

## Impact of Entomopathogenic fungi on White fly, *Bemisia tabaci* in Tomato Crop in Egypt

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**Abstract :** Impact of entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, and *Verticillium lecanii* to *Bemisia tabaci* under laboratory conditions and in the field in tomato crop were studied during two successive tomato seasons (2016 and 2017). Three concentrations were used ( $2 \times 10^3$ ,  $2 \times 10^4$  and  $2 \times 10^5$  Conidia/ ml.). Under laboratory conditions the results showed that *V. lecanii*, *B. bassiana* and *M. anisopliae* mortalities were occurred after the two days from treatment. The third concentration ( $2 \times 10^5$  Conidia / ml.) was the highly toxic in *V. lecanii*, *B. bassiana* and *M. anisopliae* to the adult of *B. tabaci* compared with the other two concentrations. Under field conditions the third concentration ( $2 \times 10^5$ ) also, was the best concentration on whitefly after the third application in *V. lecanii*, *B. bassiana* and *M. anisopliae*.  
**Keywords:** Entomopathogenic Fungi, *Bemisia tabaci*, Tomato Crop.

### 1. Introduction

White fly, *Bemisia tabaci* is a serious threat to crop production due to direct damage<sup>1</sup>. *Bemisia tabaci* is considered a cryptic species complex and was recently split into 11 groups encompassing 24 species<sup>2</sup>. The biotype B of *B. tabaci* (also known as Middle East-Asia Minor 1) is widely distributed throughout Brazil and it can cause economic losses estimated in 714 million US\$/year in crops such as beans, soybeans, cotton, tomatoes, and leafy vegetables<sup>3,4</sup>.

Moreover, they play a role in the natural mortality of whitefly populations<sup>5</sup>. The most promising mitosporic fungi include *Metarhizium anisopliae*, *Verticillium lecanii*, and *Beauveria bassiana*<sup>6-14</sup>.

*Metarhizium anisopliae*, *Verticillium lecanii*, and *Beauveria bassiana* species have been used to control whiteflies and related insects in greenhouses in Europe, Canada and Egypt. These applications have been successful in cases where environmental conditions of high relative humidity and moderate temperatures are appropriate<sup>15-17</sup>.

Impact can be indirectly measured with the activities of enzymes related to infection pathways, such as subtilisin-like (Pr1) and trypsin-like (Pr2) enzymes and chitinases<sup>18-20</sup>.

The widespread distribution of *B. tabaci* is attributed to their exceptionally wide host range and short generation time<sup>21</sup>.

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin has high activity against whitefly<sup>22</sup>. Blastospores and conidia can infect the host directly; mycelium needs to grow and from infectious propagates first. Conidia can be produced easily and are more stable in challenging environmental conditions than blastospores<sup>23, 24</sup>.

The present study aims to use of entomopathogenic fungi to control such this pest to avoid using of insecticides to reduce pollution in tomato and the as well as reduce the costs of control.

## Materials and Methods

### Preparing of the concentrations

The concentrations were used ( $2 \times 10^3$ ,  $2 \times 10^4$  and  $2 \times 10^5$  Conidia/ ml.) and add 0.5 % Tween 80. The spores were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm<sup>2</sup>).

### Laboratory inoculation

Adults whitefly, *B. tabaci* were transferred to the laboratory from the Field and put in Petri-dishes with tomato leaf disk and incubated in  $23 \pm 1^\circ\text{C}$  and  $75 \pm 5$  % RH. (Five adults / replicate) were used in all treatments. The entomopathogenic fungi were sprayed using a manual sprayer in a suspension containing  $2 \times 10^3$ ,  $2 \times 10^4$  and  $2 \times 10^5$  Conidia/ ml; while sterilized water was sprayed to the leaves disks as blank control. The mortality of whitefly was observed daily for seven day.

### Field application

An area of 4200 m<sup>2</sup> in Nubaria, El-Behira Governorate was divided into four parts; each part was divided into three plots were treated with three concentrations from *V. lecanii*, *M. anisopliae* and *B. bassiana* and the other one as control treated by water. Every plot divided into three replicates. The suspensions were sprayed early in the morning. The suspensions were sprayed three times. The live insects of *B. tabaci* per leaf/replicate were counted after all treatment.

- The percent of reduction were calculated according to Handerson and Tilton formula.
- Statistical analysis

Data were analyzed by analysis of variance (one ways classification ANOVA) and followed by a least significant difference (L.S.D at 5%) (SAS Institute Inc., 2003).

