

## Biodiversity of Entomopathogenic Fungi Naturally Infecting Cabbage Aphid, *Brevicoryne brassicae* L.

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### ABSTRACT

Ten entomopathogenic fungi were recorded naturally associated with cabbage aphid. They were five hyphomycetes and five Zygomycetes (four Entomophthorales & one Mucorales). Six genera showed good epizootic beside their safety to plants. They were: *Panadora neoaphidis*, *Conidiobolus obscurus*, *Neozygites fresenii*, *Entomophthora planchoniana*, *Cladosporium cladosporioides* and *Verticillium lecanii*. Efficiency of these entomopathogenic fungi against *Brevicoryne brassicae* was evaluated under laboratory conditions. *P. neoaphidis* was the most efficient with  $LC_{50}$ :  $2.5417 \times 10^2$  conidia/ml then, *V. lecanii* which showed  $LC_{50}$  of  $3.052 \times 10^2$  conidia/ml followed by *C. obscurus* which showed  $LC_{50}$  of  $3.161 \times 10^2$  conidia/ml, then, *N. fresenii* which recorded  $LC_{50}$  of  $8.536 \times 10^2$  conidia/ml. Then, *C. cladosporioides* showed  $LC_{50}$  of  $23.271 \times 10^2$  conidia/ml, then at last comes *E. planchoniana* with  $LC_{50}$  of  $70.593 \times 10^2$ .

**Keywords:** *Brevicoryne brassicae*, entomopathogenic fungi

### INTRODUCTION

Cabbage aphid, *Brevicoryne brassicae* L. (Homoptera: Aphididae) is a serious specialist pest attacking Brassicaceae. It causes high reduction of plant growth, when populations are large; this can stunt or kill plants. Also, it is considered an important vector of plant virus transmission leading to high loss of crop yield. Problems resulting from the extensive and continuous use of the chemical insecticides press to develop alternative control strategies.

Entomopathogenic fungi are considered one of the most promising candidates in safe management of aphids because of their good epizootic. Moreover, they don't have to be ingested by the insect host but can invade through the insect cuticle (Boucias *et al.*, 1988) which make them suitable for controlling insects with sucking mouth parts (Tanada and Kaya, 1993; Hajek and St. Leger, 1994).

This study was oriented to survey and identify entomopathogenic fungi naturally associated with cabbage aphid, *B. brassicae* in Dakahlia Governorate and evaluate their virulence against cabbage aphid under laboratory conditions.

### MATERIALS AND METHODS

#### Survey of entomopathogenic fungi associated with *A. craccivora*:

The survey was carried out in Dakahlia Governorate. The survey was weekly conducted on cabbage plants from October 2016 to September 2017.

#### 1. Collection and incubation of alive aphid samples:

Alive aphids without and/or with any symptoms of fungal infection were collected in plastic bags and transferred to the laboratory and were individually reared on sterilized cabbage leaves in Petri-dishes prepared with moistened filter paper at  $25 \pm 2$  C and photoperiod of 16:8 hrs (L:D) for 10 days. Plant leaves were replaced twice a week. Dead insects were collected in sterilized vials for fungal identification.

#### 2. Examination of insect cadavers:

Cadavers of incubated insects or that collected directly from the farm were mounted with lactophenol blue and/or aceto-orcein and examined microscopically as soon as possible after death.

#### Isolation of the entomopathogenic fungi:

Aphid cadavers were crushed and suspended with 1ml sterilized water, then one drop of this fungal suspension was incubated in one of the following media:

- Autoclaved Sabouraud dextrose yeast extract agar (SADYA) [10g/l peptone, 40g/l dextrose, 10g/l yeast extract and 20g/l agar] and incubated at  $25 \pm 2$  C and  $80 \pm 5\%$  RH until further growth.
- Autoclaved Sabouraud dextrose yeast agar supplemented with egg yolk and milk [(SADYA), 80 ml/l egg yolk (4-5 eggs) and 120ml/l sterilizing milk] was used as specific media for cultivating entomophthorales (Papierok and Hajek, 1997).

After obtaining cultures of fungi, cultures were then purified using single spore or hyphal tip technique.

#### Identification of the entomopathogenic fungi:

The isolated fungi were identified based on the spore morphology according to the keys of Humber (1989 & 1997).

