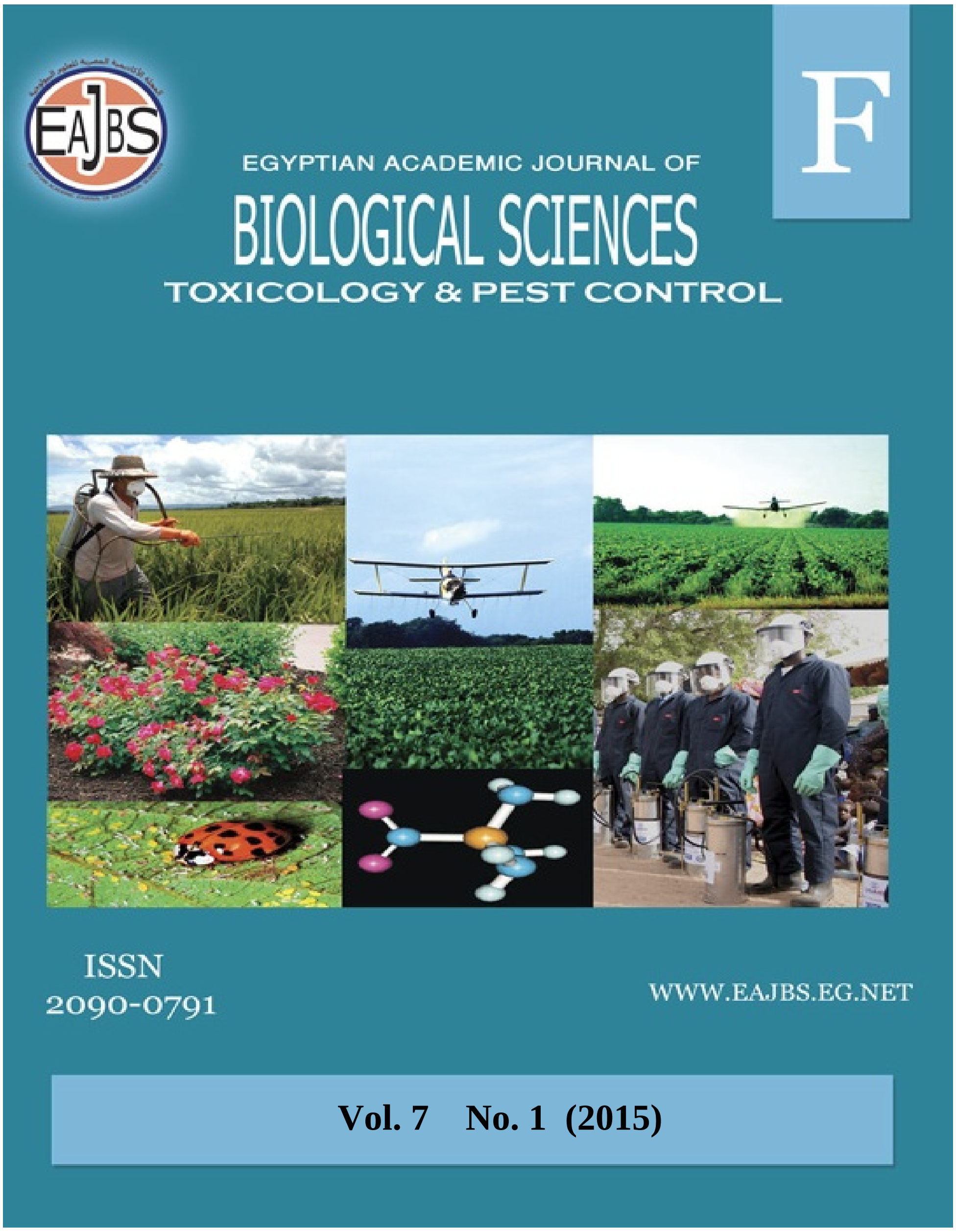

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Citation :*Egypt.Acad.J.Biolog.Sci. (F.Toxicology &Pest control) Vol.7(1)pp1-5 (2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 1-5 (2015)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN :2090 - 0791
www.eajbs.eg.net

Efficacy of some Entomopathogenic Fungi against *Bracon brevicornis* under Laboratory Conditions

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ARTICLE INFO

Article History
Received:2/1/2015
Accepted: 5/2/2015

Key words:
Biological control
Fungi
Beauvaria bassiana
Metarhizium anisoplia
Bracon brevicornis

ABSTRACT

In this work we studied the effects of the *Beauvaria bassiana* and *Metarhizium anisoplia* on *Bracon brevicornis* in the laboratory to evaluate the possibility of application of entomopathogenic fungi and the parasitoid *Bracon brevicornis* side by side in IPM programs. *Metarhizium anisoplia* was found No mortality observed at the highest concentration (4g/L) and the lowest tested concentration while the recommended concentration recorded mortality (0.1333±0.0908%) of *B. brevicornis* adult. While The recommended concentration (2g/L) of *B. bassiana* induced no mortality among the adult parasitoids while its highest tested concentration (4g/L) and the lowest concentration (1g/L) gave mortality (0.0667±.06667%) and (0.1333±0.0908%) respectively.