## Results

Three concentrations of entomopathogenic Fungi, *V. lecanii*, *M. anisopliae* and *B. bassiana* were evaluated on *B. tabaci* under laboratory and the field.

### 1- Effect of *V. lecanii*, *M. anisopliae* and *B. bassiana* on *B. tabaci* under laboratory conditions.

As Show in Table (1) there are no effect for *V. lecanii*, *M. anisopliae* and *B. bassiana* to *B. tabaci* after three day from treatment.

**Table (1): % Mortality of *V. lecanii*, *M. anisopliae* and *B. bassiana* on *B. tabaci* under laboratory conditions at  $23 \pm 1^\circ\text{C}$  and  $75 \pm 5$  % RH.**

Days	% of mortalities									
	Con.	<i>V. lecanii</i>			<i>B. bassiana</i>			<i>M. anisopliae</i>		
		$2 \times 10^3$	$2 \times 10^4$	$2 \times 10^5$	$2 \times 10^3$	$2 \times 10^4$	$2 \times 10^5$	$2 \times 10^3$	$2 \times 10^4$	$2 \times 10^5$
2 <sup>nd</sup>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 <sup>rd</sup>	0.0	10	11	12	8	9	10	7	8	10
4 <sup>th</sup>	0.0	13	25	30	11	23	25	12	20	23
5 <sup>th</sup>	0.0	33	45	55	30	35	53	25	27	35
6 <sup>th</sup>	0.0	55	75	95	37	45	65	30	41	55
7 <sup>th</sup>	0.0	80	85	100	45	65	75	45	58	70

Mortalities are occurred in the third day. The percent of mortalities are increased gradually and reached to the maximum in the 7<sup>th</sup> day from treatment. With the all concentrations, the percent of mortalities are increased with increase of concentrations. The percent of mortalities ranged between 80 to 100, 45 to 75 and 45 to 70 % with *V. lecanii*, *B. bassiana* and *M. anisopliae*, respectively, in the 7<sup>th</sup> day after treatment. Table (1) shows that there are slight differences between effect of *V. lecanii*, *M. anisopliae* and *B. bassiana* isolation to *B. tabaci*. This mean that *V. lecanii* isolation is more effective than *B. bassiana* and *M. anisopliae*. The percent of mortalities with all concentrations  $2 \times 10^3$ ,  $2 \times 10^4$  and  $2 \times 10^5$  Conidia/ ml of *V. lecanii* isolation were 80, 85.4 and 100%, respectively.

## 2- Effect of *V. lecanii*, *B. bassiana* and *M. anisopliae* on *B. tabaci* in season 2016.

Table 2; show that after the second application the number *B. tabaci* leave were decreased compared with control. That the percent of reduction by *V. lecanii*, *M. anisopliae* and *B. bassiana* after the third treatment were 50.7, 79.3 & 100, 50.2, 63.0 & 73.5 and 45.5, 50.5 & 63 %, respectively.

The statistical analysis shows that there are no significant differences between the concentrations and control after the first application all plots. After the second application there are significant differences between all concentrations and control. After the third treatment there are highly significant differences among all concentrations. The L.S.D was 2.1 after the third treatment. The statistical analysis confirmed that the third concentration ( $2 \times 10^5$ ) was the highly toxic compared the first and the second concentrations in all treatment.

**Table (2): Effect of *V. lecanii*, *B. bassiana* and *M. anisopliae* on *B. tabaci* during season 2016.**

Treatment	The percent of infestation by <i>B. tabaci</i> / leave										L.S.D
	Control	<i>V. lecanii</i>			<i>B. bassiana</i>			<i>M. anisopliae</i>			
		2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	
1 <sup>st</sup>	7 ±1.3 <sup>a</sup>	8 ±2.3 <sup>a</sup>	8 ±1 <sup>a</sup>	9 ±3 <sup>a</sup>	9 ±1.3 <sup>a</sup>	10 ±2.2 <sup>a</sup>	10 ±0.2 <sup>a</sup>	9 ±1 <sup>a</sup>	9 ±1.5 <sup>a</sup>	8 ±2.3 <sup>a</sup>	3.1
2 <sup>nd</sup>	8 ±1.2 <sup>a</sup>	5 ±1 <sup>bc</sup>	4 ±1 <sup>bc</sup>	3 ±2 <sup>c</sup>	7 ±1 <sup>bc</sup>	6 ±2 <sup>bc</sup>	5 ±1.5 <sup>bc</sup>	8 ±1 <sup>b</sup>	7 ±1.7	4 ±2 <sup>bc</sup>	2.8
3 <sup>rd</sup>	9 ±1 <sup>a</sup>	4 ±1.5 <sup>bc</sup>	2 <sup>bc</sup>	0.0 <sup>c</sup>	4 ±2 <sup>bc</sup>	3 ±0.2 <sup>bc</sup>	2 ±1.3 <sup>bc</sup>	5 ±1 <sup>b</sup>	4 ±1.7 <sup>bc</sup>	3 <sup>bc</sup>	2.1
Percent of reduction %	-----	50.7	79.3	100	50.2	63.0	73.5	45.5	50.5	63.0	----

