#### A BIOLOGICAL SPORES BAIT TRAP TECHNIQUE AS A NEW APPROACH FOR TRAPPING, MONITORING AND CONTROLLING THE MEDFLIES ADULTS, Ceratitis capitata (WIEDEMANN) (DIPTERA : TEPHRITIDAE).

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#### ABSTRACT

Laboratory and field assessments were carried out to determine the efficiency of three biological control agents *Metarhisium anisopliae*, *Verticillium lecanii* and *Beauveria bassiana* locally isolated from dead pupae and adults medflies *Ceratitis capitata* against adults of *C. capitata*. Serial dilutions were prepared at  $10^5$ ,  $10^6$  and  $10^7$  spores/ ml and applied as poisonous suspension for 5,7 and 10 days.

Increased mortality percentages were obtained for the three agents chosen, at <sup>7</sup>10 spores / ml, being 92.4, 80.8 and 76.0 %, respectively after 10 days. Calculated LC<sub>50</sub> after 72 hr. were 1.4 x  $10^2$ , 4.6 x  $10^2$  and 2.4 x  $10^3$  spores / ml, respectively. A new insect spores bait trap was designed for field experiments, and applied in the field trails in Ibshway district, Fayoum Governorate, Egypt on apple tress *Malus pumila*. In such trails, double concentrations of the aforementioned once were used. Higher percentages of mortality were obtained from April to September. Reduction percentages of 93.2, 85.0 and 84.9% at the concentration 2 x  $10^7$  spores/ml for *M. anisopliae*, *V. lecanii and B. bassiana*, respectively.

#### Key words: Ceratitis capitata Metarhisium anisopliae, Beauveria bassinet, Verticillium lecanii, entomopathogen, Bioinsecticides, Bait trap.

#### INTRODUCTION

*Ceratitis capitata* (Wiedemann) is well known as dangerous highly polyphagous pest against many fruitful crops all over the world (Garcia-Mari **2008**). The damages caused by Medflies result first from oviposition in fruits followed by larvae feeding in addition to decomposition of plant tissue by invading secondary micro-organisms. Larval feeding damage in fruits is the most important. Mature attacked fruits may develop a water soaked appearance. Young fruits become distorted and usually drop. The larval tunnels provide entry points for bacteria and fungi that cause the fruit to rot (Bachrouch et al., 2008). In addition Ceratitis capitata which is thought to have originated in Africa. It is well adapted to diverse climates and has in excess of 350 host fruit species (Layne and Bassi 2008), and were shown to transmit E. coli to wounded fruit apples (Bailey et al 2006), Therefore, the present study was carried out in Fayoum Governorate, to Egypt, 2010, to evaluate the spores trap capacity in monitoring and controlling the C. capitata on mango trees where the female seek suitable sites for oviposition and puncture mango fruit early in the season, before the fruit has rinened, forming great damage (Rohde et al., 2010). Metarhizium anisopliae, Beauveria bassiana and Verticillium lecanii were applied as spore's of suspensions. Evaluation the efficiency of the chosen entomopathogens was made in the laboratory as well as in the field.

# MATERIALS AND METHODS

## A-<u>Laboratory studies</u>

## 1. Stock culture of C. capitata

Adults of the this insect pest were collected from heavily infested fruits of apple collected from orchards and transferred to the laboratory, and left on a sandy soil, in muslin cages ( $25 \times 25 \times 25$ cm) till emergence (**Manrakhan and Lux 2006**). The experimental insect, were reared on fresh apple fruits for three generations.

### 2. Preparation of spore bait;

The entomopathogenic fungi *M. anisopliae*, *V. lecanii* and *B. bassiana*, were isolated from naturally infested dead pupae and adults of *C. capitata*. Serial dilutions of each of these fungi were prepared at the concentrations of  $10^5$ ,  $10^6$  and  $10^7$  spores /ml. Each was added to 1 ml tween 40, 20 ml molasses and 1 ml glycerin. (Castillo et al., 2000).

#### 3. Efficiency tests:

Twenty five adults of *C. capitata* were exposed in a cage to each of the suspension tested which was applied in a glass of 60 ml supplied with a wick for 5, 7 or10days. The insects were, then transferred to complete feeding on 10% sucrose solution. Four replicates were prepared for each concentration and for control which was given (10% sucrose), only.

### 4. <u>Design of the spore bait trap:</u>

The spores bait trap is made of a yellow hard polypropylene, as lantern with four perforated sides for easy move of flies. The lantern is covered by an umbrella for shading and has one door for easy access of spore suspension glass, provided with a wick, was fixed on the base which was also perforated to allow the dead adults to pass down into a plastic bag connected a cylindrical tube inside the lantern. A ring and hanging wire were fixed on top of the trap for field use. Detailed of the design are shown in fig. No.1.

