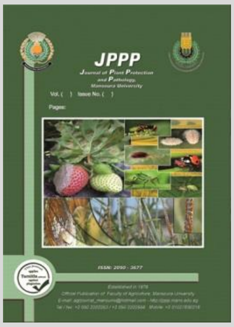
Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Comparative Effect of Three Entomopathogenic Fungi against Whitefly *Bemisia tabaci* (Gennadius) Infesting Eggplant under Field Conditions at Kafr El-Sheik Gov., Egypt

Maha S. Nada[1]; Hala A.[2*]; S. A. Gaffar[2*] and Ahmed Taman[1]

[1] Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt
[2] Central Laboratory for Organic Agriculture, Agricultural Research Center, Cairo, Egypt



ABSTRACT

Using entomopathogenic fungi, as a powerful bio-control agent, is considered one of the promising methodologies in IPM programs. The current investigation aimed to test the efficiency of *Metarhizium anisopliae, Verticillium lecanii, and Beauveria bassiana* against whitefly on Eggplant (variety Anann) in Kafr El Sheikh Governorate, Egypt. The experiment was laid in a randomized complete block design, in an area of one feddan, divided into 4 plots (5 replicates each). The study extended from July till September, during two successive seasons (2019 and 2020) and each fungal product was sprayed four times (one spray/week). Temperature fluctuated from 29 to 35℃ and RH ranged from 49 to 52%. Results indicated that using entomopathogenic fungi significantly reduced *B. tabaci* population. *V. lecanii* was the most influential fungus, as it caused the highest control percentage compared to the other two fungi and control treatment. Repeating application during 4 successive weeks raised fungi effectiveness and caused ascending mortality levels in *B. tabaci* population. Production rates were in line with percentage reductions of whiteflies. The largest yield of eggplant's fruits was produced in the plots treated with *V. lecanii*. Accordingly, the study recommends using *V. lecanii* in whiteflies control strategies in Egypt. Moreover, further investigations are needed to study compatibility of *V. lecanii* with other bio-agents to avoid inconsistency when applied together in pest management programs.

Keywords: *Metarhizium anisopliae, Verticillium lecanii, Beauveria bassiana, B. tabaci,* eggplant, biocontrol.

INTRODUCTION

Eggplant, *Solanum melongena* (Solanales: Solanaceae) is one of the most important vegetable corps worldwide, particularly in Egypt and especially during summer season (Abd El-Al *et al*., 2008). It is also one of the healthiest vegetable due to its high content of bioactive compounds, minerals and vitamins (Plazas *et al*., 2013). In 2019, Egypt's total cultivated area of eggplant was estimated by 106531 feddans (1 feddan = 0.42 ha). The Food Agriculture Organization Corporate Statistical Database (FAOSTAT, 2019), mentioned Egypt as the third Eggplant producer worldwide on the years 2016-2018. Total production increased from 1.37 million tons in 2017 to 1.4 million tons in 2018 and exports recorded about 1.57 tons (Saifaddin, 2021). These data reflect the importance of eggplant as a strategic vegetable in Egypt.

Yet, eggplant is attacked by several pests; one of which is the whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). It is considered to be one of the eggplant's most destructive pests as infestation causes percentage reductions of leaf area and leaf fresh and dry weight that reach 26.6, 21.8, and19.27%, respectively (Islam *et al*., 2010). In addition, they also reported a percentage reduction in chlorophyll content (9.7%) and photosynthesis rate (65.9%). The whitefly can lead to either direct or indirect injury as it transmits about 150 plant viruses (Oliveira *et al*., 2001; Stansly and Naranjo, 2010).

Moreover, *B. tabaci* has a tremendous potential to develop resistance to insecticides; it has shown resistance to more than 40 active ingredients of insecticides (Naveen *et al*., 2017). Repetition of using compounds with the same active ingredients and excessive doses during the same season has caused *B. tabaci* resistance against Organophosphates and pyrethroids as reported by Kranthi, *et al*. (2002). Thus, applying microbial bio-pesticides, such as entomopathogenic fungi, can be a powerful alternate (Ghongade *et al*., 2021). The most promising fungi includes *Metarhizium anisopliae*, *Verticillium lecanii* (*Lecanicillium* spp.) of the family Clavicipitaceae (Hypocreals) and *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) (Faria and Wraight 2001). These species have been used to control whiteflies and related insects in greenhouses in Europe, Canada and Egypt. They are normally applied in spray formulations as pest control agents for a short period of time (Inglis *et al*., 2001). Studies proved that *B. bassiana*, has high activity against whitefly (Al-Deghairi, 2008). Not only that but several research works stated entomopathogenic fungi can protect plants against different insects and diseases (Jaber and Ownley, 2017). In addition, there is growing evidences that entomopathogenic fungi also enhance plant growth by improving nutrient uptake, hormone production, and tolerance to different abiotic and biotic stresses [Sanchez Rodriguez *et al*., 2017). Hence, some fungi species can enhance plant growth and at the

[*] **Corresponding author.**
E-mail address: halaadel3528@gmail.com - saad_bio_organic@yahoo.com

DOI: 10.21608/jppp.2021.157404

same time reduce insect pests' development when colonized into plants.

