CONTROLLING CABBAGE APHID (Brevicoryne brassicae L.) USING ISOLATED MYCOINSECTICIDES
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ABSTRACT
This study aims to isolate and identify entomopathogenic fungi and evaluate their virulence against the cabbage aphid, Brevicoryne brassicae L. A weekly survey of pests present on cabbage crop at El Dair region, Qualubia Governorate was carried out during 2014 – 2015 season. The results indicated that, cabbage aphid, B. brassicae, was moderate collected. The susceptibility of cabbage aphid B. brassicae, for two entomopathogenic fungi, i. e., Beauveria bassiana and Metarhizium anisopliae, were investigated. Where, B. bassiana gave higher levels of mortality against adults of cabbage aphid with low lethal time (LT50). The mortality percents of both entomopathogenic fungi were increased with increasing the period after treatment.

INTRODUCTION
Cabbage aphid, Brevicoryne brassicae (L.) (Hemiptera: Aphididae) is an important agricultural insect pest that attack many vegetable crops all over the world (Irshad, 2001). In some cases, the vegetable crop can be completely lose due to heavy infestation, while the aphid can reduce the crop yields due to sucking the plant sap besides excretion on honey dew which inhibit photosynthesis process leading to retardation in the plant growth. Also, aphid is well known as a main plant pathogen transmitter (Strauss et al., 2002 and Asi et al., 2009).

The indiscriminate use of insecticides for controlling these devastating pests caused several environmental problems such as pollution besides the negative effects on the other beneficial insects (Asi et al., 2009). These problems derived the scientists for finding more safe methods in controlling these pests. Entomopathogenic fungi, especially B. bassiana and M. anisopliae seems to be more safe and good alternatives in controlling these pests (Loc et al., 2010). The results of many researches assured successful safely use of the previous species of entomopathogenic fungi in controlling several insect pests rather than the pest under studying (Khashaveh et al., 2008 Barra et al., 2013). In this research infested aphids with B. bassiana and M. anisopliae were collected from the field. The two species of entomopathogenic fungi were propagated in the laboratory. Mortality effects and virulence of both species were evaluated against cabbage aphid.

MATERIALS AND METHODS
Survey:
Cabbage plants growing at El-Dair region, Qualubia Governorate, Egypt were surveyed during 2014 – 2015 season. Three plots (A, B & C) were sampled weekly and all cabbage aphid, B. brassicae adults were collected from 724 randomly selected plants (Embaby and Lotfy, 2015). Several aphid colonies were surveyed according to (Strauss et al., 2002) method. The collected aphids were placed individually as adult into empty plastic tubes with a small slice of leaf from the host plant. All collections were held in the laboratory at National Research Centre, Plant Protection Dept., Egypt, to record the identification of the insects. Collections were checked every 2-3 days to record the number of dead adults and to add fresh food material if required. The dead insects were collected.

Isolation and identification of fungi: The fungi were isolated and identified to culture according to methods of Boikova and Novikova, (2001). The cultures of fungi were grown in sterilized Petri dishes on agarized media at 24± 2°C using Sabouraud’s nutrient media then identified according to the manual of Polovinkoa et al. (2010).

Fungus Culture: Two isolates of entomopathogenic fungi namely Beauveria bassiana and Metarhizium anisopliae were cultured on potato dextrose agar medium containing 20g glucose, 20g starch, 20g agar, and 1000 ml of distilled water in test tubes. Then autoclaved at 121°C (15 Psi) for 15-20 minutes and incubated for two weeks at 26±1°C. Conidia were harvested by brush, used as stock and stored in refrigerator at 4°C, from which the fungi were used as inoculum for laboratory experiments. Bioassay procedure for efficacy of entomopathogenic fungi against B. brassicae was followed.

Pathogenicity: The 16-days-old cultures of M. anisopliae and B. bassiana were used for the test. Adult stage of the cabbage aphid, B. brassicae was bioassayed. The majority of the test-insects fed on the leaves of their host plants. The tested insects were infected using the contact method by placing 45 adult aphids in Petri dishes directly on the surface of conidiial layer of each fungus isolate alone for 60 seconds. The development of mycosis in the tested-insects was observed at 20-25°C. The virulence of the isolates were estimated by two parameters: death rate of the infected insects (%) and time of their death (days) according to (Polovinkoa et al., 2010)

Data analysis: The lethal time (LT50), the number of days until death of 50% the adult was computed through probit analysis using the Propan Proggram according to (Finney, 1970).

RESULTS AND DISCUSSION
1-Survey:
Survey of cabbage aphid on cabbage plants were recorded in Table (1). Seven hundred and twenty four
plants were surveyed and incidence of infested cabbage plants by cabbage aphid was 22.42%. On the other hand, (B) showed higher infection than others which recorded 10.20%, followed by (A) which gave 7.604%, while location (C) was with less incidence and recorded 4.62%.