## 3- Effect of *V. lecanii*, *B. bassiana* and *M. anisopliae* on *B. tabaci* in season 2017.

Table 3; show that after the second treatment the number *B. tabaci* leave were decreased compared with control. That the percent of reduction by *V. lecanii*, *B. bassiana* and *M. anisopliae* after the third treatment were 55.1, 72.2 & 100, 52.0, 60.0 & 75.2 and 45.9, 51.5 & 64 %, respectively.

The statistical analysis shows that there are no significant differences between the concentrations and control after the first treatment all plots. After the second treatment there are significant differences between all concentrations and control. After the third treatment there are highly significant differences among all concentrations. The L.S.D was 2.1 after the third application. The statistical analysis confirmed that the third concentration ( $2 \times 10^5$ ) was the highly toxic compared the first and the second concentrations in all treatment.

**Table (3): Effect of *V. lecanii*, *B. bassiana* and *M. anisopliae* on *B. tabaci* during season 2017.**

Treatments	The percent of infestation by <i>B. tabaci</i> / leave										L.S.D
	Control	<i>V. lecanii</i>			<i>B. bassiana</i>			<i>M. anisopliae</i>			
		2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>3</sup>	2 x 10 <sup>4</sup>	2 x 10 <sup>5</sup>	
1 <sup>st</sup>	7 ±2.1 <sup>a</sup>	8 ±2.1 <sup>a</sup>	8 ±2 <sup>a</sup>	9 ±1 <sup>a</sup>	9 ±2 <sup>a</sup>	8 ±21.1 <sup>a</sup>	10 ±3.2 <sup>a</sup>	10±1 <sup>a</sup>	9±1.3 <sup>a</sup>	9 ±2.2 <sup>a</sup>	3.2
2 <sup>nd</sup>	8 ±2.2 <sup>a</sup>	7 ±1 <sup>bc</sup>	6 ±2 <sup>bc</sup>	3 ±1 <sup>c</sup>	8 ±1 <sup>bc</sup>	7 ±1.2 <sup>bc</sup>	4 ±1.2 <sup>bc</sup>	8 ±1 <sup>b</sup>	7 ±1.7	4 ±2 <sup>bc</sup>	2.5
3 <sup>rd</sup>	9 ±1 <sup>a</sup>	5 ±2 <sup>bc</sup>	3±1 <sup>bc</sup>	0.0 <sup>c</sup>	5 ±1 <sup>bc</sup>	4 ±2.3 <sup>bc</sup>	3 ±1 <sup>bc</sup>	5 ±1 <sup>b</sup>	4 ±2.2 <sup>bc</sup>	3±1 <sup>bc</sup>	2.3
Percent of reduction %	-----	55.1	75.2	100	52.0	60.0	75.2	45.9	51.5	64.0	----

## Discussion

These data showed that the entomopathogenic fungi *V. lecanii*, *B. bassiana* and *M. anisopliae* can be used as agent in pest control and integrated pest management programs.

The effect of *V. lecanii*, *B. bassiana* and *M. anisopliae* may be depending on the type of host plant. While the percent of mortality after 7<sup>th</sup> day by *V. lecanii*, *B. bassiana* and *M. anisopliae* ranged between 100, 75.2 and 60%, respectively<sup>25</sup>. This result compatible with<sup>26</sup> who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively.<sup>27</sup> reported that *B. bassiana* as an entomopathogenic fungi showed high effects on the aphid *Aphis craccivora* and the white fly *B. tabaci* infesting cucumber.<sup>28</sup> reported that *V. lecanii* caused higher virulence in the early stages of whitefly and reduced with older instars.<sup>29</sup> mentioned that entomopathogenic fungi caused good mortality to whitefly.