The same procedures were fallowed with any of each other pathogen.

#### B. Field trials:

In an apple orchard in Fayoum Governorate, district of Ibshway an area of about twelve kirrate (2100) m<sup>2</sup>. Three replicates as control were prepared per each concentration, as control, distributed randomly. Each replicate has five trees per concentration as control. In the control treatment a sticky yellow traps were used, double concentrations of the spores suspension of M. *anisopliaee*, *V. lecanii* and *B. bassiana* were prepared at the concentrations of (2x10<sup>5</sup>, 2x10<sup>6</sup> and 2x10<sup>7</sup>) were applied per treatment. The experiments were carried out on apple trees and the biological spores bait traps were connected to an apple trees from April till the end of September, (Garcia-Mari, 2008).

The field trials were inspected weekly and the dead adults in the plastic pages of the spore bait traps were collected in paper pages, and transferred to the laboratory. Percent reduction of infestation was calculated.

The data were subjected to the (ANOVA) test and the L.S.D. 5% was obtained The  $LC_{50s}$  and reduction percentages for different treatments.



Fig (1): Design of the Insect Spores Bait Trap.

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### **RESULTS AND DISCUSSION**

For *M.anisopliae*, data in Table (1) and Figs (2,3 and 4) showed that the mortality percentages of *C. capitata* were 50.4, 55.6 and 69.2 and 58.4, 80.4 and 80.4 and 80.0, 80.4 and 92.4% with the concentrations of  $10^5$ ,  $10^6$ , and  $10^7$  spores/ ml of *Metarhizium anisopliae* after 5, 7 and 10 days, respectively, with no mortality in the check treatment.

For *V. lecanii* mortality percentages were 44.4, 52.0 and 60.8 and 55.2, 56.0 and 61.6 and 66.8, 78.0 and 80.8 with the concentrations of  $10^5$ ,  $10^6$ , and  $10^7$  spores / ml after 5, 7 and 10 days, respectively. and 0.0% in the check.

For *B. bassiana*, the mortality percentages were 41.6, 50.0 and 56.0 and 52.8, 61.6 and 61.6 and 61.6, 74.0 and 76.0 % with the concentrations of  $10^5$ ,  $10^6$ , and  $10^7$  spores/ml after 5, 7 and 10 days, respectively. and also 0.0% in the check, respectively.

The highest dead counts were found with *M. anisopliae* at the concentration of <sup>7</sup>10 spores/ml. with a gradual increase over inspection period recording the highest value 92.4 % after 10 days of treatment followed by *V. lecanii* value 80.8 % while *B. bassiana* recorded the lowest reduction percentage which was 76.0%. LC<sub>50</sub> lowest values were after 10 days of treatment for *M. anisopliae* (1.4 x <sup>2</sup>10 spores /ml.) followed by *B. bassiana*, (2.4 x<sup>3</sup>10 spores / ml.) and *V. lecanii* (2.7 x 10<sup>3</sup> spores/ml.).

Statistical analysis of the showed significant differences in reducing *C. capitata* at different concentrations of the three entomopathogens after 5, 7 and 10 days.

This study revealed that the tested entomopathogenic fungi could be arranged according to their efficiency in the reduction of population density, of *C. capitata* in this descending order: *M. anisopliae* followed by *V. lecanii* and *B. bassinet*.

In this respect, (Ortiz-Urquiza *et al.*, 2008) found that *M. anisopliae* isolate secretes a protein with insecticidal and antifeedant properties. The oral toxicity of this crude protein soluble extract was tested in a diet test against adults of *C. capitata*, which caused mortality which progressively increased with concentration and exposure time.

Table 1



Fig (2): Efficiency of *M. anisopliae V.lecanii and B. bassiana* at the concentration of  $10^5$  on *C. capitata* adults in the laboratory.



Fig (3): Efficiency of *M. anisopliae*, *V. lecanii and B. bassiana* at the concentration of 10<sup>6</sup> on *C. capitata* adults in the laboratory.



**Fig** (4): Efficiency of *M. anisopliae*, *V. lecanii and B. bassiana* at the concentration of 10<sup>7</sup> on *C. capitata* adults in the laboratory.

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Table 2

Table 3

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Table 3



Fig (5): Monthly% reduction of C. capitata adults attracted to spore bait traps of M. anisopliae, V. lecanii and B. bassiana at the concentration of 10<sup>5</sup>.