Table (1): Percent infestation of cabbage plants by cabbage aphid in different sampled locations

<table>
<thead>
<tr>
<th>Percentage of Infestation</th>
<th>Total No of Infested plants</th>
<th>Total No of examined plants</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy plants</td>
<td>243</td>
<td>20</td>
<td>A</td>
</tr>
<tr>
<td>7.60%</td>
<td></td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>10.20%</td>
<td>220</td>
<td>25</td>
<td>B</td>
</tr>
<tr>
<td>4.62%</td>
<td>206</td>
<td>216</td>
<td>C</td>
</tr>
<tr>
<td>22.42%</td>
<td>669</td>
<td>724</td>
<td></td>
</tr>
</tbody>
</table>

2-Fungal infestation:

Naturally occurring entomopathogenic fungi that were isolated from adult cabbage aphid tabulated in Table (2). *Metarhizium anisopliae*, was the isolated entomopathogenic fungus, whereas the representatives of the genera *Aspergillus*, *Mucor*, and *Rhizopus* can be conditionally attributed to saprotrophic fungi that more often develop on the died insects. On the other hand, *M. anisopliae* was the most occurring fungus comparing with other isolated fungi recording 92.25% followed by *Rhizopus* sp. (5.53%) and *Mucor* sp. (1.85%), and *Aspergillus niger* (0.37%). Polovinkova et al. (2010) reported that, naturally occurring entomopathogenic fungi have been shown to occasionally cause high mortality of lepidopterous larvae in cabbage crop. The mycobiota of the collected cadavers of insects lists ascomycetes anamorphs of 13 genera. Such species as *B. bassiana* and *M. anisopliae*, are entomopathogenic fungi, whereas the representatives of the genera *Aspergillus*, *Fusarium*, and *Rhizopus* can be conditionally attributed to saprotrophic fungi that more often develop on the insects who died due to other reasons. Although in some cases the species and strains *Aspergillus* and *Fusarium* are experimentally shown to be highly pathogenic for insect, it is known that the given fungi usually develops on the insect that died due to mechanic damage to cuticle.

Table (2): Percentage of fungal frequency associated with adult aphids.

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Total of fungi</th>
<th>% of fungal frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>2</td>
<td>0.37</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em></td>
<td>500</td>
<td>92.25</td>
</tr>
<tr>
<td><em>Mucor</em> sp.</td>
<td>10</td>
<td>1.85</td>
</tr>
<tr>
<td><em>Rhizopus</em> sp.</td>
<td>30</td>
<td>5.53</td>
</tr>
<tr>
<td>Total</td>
<td>542</td>
<td>100.00</td>
</tr>
</tbody>
</table>

3- Pathogenicity to cabbage aphid:

percentages of cabbage aphid, *B. bassiana* adult mortality were recorded in Table (3) and represented in Figs. (1, 2 and 3). Data indicated that, *B. bassiana* gave higher levels of mortality while, low level of mortality was recorded with *M. anisopliae* as follow:-

a- After Four days from treatment, *B. bassiana* and *M. anisopliae* resulted in 88.89%, and 51.11% mortality of cabbage aphid adults, respectively.

b- After Seven days from treatment, both fungal isolates caused mortalities. But *B. bassiana* was more effective (100% mortality) than *M. anisopliae* (80% mortality) against adult aphids.

c- After ten days from treatment, all fungal isolates again caused mortalities after 10 days of treatments. *B. bassiana* isolate, was highly effective against aphid with 100% mortality than *M. anisopliae* (91.11% mortality)

On the other hand, the same Table (3) shows that, the levels of mortality percent were increased with increasing the period after treatment. Mortality percent of cabbage aphid was found to be increased from 88.89% after 4 days to 100% after 7 days when treated by *B. bassiana* fungus. Percentage of cabbage aphid mortality was found to be increased from 51.11% after 4 days to 80.00 and 91.11% after 7 and 10 days respectively, when treated by *M. anisopliae*. This result agreed with (Kumar and Chowdhry, 2004) who reported that *M. anisopliae* gave mortality percentage ranged between 50-92.5%. Thompson and Brandenburg (2005) reported that death caused by the fungi usually was more than 48 post infection after attachment of conidia to the insect cuticle.

Table (3): Cumulative corrected mortality percentage of cabbage aphid, (*B. brassicae*) using some mycoinsecticide (as a biopesticide)

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Total Number of treated adult aphids</th>
<th>Total No. of dead adults</th>
<th>% mortality</th>
<th>Total No. of dead adults</th>
<th>% mortality</th>
<th>Total No. of dead adults</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of dead adults</td>
<td></td>
<td>4</td>
<td></td>
<td>7</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td><em>B. bassiana</em></td>
<td>45</td>
<td>40</td>
<td>88.89</td>
<td>45</td>
<td>100</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td><em>M. anisopliae</em></td>
<td>45</td>
<td>23</td>
<td>51.11</td>
<td>36</td>
<td>80.00</td>
<td>41</td>
<td>91.11</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>63</td>
<td>70.00</td>
<td>81</td>
<td>90.00</td>
<td>86</td>
<td>95.56</td>
</tr>
</tbody>
</table>
Fig.1: Pathogenicity: Cabbage aphid, adult instars were infected using the contact method on the surface of conidial layer of both the entomopathogenic fungi *B. bassiana* (a) and *M. anisopliae* (b).