On the other hand,<sup>30-33</sup> described the control of the silver leaf whitefly *Bemisia argentifolii* on several plants (including cucurbits, broccoli, tomatoes, and cotton) using *B. bassiana*; they observed only a few fungus-killed adults on the plants did not exceed 1 %.

## References

- Oliveira, M.R.V., Henneberry, T.J., Anderson, P. History current status and collaborative research projects for *Bemisia tabaci*. Crop Prot., 2001, 20: 709–723.
- De Barro, P.J., Liu, S.S., Boykin, L.M., Dinsdale, A.B. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. , 2011, 56: 1–19.
- Fontes, F.V.H.M., Colombo, C.A., Lourenço, A.L. Structure of genetic diversity of *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) populations in Brazilian crops and locations. Sci. Agric., 2012, 69 (1): 47–53.
- Oliveira, C.M., Auad, A.M., Mendes, S.M., Frizzas, M.R. Economic impact of exotic insect pests in Brazilian agriculture. J. Appl. Entomol. , 2013, 137: 1–15.
- Lacey, L.A., Fransen, J.J., Carruthers, R.I. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: Gerling, D., Mayer, R.T. (Eds.), *Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management. Andover, UK, 1996: 356–456, Intercept.
- Faria, M., Wraight, S.P. Biological control of *Bemisia tabaci* with fungi. Crop Prot., 2001, 20: 767–778.
- Wraight, S.P., Inglis, G.D., Goettel, M.S. Fungi. In: Lacey, L.A., Kaya, H.K. (Eds.), *Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests*. Springer, Dordrecht, The Netherlands, 2007: 223–248.
- Lacey, L.A., Wright, S.P., Kirk, A.A. Entomopathogenic fungi for control of *Bemisia tabaci* biotype B: foreign exploration, research and implementation. In: Gould, J., Hoelmer, K., Goolsby, J. (Eds.), *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*. Progress in Biological Control, 2008, 4:33–69.
- Zaki, F.N. and Abdel-Raheem, M.A. Using of Entomopathogenic fungi and insecticide against some insect pests attacking peanuts and sugar beet. Archives of phytopathology and plant protection, 2010, 43(18): 1819-1828.
- Sabry, K. H., M. A. Abdel-Raheem and Monira M. El-Fatih. Efficacy of the Entomopathogenic Fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on some pests under Laboratory Conditions. J. Biol. Pest control, 2011, 21(1):33-38.