Fig (6): Monthly% reduction of C. capitata adults attracted to spore bait traps of M. anisopliae, V. lecanii and B. bassiana at the concentration of  $10^6$ .



Fig (7): Monthly% reduction of C. capitata adults attracted to spore bait traps of M. anisopliae, V. lecanii and B. bassiana at the concentration of  $10^7$ .

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The same authors, 2010, reported, as new insecticide, a compound, which is the crude soluble protein extract from the fermentation product of the entomopathogenic fungus, *M. anisopliae*, a promising insecticide for the control of the Mediterranean fruit fly, *C. capitata*.

Konstantopoulou and Mazomenos (2005) revealed that moderate to high mortality rates for *C. capitata* adults occurred when the adults were exposed to conidia of *B. bassiana* causing 85.6% mortality. Dimbi *et al.*, 2003 also found that *M. anisopliae* and *B. bassiana* were the most dominant pathogens on *C. capitata* where *M. anisopliae* was determined to be highly virulent and caused mortality between 7 to 100% after 4 days.

**Goble** *et al* (2011) tested *M. anisopliae* and *B. bassiana* under laboratory conditions against the subterranean life stages of the citrus pests, *C. rosa* Karsch, *C. capitata* Wiedemann and Thaumatotibia leucotreta Meyrick. (Lepidoptera: Tortricidae), treatment with a concentration of  $1 \times 10^7$  conidia ml, fungal isolates had a significantly greater effect on the adults of *C. rosa* and *C. capitata* than on the puparia of these two fruit fly species.

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المصيدة البيولوجية الجرثومية تقنية جديدة في الجذب والتنبؤ والمقاومة لحشرة ذبابة فاكهة البحر المتوسط سيد محمد مصطفي الجارحي ١ ، نارمين أحمد يوسف ٢ معهد بحوث وقاية النبات- مركز البحوث الزراعية – الدقي – جيزة قسم وقاية النبات- كلية الزراعة – جامعة الفيوم

تهدف هذه الدراسة لمكافحة حشرة ذبابة البحر المتوسط Ceratitis capitata حيويا بإستخدام ثلاثة أنواع من الفطريات والتي تم عزلها من العذاري والحشرة الكاملة وهما Metarhizium وanisopliae Eecuvi lecani و Verticillium lecani حيث استخدما بتركيزات ١٠ و ٢٠١ و ٢٠١ جراثيم / مل علي الترتيب بالاضافة إلي الكنترول. لقد تم تحضير التركيزات السالفة الذكر وذلك بإضافة ٢٠ مل مولاس قصب السكر و ١٠ مل جليسرين و ١ مل 40 Tween ثم وضعت في زجاجات تسع كل منها ٢٠ مل ثم تم تم تحضين الزجاجات علي درجة حرارة ٢٢± ١ م المصيدة البيولوجية الجديدة والتي صممت لغرض جذب وكذلك التنبؤ بتعداد الحشرة ومقاومتها. تم وضع مصيدة لكل خمس شجرات كمقرر بالإضافة إلي الكونترول الذي وضع به محلول سكري من المولاس و 1 مل 40

M. دلت النتائج علي أن التركيز ١٠ أحدث نسبة عالية في القتل للثلاثة ممرضات M. دلت النتائج علي أن التركيز ١٠ أحدث نسبة عالية في القتل للثلاثة ممرضات M. ومانعونة النسبة المئوية B. bassiana و V. lecani ومانعونة الفي الترتيب. حيث كانت النتائج النسبة المئوية الموت هي ٢.٤ و ٨٠.٨ و ٢٠٠ بعد مرور ١٠ أعداد المقتولة في الكنترول هي ٢.٠ بعد مرور ١٠ أيام. كما دلت النتائج أيضا علي أن قيمة  $LC_{50}$  هي ٢.٤ م.٤ و ٢.٤ علي أن قيمة المتوية المتوية بينا علي أن ما ترتيب. حيث كانت التائية ما ترتيب النتائج النسبة المئوية الترتيب. حيث كانت الترابي الترتيب.

كما أظهرت النتائج أن أعلي نسبة خفض في التعداد قد تحققت بإستخدام ضعف التركيز وهو ٢ × ١٠ من الثلاثية ممرضات علي الترتيب حيث كانت ٢ ٣٠ و ٥٠٠ و ٨٤. %. وأن المتوسط العام للخفض في التعداد هو ٨٦. و ٥٢٠ و ٨١.٤ حشرة من بداية شهر أبريل وحتي نهاية شهر سبتمبر.