#### Rearing of cabbage aphid, *B. brassicae*:

The strain of *B. brassicae* was obtained from the farm of Faculty of Agriculture, Mansoura University, and had been known to be free from insecticidal contamination. Aphid strain was reared on cabbage plants (3-4 weeks old) planted in small pots (30 cm) and kept under plastic greenhouse conditions of  $25 \pm 5$  C,  $75 \pm 5$  RH and 16:8 L: D. Plants were changed as needed once or twice per week by artist's brush.

#### Preparation of series of different concentrations of fungal spore suspensions:

Spores of the tested fungi of 12 days old, were harvested by rinsing in sterilized 0.5% Tween 80. The spore suspensions were filtered through nylon mesh to get rid of mycelia. Then the spores were counted using a haemocytometer Neeubauer. Three concentrations;  $1 \times 10^3$ ,  $1 \times 10^4$  and  $1 \times 10^5$  of spore suspensions of each tested fungi were prepared.

#### Bioassay Study:

#### Evaluation of the tested fungi against cabbage aphid:

Cabbage leaves were sterilized by fast immersing in 70% alcohol then in sterilized water followed by 5% sodium hypochlorite for 90 seconds, then in three changes of sterile water (Clair *et al.*, 1997). Plant leaves were dried and transferred to 15 cm Petri – dishes. Ten adult aphids were sterilized by 1% sodium hypochlorite

then washed by sterile water and transferred to a Petri-dish containing cabbage leaves then sprayed with the tested fungal concentration and incubated at  $22 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  RH., and photoperiod 16:8 hrs L:D. Each concentration had 3 replicates and another three replicates sprayed only with water with 0.05% aqueous Tween 80 to be considered as control. Cabbage leaves were replaced by fresh ones after the first three days of the treatment to provide a source of nutrition. Observations were recorded daily and the experiment continued for seven days.

**Data analysis:**

At the end of the test period, the average of mortality percentages of *B. brassicae* was estimated and corrected using Abbott's formula (1925). The corrected mortality percentage of each extract was statistically

calculated according to Finney (1971). The corresponding concentration probit lines (LC-p lines) were estimated in addition to determination of LC50, LC90 and slope values.

**RESULTS AND DISCUSSION**

The present study recorded 10 fungal species infected cabbage aphid, *B. brassicae* from October (2016) to September (2017) as shown in Table 1. They belong to two main phylum, Ascomycota (five hyphomycetes\*) and Zygomycota (four Entomophthorales\*\* & one Mucorales\*\*\*). Six genera showed good epizootic beside their safety to plants. These genera were:

**Table 1. Fungal species naturally infected cabbage aphid, *B. brassicae* on cabbage plants from October (2016) to September (2017) in Dakahlia governorate.**

Fungi	Safety to the plant	Date
<i>Conidiobolus obscurus</i> **	Safe	March and April
<i>Panadora neoaphidis</i> **	Safe	November, December, January, February and March.
<i>Neozygites fresenii</i> **	Safe	December, January and February.
<i>Entomophthora planchoniana</i> **	Safe	December, January February and March.
<i>Cladosporium cladosporioidis</i> *	Safe	December, January, March, April, May and June.
<i>Verticillium lecanii</i> *	Safe	January, February and March
<i>Fusarium sp.</i> *	Not Safe	September, April, May, June and July.
<i>Aspergillus niger</i> *	Not Safe	May, June, July and August
<i>Mucor sp.</i> ***	Not Safe	March and April
<i>Alternaria sp.</i> *	Not Safe	May, Jun and July.

► ***P. neoaphidis*:**

Conidiophores are digitately branched at their apices. Primary conidia are clavate to obovoid, uninucleate with basal papillae discharged laterally from the spore axis, forcibly discharged by papillary eversion (Fig.1). Secondary conidia nearly globose and they are produced on primary conidia. Cystidia tapering toward bluntly pointed apex, thicker than hyphae at base. Freshly killed cadavers showing typically pale brown in color then turning a rusty red color upon desiccation.

► ***C. obscurus*:**

Primary conidia globose with hemispherical papilla emerging abruptly from the spore. Conidiophores are simple and unbranched (Fig.2). The mature resting spores spherical to subspherical and characterized by their thick cell walls. Cadavers were surrounded with few white mycelium growths.

► ***N. fresenii*:**

Conidiophores are simple unbranched. Primary conidium is nearly spherical to ovoid with a flattened basal papilla, varying in size and forcibly discharged (Fig.3). Secondary conidia are capilliconidia carried on capillary conidiophores arising from primary conidia, almond-shaped with a mucoid drop at the tip. Resting spores are black to smoky- gray in color arising from conjugation between two spherical gametangia. The mycosed aphids were typically dark brown to gray color.