11. Mahmoud M. M. Soliman, Abdel-Moneim, A.S.H. and Abdel-Raheem, M. A. Impact of some insecticides and their mixtures on the population of tomato borers, *Tuta absoluta* (Meyrick)(Lepidoptera:Gelechiidae) and *Helicoverpa armigera*(Hubner)(Lepidoptera: Noctuidae) in tomato crop at upper Egypt. Archives of Phytopathology and Plant Protection, 2014, 47(14): 1764–1776.
12. Abdel-Raheem, M.A., K.H.Sabry and Zakia A.Ragab. Effect of different fertilization rates on control of *Bemisia tabaci* (Genn.) by *Verticillium lecanii* and *Beauveria bassiana* in potato crop. Journal of Biological Pest control, 2009, 19(2):129-133
13. Ismail, I.A. and R.S. Abdel-Rahman and M.A. Abdel-Raheem. Utilization of certain plant extracts and entomopathogenic fungi for controlling the black fig fly, *Lonchaea aristella* on fig trees. International Journal of ChemTech Research, 2016, 9 (4):35-42.
14. Saleh, M.M.E., M.A. Abdel-Raheem., I.M. Ebadah and Huda H. Elbeheri. Natural Abundance of Entomopathogenic Fungi in Fruit Orchards and their Virulence against *Galleria mellonella* larvae. Egyptian Journal of Biological Pest Control, 2016, 26(2):203-207.
15. Fransen, J.J., Winkelman, K., van Lenteren, J.C. The differential mortality at various life stages of the greenhouse whitefly, *Trialeurodes* (Homoptera: Aleyrodidae), by infection with the fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes). J. Invertebr. Pathol.1987, 50:158–165.
16. Chandler, D., Heale, J.B., Gillespie, A.T. Effect of osmotic potential on the germination of conidia and colony growth of *Verticillium lecanii*. Mycol. Res., 1994, 98:84–88.
17. Abdel-Raheem, M. A. Possibility of Using the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* for controlling the Sugar-Beet Insects *Cassida vittata* Vill. and *Scrobipalpa ocellatella* Boh. in Egypt, Ph.D. Faculty of Agriculture, Cairo University, Cairo,2005: 86.
18. Shah, F.A., Wang, C.S., Butt, T.M. Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiol. Lett. , 2005, 251: 259–266.
19. Shah, F.A., Allen, N., Wright, C.J., Butt, T.M. Repeated in vitro subculturing alters spore surface properties and virulence of *Metarhizium anisopliae*. FEMS Microbiol. Lett., 2007, 276: 60–66.
20. Jackson, M.A., Dunlap, C.A., Jaronski, S. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. BioControl, 2010, 55: 129–145.
21. WANG, L.; J. HUANG; M. YOU; X. GUAN; and B. LIU .Toxicity and feeding deterrence of crude toxin extracts of *Lecanicillium* (*Verticillium*) *lecanii* (Hyphomycetes) against *Bemisia tabaci* (Homoptera: Aleyrodidae). (Pest Management Science, 2007, 63: 381 – 387).
22. AL-DEGHAI, M.A. Bioassay evaluation of the Entomopathogenic Fungi, *Beauveria bassiana* Vuillemin against eggs and nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). (Pakistan J. Bio. Sci, 2008, 11: 1551-1560).
23. DEL PRADO, E.N., J. IANACONE and H. GÓMEZ .Effect of two entomopathogenic fungi in controlling *Aleurodicus cocois* (Curtis, 1846) (Homoptera: Aleyrodidae). (Chilean Journal of Agricultural Research, 2008, 68(1):21-30).
24. SCORSETTI, A.; R. HUMBER; C. DE GREGORIO and C. LASTRA. New records of entomopathogenic fungi infecting *Bemisia tabaci* and *Trialeurodes vaporariorum*, pests of horticultural crops, in Argentina. (BioControl, 2008, 53: 787-796).
25. WRAIGHT, SP; RI CARRUTHERS; ST JARONSKI; CA BRODLEY; CJ GARZA; and SG WRAIGHT. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly *Bemisia argentifolii*. (Biol. Control, 2000, 17:203–217).
26. MANIANIA, N.K. Susceptibility of *Chilo partellus* Swinhoe (Lep., Pyralidae) eggs to entomopathogenic hyphomycetes. (J. Appl. Entomol., 1991, 112: 53-58).
27. ZAKI, F. N. Efficiency of the entomopathogenic fungus, *Beauveria bassiana* (Bals), against *Aphis craccivora* Koch and *Bemesia tabaci*, Gennandius. (J. of Applied Entomol., 1998, 122: (7) 397-399).
28. GINDIN, G.; N.U. GESCHTOVT; B. RACCAH and I. BARASH Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf, *Bemisia argentifolii*. (Phytoparasitica, 2000, 28: 229-239).
29. ABDEL-BAKY, N.F.; H.A. EL-FADALY, M.E. EL-NAGAR, N.S. ARAFAT and R.R.H. ABD-ALLAH .Virulence and enzymatic activities of some entomopathogenic fungi against whiteflies and aphids. (J. Agrie. Sci., Mansoura Uni., 2005, 30: 1153-1167).

30. Abdel-Raheem, M.A. ; Ismail, I.A. ; Abdel-Rahman, R.S. ; Abdel-Rhman, I. E. and Naglaa F. Reyad. Efficacy of Three Entomopathogenic Fungi on Tomato leaf miner, *Tuta absoluta* in Tomato crop in Egypt. Swift journals of Agricultural Research, 2015, 1(2): 015-020 June, 2015.
31. Abdel-Raheem, M. A.; Naglaa F. Reyad ;Abdel-Rahman, I. E.and Al-Shuraym, Laila. A. Evaluation of some isolates of entomopathogenic fungi on some insect pests infesting potato Crop in Egypt. International Journal of ChemTech Research, 2016, 9(8):479-485.
32. Ismail, I.A.; R.S. Abdel-Rahman and M.A. Abdel-Raheem . Economical evaluation of different methods for controlling fig longihorne beetle, *Hesperophanes griseus* (Coleoptera: Cerambycidae) on fig trees. International Journal of ChemTech Research, 2016, 9 (4):122-125.
33. Abdel-Raheem, M.A.; and Lamya Ahmed Al-Keridis.A. Virulence of three Entomopathogenic Fungi against White fly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) in Tomato Crop. Journal of Entomology, 2017, 14(4):155-159.

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