INTRODUCTION

The program of IPM includes the simultaneous use of different methods of control and these methods of control must be examined together to finally be able to utilize them for pest control. So the Potential effects of microbial control agents on the parasitoids must be studied (Hajek and St. Leger, 1994).

Entomopathogenic fungi were among the first organisms to be used for the biological control of pests. More than 700 species of fungi from around 90 genera are pathogenic to insects (Khachatourians and Sohail, 2008). Entomopathogenic fungi are widely distributed with both restricted and wide host ranges which have different biocontrol potentials against arthropods insects and plant pathogenic fungi. These fungi have a lower risk on the environment and humans.

Beauveria bassiana and *Metarhizium anisopliae* are intensively studied as common natural enemies and important epizootics of wide range agricultural pests (Roberts and St. Leger, 2004; Thomas and Read, 2007; Wang *et al*., 2004; Li and Sheng, 2007).

In the present study the effects of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* were evaluated on of *Bracon brevicornis* in the laboratory, in order to evaluate the possibility of simultaneous application of entomopathogenic fungi and on the parasitoid *Bracon* spp in the field.

MATERIAL AND METOUDS

Culture of *Bracon brevicornis*:

One couple (female and male) of *Bracon brevicornis* were placed in cylindrical glass tube (2.0 x 10 cm) maintained on the full grown larvae of, *Galleria mellonella* and covered with muslin cloth then fitted with rubber lids. After 24h, parasitized larvae were removed from the tube, transferred to Petri dishes and kept under the previously mentioned conditions until adult emergence. One-two day old adults were used in the current experiments.

Culture of *Galleria mellonella* (Greater wax moth):

Adults of *G. mellonella* were collected from the infested bee hives. The adults were released in plastic jars (10 X 30 cm) for mating and comprised folded sheets for the deposition and collection of eggs. The hatched larvae were reared on a semi-natural diet comprising: Wheat flour 350 g, corn flour 200 g, milk powder 130 g, packing yeast powder 70 g, honey 100 ml, and sorbitol 150 ml (Metwally *et al.*, 2013). These jars were incubated under the previously mentioned conditions till larvae reached the target or proposed instars.

Entomopathogenic Fungi:

1- *Metarhizium anisopliae* is usually called as the green fungus of insects. The fungus has white mycelia within the body, but when it is ready to form spores, the spores coated the host cadaver covering with a velvety of olive-green material.

2- *Baeuvaria bassiana* (called the white fungus) is an important natural pathogen of insects and it has been developed as a microbial insecticide for use against many major arthropod pests in agricultural, urban, forest, livestock and aquatic environments (Charnley and Collins, 2007; Faria and Wraight, 2007). It has been developed as a microbial insecticide for use against many major pests; including *lepidopterans* and *orthopterans* most strains produce external spores that make the infected insect (cadaver) appeared to be coated with a white powder or cottony material.

Commercial formulations Bioranza and Biovar were used in this investigation; they were manufactured and produced by The Kingdom of Bahrain, Ministry of Municipalities Affairs and Agriculture Wealth Directorate.

The active ingredient of Bioranza is *Metarhizium anisopliae* (10%) formulated as WP, while the inert ingredient represented 90%; the recommended application concentration is 200g/100L water.

Biovar active ingredient is *Beauveria bassiana* formulated as WP. The recommended application concentration is 200g/100L water.

Concentrations used:

Three concentrations of each fungus were tested against the adult parasitoid, 200g/100L water (the recommended concentration), its double (400g/100L) and its half (100g/100L), *i.e.*, 4, 2 and 1g/L water were used.

Bioassays:

The adult stages of the parasitoid 24-h(15 individuals) were placed paired in a plastic cup (120ml volume) with two larvae of *Galleria*, then sprayed with tested concentrations of each fungus. Other group of parasitoid adults was sprayed with water only as a control. Two days later, each of the parasitoid adult was transferred to new plastic cup with fresh larval host and incubated at 25±2ºC, 18L: 6 D and 65±5% RH.