► ***E. planchoniana*:**

Conidiophores are simple unbranched. Conidiogenous cells are club-shaped. Primary conidia are bell-shaped, with broad flat papilla and pointed apex, forcibly discharged (Fig.4). Secondary conidia are budding from the primary conidia, slightly smaller, non apiculate with more rounded papillae. Aphid cadavers typically buff initially then became brown in color upon sporulation.

► ***Cladosporium cladosporioidis*:**

Colonies are mostly olivaceous-green to olivaceous- brown. Conidiophores are branching acropleurogenously and bearing numerous conidial chains arising below septa, but without swellings and sympodial elongations. Conidia ellipsoidal to lemon shaped, mostly smooth-walled, rarely minutely verruculose, olivaceous-brown (Fig.5). Freshly killed cadavers were grey in color then becoming olivaceous-grey.

► ***Verticillium lecanii*:**

Phialides arranged in whorls or pairs. Conidia are cylindrical shaped with round apices carried in slime droplets on conidiogenous cells (Fig.6). Cadavers were surrounded with dense white mycelium growths.

**Evaluation of the efficiency of the tested fungi against cabbage aphid:**

Efficiency of the tested fungi against cabbage aphid, *B. brassicae* was shown in Table 2.

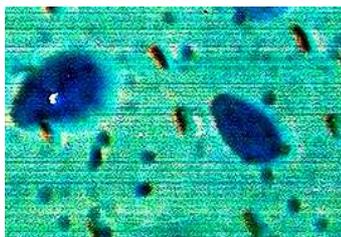


Fig. 1. Light micrograph showing primary ovoid conidia of *P. neoaphidis*.

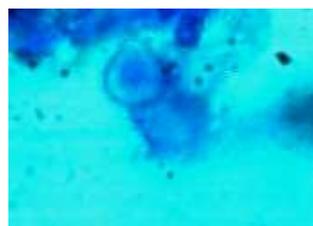


Fig. 2. Light micrograph showing primary globose conidia with hemispherical papilla of *C. obscurus*.



Fig. 3. Light micrograph showing almond-shaped capill-conidium with a mucoid apical droplet of *N. fresenii*



Fig. 4. Light micrograph showing unbranched conidiophores and primary conidia of *E. planchoniana*.

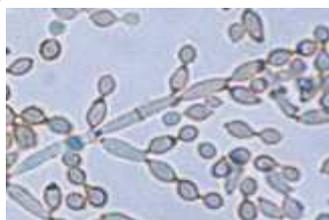


Fig. 5. Light micrograph showing branched conidiophores of *C. cladosporioides* bearing numerous conidial chains. Conidia ellipsoidal to lemon shaped.

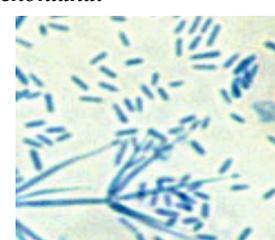


Fig. 6. Light micrograph showing mucoid conidial balls of *V. lecanii* formed apically on individual phialides.

Table 2. Efficiency of the tested fungi against cabbage aphid, *B. brassicae* under laboratory conditions of  $25 \pm 2C^{\circ}$ ,  $80 \pm 5\%$  RH.

Treatment	Conc. (Conidia /ml)	Mortality % at the end of treatment.	LC <sub>50</sub>	LC <sub>90</sub>	Slope	Toxicity Index
<i>P. neoaphidis</i>	1x10 <sup>3</sup>	66.67	2.5417x10 <sup>2</sup>	214.53x10 <sup>2</sup>	0.665	100
	1x10 <sup>4</sup>	83.33				
	1x10 <sup>5</sup>	96.67				
<i>V. lecanii</i>	1x10 <sup>3</sup>	56.67	3.052x10 <sup>2</sup>	1927.6x10 <sup>2</sup>	0.337	83.28
	1x10 <sup>4</sup>	70.00				
	1x10 <sup>5</sup>	80.00				
<i>C. obscurus</i>	1x10 <sup>3</sup>	63.33	3.161x10 <sup>2</sup>	531.108x10 <sup>2</sup>	0.576	80.40
	1x10 <sup>4</sup>	80.00				
	1x10 <sup>5</sup>	93.33				
<i>N. fresenii</i>	1x10 <sup>3</sup>	53.33	8.536x10 <sup>2</sup>	1367.106x10 <sup>2</sup>	0.581	29.78
	1x10 <sup>4</sup>	70.00				
	1x10 <sup>5</sup>	90.00				
<i>C. cladosporioides</i>	1x10 <sup>3</sup>	46.67	23.271x10 <sup>2</sup>	2924.6x10 <sup>2</sup>	0.414	10.92
	1x10 <sup>4</sup>	60.00				
	1x10 <sup>5</sup>	76.67				
<i>E. planchoniana</i>	1x10 <sup>3</sup>	36.67	70.593x10 <sup>2</sup>	14919.13x10 <sup>3</sup>	0.385	3.60
	1x10 <sup>4</sup>	53.33				
	1x10 <sup>5</sup>	66.67				