Fig.2: Cabbage aphids on cabbage leaf, insect mortality and the mummies (a) caused by *B. bassiana* fungus, insect body after death, the fungus forms aerial mycelia and sporulation. The insects which emerged from the mummies were killed (b).

Fig.3: Adult aphids dead by *M. anisopliae* fungus
Fig (4): log probit toxicity lines of of *M. anisopliae* and *B. bassiana* against the adult aphid expressed as LT50 values. Where, lethal time required to kill 50% of the aphid for *M. anisopliae* was 3.92 days and 3.53 days for *B. bassiana* as Fig (4).

Ramanujam *et al.* (2014) stated that, entomopathogenic fungi from hypomycetes group are opportunistic pathogens and usually cause insect mortality by nutritional deficiency, destruction of tissues and by release of toxins. Cuticular degrading enzymes of entomopathogens like chitinase, protease and lipase play an important role in the pathogenicity of these organisms on insects in the breakdown of insect cuticle for penetration of fungal germ tube into the insect body. The entry of entomopathogenic fungi through the insect cuticle is considered to occur by a combination of mechanical pressure and enzymatic degradation. Several mycotoxins like, Beauvericin, Beauveriolides Bassianolide (by *B. bassiana*, *V. lecanii*, *Paecilomyces* spp.) and Destruxins A, B, C, D, E, F (by *M. anisopliae*) are produced during pathogenesis and these act like poisons for the insects. After the death of the insects, the fungus breaks open the integument and forms aerial mycelia and sporulation on the cadavers. The fungi of entomopathorales group are obligate pathogens of insects and cause host death by tissue colonisation with little or no use of toxins. Hajek, and Leger (1994) reported that, entomopathogenic fungi involve an infective spore stage, in which it germinates on the host cuticle, forming a germ tube that penetrates the cuticle and invades the haemocoel of the insect host. (Goettel *et al.*, 2010); (Rohlfis and Churchill, 2011) and (Safavi, 2013) stated that, the fungus then multiplies within the insect body and kills it. Death occurs due to toxin production by the fungus and/or multiplication to inhabit the entire insect body. Entomopathogenic fungi are prolific producers of bioactive secondary metabolites, which are predicted to play key roles as virulence factors for fungi, infecting arthropods. Metabolites produced by entomopathogenic fungi would serve one or more of the following functions: (1) toxic to the host and help to cause death; (2) to aid the fungus overcome host defence; (3) to suppress competition from other pathogens and saprophytes on the insect cadaver; (4) to provide a defence outside the host against mycophagous organisms. Accordingly, many secondary metabolites tend to be compounds that bear toxic or inhibitory effects on other organisms. Also, (Zimmermann, 2007 and Safavi, 2013) reported that, entomopathogenic fungi produce secondary metabolites which may bioactively help fungus in its virulence toward insect hosts. A majority of these insecticidal molecules are low molecular weight secondary metabolites. Beauvericin, bassinian, bassianolide, beauverolides, beauveriolides, tenellin, oosporein, oxalic acid basiacidrin are some of the important metabolites of *B. bassiana*. Among them, Beauvericin is the most important compound which was reported first from *B. bassiana*. Beauvericin is a toxic cyclic hexadepsipeptide and comprising a cyclic repeating sequence of three molecules of N-methyl phenylalanine that alternate with three molecules of 2-hydroxyisovaleric acid. Not all isolates of *B. bassiana* produce beauvericin in vitro. Nevertheless, there are some reports of no toxicity against certain insects.

**CONCLUSION**

The obtained results in this study explore the pathogenicity of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* as safe and good insecticides alternatives for controlling against Cabbage aphid (*Brevicoryne brassicae* L.)

**REFERENCES**


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Loc, N. T.; Chi, V. T. B; Nhan, N. T. and Hong, T. T. B (2010). Exploitation of Beauveria bassiana and Metarhizium anisopliae as potential biocontrol agents in integrated pest management (IPM) on citrus, Omonrice 17: 152-163.


かれんを防ぐためには、アリ群を用いた自然法が有効である。アリ群は、葉を食べることで、コーヒーの成長を阻害する。さらに、アリ群は、コーヒーの成長を阻害する細菌を抑制することもできる。この研究によれば、アリ群は、コーヒーの成長を阻害する細菌を抑制することが可能である。

この研究は、コーヒーの成長を阻害する細菌を抑制するための自然法を提案するものである。この研究から、アリ群を用いた自然法が、コーヒーの成長を阻害する細菌を抑制することができる。この研究は、自然法を用いたコーヒーの成長を阻害する細菌の抑制法を提案するものである。

この研究は、コーヒーの成長を阻害する細菌を抑制するための自然法を提案するものである。この研究から、アリ群を用いた自然法が、コーヒーの成長を阻害する細菌を抑制することができる。この研究は、自然法を用いたコーヒーの成長を阻害する細菌の抑制法を提案するものである。

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