The cups were check daily, for adding fresh hosts and/or removing the dead parasitoid's adult. The cadavers were removed, then surface sterilized in 5% sodium hypochlorite and 75% ethanol solution and rinsed in plenty of sterile distilled water, then left to dry for 48h (Dourou-kpinduo *et al.*, 1995). After drying, they were kept in humid conditions in clean desiccators at room temperature to examine whether they died because of fungus infection or not according to Luz and Farques (1998).

The percentage mortality was calculated for those killed by the fungi action or the others that died normally. Percentage mortality was corrected using Abbott formula (Abbot, 1925) for determining $LC_{25,}$ LC_{50} values and by using probit analysis (Finney, 1952).The whole experiment was replicated three times at each concentration of each fungus.

RESULT

1- *Metarhizium anisoplia:*

Data in Table (1) showed a negligible mortality among the parasitoid adults. The highest percent mortality (0.1333±0.0908%) of *B.brevicornis* adult was recorded at recommended concentration used (2g/L). While no mortality observed at the highest concentration (4g/L) and the lowest tested concentration (1g/L). Statistical analysis showed a no significant differences among the tested concentrations (*P*>0.05).

Beauvaria bassiana:

The corresponding figure for *B. bassiana* (Table 1) showed that the highest tested concentration(4g/L) and the lowest concentration (1g/L) gave mortality(0.0667±.06667) and (0.1333±0.0908) respectively compared with the recommended concentration (2g/L), where the latter induced no mortality among the adult parasitoids . Statistically, no significant different was found among each other (*P*>0.05).

From the aforementioned results, it could be concluded that the application of the entomopathogenic fungi (*Metarhizium* and *Baeuvaria*) at the recommended concentrations are safe enough and without risks towards the *B.brevicornis*.

Table 1: Percentage of mortality caused by the entomopathogenic fungi to the adult parasitoid at indicated concentration

Fungi	(%) Mortality due to fungi infection				F- value
	4g/L	2g/L	1g/L	Control	
M. anisoplia	0.00±0.00a	0.1333±0.091 a	0.00±0.00a	0.00±0.0a	2.154^{NS}
B. bassiana	0.067±.0667a	0.0±0.0a	0.133±0.091a	0.00±0.0a	1.283^{NS}

Means in a raw followed with the same letter are not significantly different at 5% level of probability.
Ns: Not significant

DISCUSSION

The present result showed that the effect of entomopathogenic fungi, *Metarhizium anisoplia* and *Beauvaria bassiana*, was low or negligible when compared with the control; this may be due to what mentioned by Thungrabeab and Tongma (2007) who concluded that some genera of fungi could be specific and might inflict only on certain types of hosts. Goettel *et al*., (1990) found that some commercial formulations of the entomopathogenic fungi can control aphids and thrips with low impact on non-target insects. Matter and Sabbour (2013) stated that the differences in the safety of entomopathogenic fungi stated by many different authors might be due to the relative efficacy of the fungus or its isolates on pests which exhibit different susceptibilities, bionomics, and characters. Also may be due to the types of assessment and application rates.

The obtained results were in accordance with Ahmad *et al.*, (2013) and Vahid Mahdavi *et al*. (2013) who concluded that various fungal isolates of *B. bassiana* and *M. anisopliae* had little adverse impact on *Bracon hebetor* wasp. Also the result is consistent with the results of Stolz *et al*. (2002) that different isolates of *M. anisopliae* fungus had very little risk on parasitoids when evaluating the susceptibility of the parasitoids *Apoanagyrus lopezi* and *Phanerotoma sp.* against the entomophatogenic this fungus. Rashki *et al*. (2009), in studying the effect of *B. bassiana* on *Aphidius matricariae* and its host *Myzus persicae*, showed that this pathogen had no effect on biological parameters of the parasitoid . Rosa *et al*., (2000) reported that various isolates of *Beauveria* and M*etarhizium* have little negative impact on the parasitoid *Prorops nasuta*. Akmal *et al*., 2013 *B. bassiana* showed little or no detrimental effects to *C. septempunctata*.

On the other hand, there were some studies indicated several adverse effects of some entomopathogenic fungi against some natural enemies. Haseeb and Murad (1997) and Delete *et al.* (1995) consider *C. septempunctata* to be somewhat susceptible to *B. bassiana*. While, Farag (2008) consider that some entomopathogenic formulations of *B. bassiana* have deleterious effects on *C. undecimpunctata* if applied at high concentration levels.

From the aforementioned results, it could be concluded that the application of the entomopathogenic fungi (*Metarhizium* and *Baeuvaria*) at the recommended concentrations are safe enough to be used along with these parasitoids in an integrated pest management programs (IPM)

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