In spite of slow action of the tested fungi that didn't reveal activity against aphid adults during the first day of application, the mortality percent was clear after the third day of treatment then increased dramatically. Also, results indicated that *P. neoaphidis*

was the most effective against adults of *B. brassicae* with LC<sub>50</sub> of 2.5417x 10<sup>2</sup> conidia/ml and toxicity index at LC<sub>50</sub> of 100%, then, *V. lecanii* which showed LC<sub>50</sub> of 3.052x10<sup>2</sup> conidia/ml, and toxicity index of 83.28%, followed by *C. obscurus* which recorded LC<sub>50</sub> of

$3.161 \times 10^2$  conidia/ml and toxicity index 80.4 then, *N. fresenii* which showed  $LC_{50}$  of  $8.536 \times 10^2$  conidia/ml and toxicity index 29.78. Then, *C. cladosporioides* showed  $LC_{50}$  of  $23.271 \times 10^2$  conidia/ml and toxicity index 10.92, At last comes *E. planchoniana* with  $LC_{50}$  of  $70.593 \times 10^2$  and toxicity index of 3.6.

Previous data indicated that there was a clear variation of virulence of the tested fungi even when belong to the same orders or families and the insect host was the same. This is due to the pathogenicity genes of each fungal specie, which encode enzymes that allow fungus to overcome the host barrier (St. Leger, 1995).

The great diversity of entomopathogenic fungi attacking *B. brassicae* provides many promising candidates of insect microbial control. One of these candidates, is Entomophthorales which worth to be focused and studied to make the most of it.

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## التنوع البيولوجي للفطريات الممرضة للحشرات التي تصيب من الكرنب طبيعياً.

هبة يوسف السيد ابراهيم

معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى- الجيزة- مصر.

تم حصر الفطريات الممرضة للحشرات والتي تتواجد طبيعياً مع من الكرنب و تصيبه بداية من شهر اكتوبر 2016 وحتى سبتمبر 2017. وجد أن هناك عشرة أنواع من الفطريات تصيب من الكرنب طول العام. وقد تم عزل وتعريف هذه الفطريات ووجد أنها تنتمي لقبيلتين رئيسيتين هما الفطريات الأسكية (الخيضية) و الفطريات الزيجية (الانتومفثورالس والميوكورالس). وقد وجد من بينهم ستة أجناس لها قدرة كبيرة على افشاء المرض بين تجمعات من الكرنب علاوة على أمانها الحيوي على النبات وهى: *Panadora neoaphidis*, *Conidiobolus obscurus*, *Neozygites fresenii*, *Entomophthora planchoniana*, *Cladosporium cladosporioides*, *Verticillium lecanii*. البالغة لحشرة من الكرنب، فكان فطر *P. neoaphidis* الأكثر كفاءة حيث أظهر تركيز نصف مميت:  $2 \times 10^2 \times 2.5417$  كونيده/مل يليه فطر *V. lecanii* بتركيز نصف مميت  $2 \times 10^2 \times 3.052$  كونيده/مل ثم فطر *C. obscurus* بتركيز نصف مميت:  $2 \times 10^2 \times 3.161$  كونيده/مل. أيضاً، فطر *N. fresenii* الذي أظهر تركيز نصف مميت:  $2 \times 10^2 \times 8.536$  كونيده/مل يليه فطر *C. cladosporioides* بتركيز نصف مميت:  $2 \times 10^2 \times 23.271$  كونيده/مل وأخيراً فطر *E. planchoniana* بتركيز نصف مميت:  $2 \times 10^2 \times 70.593$  كونيده/مل.