *Brevicoryne brassica* L. Conidio spores of *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* were applied to cabbage plants by using the concentrations of 1 x 10^2, 1x 10^3 and 1 x 10^4 spores / ml.

An area was divided into four plots each plot consists of five replicates (two replicates were treated with *Verticillium lecanii*, *Metarhizium anisopliae* and the others were treated with *Beauveria bassiana*) and one

replicate was used as control. Culture 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 *Brevicoryne brassica* L. per leaf / replicate. The suspension was sprayed early in the morning.

Statistical analysis

- Data of the current results was statistically processed by analysis of variance, (One – way ANOVA using SAS statistical package programme (SAS Institute Inc. 2003). The percent of reduction was calculated according to Handerson and Tilton formula. Data are presented as means and the statistical differences were indicated using the least significant differences at a probability level of 5%

Results

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

1- Effect of *V. lecanii*, *M. anisopliae* and *B. bassiana* on *Brevicoryne brassica* L. under laboratory conditions:

The data in Table (1) indicated that *V. lecanii*, *M. anisopliae* and *B. bassiana* have no effect on *Brevicoryne brassica* L after two days from treatment.

Table (1): Effect of *Verticillium lecanii*, *M. anisopliae* and *Beauveria bassiana* on Cabbage Aphid survival under laboratory conditions:

Table (1): % Mortality of *Brevicoryne brassica* L. infection with *V. lecanii*, *M. anisopliae* and *B. bassiana* at 20 ± 2 ˚C and 65 ±5 % RH.

Date	Cont.	*V. lecanii*			*M. anisopliae*			*B. bassiana*		
		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	4.0	9.0	15.0	2.0	3.0	5.0	2.0	2.0	10.0
4th	0.0	14.0	19.0	25.0	5.0	9.0	14.0	10	15.0	17.0
5th	0.0	27.0	30.0	38.0	15.0	20.0	25.0	20.0	27.0	30.0
6th	0.0	29.0	45.0	48.0	21.0	20.0	35.0	25.0	40.0	47.0
7th	0.0	43.0	48.0	60.0	35.0	41.0	55.0	37.0	45.0	59.0
8th	0.0	50.0	66.0	75.0	41.0	59.0	63.0	45.0	60..0	63.0
9th	0.0	68.0	75.0	95.0	47.0	70.0	75.0	53.0	73.0	76.0
10th	0.0	80.0	95.0	100.0	62.0	80.0	83.0	65.0	79.0	85.0

Mortalities occurred in the 3rd day. Thus, it is clear that the entomopathogenic fungi weather *V. lecanii*, *M. anisopliae* or *B. bassiana* have a latent effect not acute. The percent of mortalities are increased gradually and reached to the maximum in the 10th day from treatment. Data also showed a positive correlation between concentrations of fungi and the percentage of aphids mortality. The percent of mortalities ranged between 80.0 to 100, 62.0 to 83.0 and 65.0 to 85.0 % with *V. lecanii*, *M. anisopliae* and *B. bassiana*, respectively, in the 10th day after treatment. This mean that *V. lecanii* isolation is more effective than *M. anisopliae* and *B. bassiana*.

A - Season (2014-2015):

During (2014-2015) season (Table 2) showed that there are significant differences 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 3rd concentration (C3) in *V. lecanii* was the best concentration against *Brevicoryne brassica* L. followed by the 3rd concentration in *M. anisopliae* and the third concentration in *Beauveria bassiana*

Table (2): Average number of *Brevicoryne brassica* L on Cabbage sprays with *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* in season 2014-2015.

B - Season (2015-2016):

During (2015-2016) season (Table 3) showed that there are significant differences also between 1st (c1) and 3rd (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 *V. lecanii* was the best concentration against ***Brevicoryne brassica*** L. followed by the third concentration in *M. anisopliae* and the third concentration in *Beauveria Bassiana*. The Percent of reduction in all treatment was ranged between 91.0 to 97.0% in all concentrations.

Table (3): Average number of *Brevicoryne brassica* L. on Cabbage sprays with *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* in season 2008-2009.

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	115.2 ±7.5[a]	120.5± 6.5 [a]	128.3± 7.6 [a]	102.2± 14.2 [a]	120±7[a]	99.4± 20.2 [a]	113.2± 13.2 [a]	103.5± 15.5 [a]	116.7± 15.2 [a]	113.2± 12.2 [a]	17.6
2nd	117± 9[a]	61.3± 10.0[b]	81.7± 10.4	31 ± 9[b]	89±1.0[b]	71±11[b]	68.2± 2.8[b]	63.5± 11.2[b]	56.5± 15.2[b]	36.6± 5.6[b]	31.5
3rd	115.2 ± 4.2[a]	45.2± 5.5[b]	26.7± 5.8[b]	18.5± 2.2[b]	40±11[b]	41±11[b]	27.5± 8.5[b]	33.5± 4.5[b]	26.6± 5.8[b]	21.6± 2.6[b]	16.8
4th	114.7 ± 11.2[a]	8 ± 2	6.5±1.0	2.2 ± 2.2	9.5±0.5	5.5±2.7	3.5±0.5	8.8±1.2	6.6± 1.3	4.2± 3.0	14.9
Percent of reduction	----	92.0	94.0	97.0	91.0	94.0	97.0	91.0	94.0	96.0	------

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

Discussion

This study obtained the percent of mortalities with all concentrations (C_1, C_2 and C_3) of *V. lecanii* isolation were 80.0, 95.0 and100 %, respectively. The corresponding results with *B. bassiana* isolation were 65.0, 79.0 and 85.0 %, respectively. Our results are in agreement with those in some previous studies [28-31]. Those results showed 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®) has resulted in efficient control of aphids and whitefly. [32, 33] who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. [34]reported that *B. bassiana* as an entomopathogenic fungi showed high effects on the aphid *Aphis craccivora* and the white fly *B. tabaci* infesting cucumber. [35] and [36] reported that *V. lecanii* caused higher virulence in the early stages of whitefly and reduced with older instars. [37] mentioned that entomopathoginic fungi caused mortality to whitefly.

The 3rd concentration (C3) in *V. lecanii* was the best concentration against *Brevicoryne brassica* L. followed by the 3rd concentration in *M. anisopliae* and the third concentration in *Beauveria Bassiana*. These results are in accordance with those found by [38, 39] 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 concentrations against *Brevicoryne brassica* [40-48].

Conclusion

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

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