Accordingly, the current investigation aims to test the effectiveness of 3 entomopathogenic fungi, i.e. *Metarhizium anisopliae, Verticillium lecanii,* and *Beauveria bassiana* against whitefly in Eggplant fields to determine which fungus is more efficient to be integrated in *B. tabaci* control strategy in Egypt. In addition, the effect of using the fungi products several successive weeks *on B. tabaci* population and the relationship between pest population reduction and production rates was also investigated.

MATERIALS AND METHODS

Experiment location, conditions and Dates:

The experiment was carried out in a private farm located in Qulien district, Kafr El Sheikh Governorate, Egypt. This governorate lies in the northern part of Egypt, along the western branch of the Nile in the Nile Delta with 31.31° N latitude and 30.80° E longitude. The study extended from July till September, during two successive seasons, i.e. 2019 and 2020. Weather conditions including average temperature, average relative humidity, and average wind velocity (Km/h) were daily recorded with the assistance of colleagues in the Rice Research and Training Center, Sakha, Agriculture Research Center. The climate was arid, characterized by no precipitation during the study period. Temperature ranged between 29-35℃, whereas relative humidity was moderate ranging between 49-52%. Wind speed was classified as light air where it did not exceed 4 Km/h.

Entomopathogenic fungi products:

Three different entomopathogenic fungi types were used in this study. Table (1) presents the fungi scientific name, trade name, source and formulation. The application rate used for all products was 5 cm^3/ liter of water. Products were applied 60 days post cultivation and repeatedly sprayed for 4 successive weeks (one spray/ fungus/ week).

Table 1. Entomopathogenic Fungi products trade name, scientific name, source, and formulation

Scientific name	Trade name	Source	Formulation
Beauvaria bassiana	Bio-Power		
Verticillium lecanii	Bio-Catch	Gaara	1×10^8
Metarhizium anisopliae	Bio-Magic	Est.	C.F.U./ ml

Area and Experiment's design:

An area of one feddan was cultivated, on the beginning of July, with Eggplant variety Anann. The experimental area was divided into 4 plots (1000 m^2/treatment) three of them were sprayed with entomopathogenic fungi and the 4^{th} was used as control (treated with water only). The experiment was laid in a randomized complete block design. Each plot was divided into 5 replicates (each of 200 m^2) and planted with about 900 tomato seedlings (total seedlings' no. was 3600/ feddan). Fertilizers were used as recommended, and plants were irrigated regularly. Weeding and all necessary agronomic processes were maintained constantly when required. Each entomopathogenic fungi product was adjusted, calibrated and sprayed weekly according to the plot area, using a twenty liters volume sprayer (4 times/ plot/ season). Numbers of live whiteflies were counted on plants (5 rep. X 5 plants/ treatment). Number of whiteflies was registered before the first spray to estimate population homogeneity and calculated percentage reduction after each spray.

Control index and potency levels:

Efficiency of the tested entomopathogenic fungi was estimated according to Khidr *et al.*, 2004 following the next equations:

$$\text{Control index} = \frac{\text{General reduction in the tested treatment \%}}{\text{General reduction in the most promising treatment \%}} \times 100$$

$$\text{Potency level} = \frac{\text{General reduction in the tested treatment \%}}{\text{General reduction in the least effective treatment \%}}$$

Eggplant's production rates/treatment

Eggplant was harvested weekly and fruit yield/plot was weighted in both seasons for each treatment separately. Production rate was calculated as Kg per feddan.

Statistical Analysis

Results were statistically analyzed using SPSS statistical package according to the analysis of variance (ANOVA) to explain the significant differences between the various treatments at $p < 0.05$.

RESULTS AND DISCUSSION

Results

1. Season one

Numbers of whiteflies were registered before the first application of the three entomopathogenic fungi. *B. tabaci* population showed homogeneity, mainly in the *B. bassiana*, *V. lecanii*, and control plots, whereas in *M. anisopliae* plot numbers were higher to some extent as shown in Table (2). After the first application, mean numbers of whiteflies decreased from 126± 2.8 to 62.8 ± 3.3, from 130 ± 3.8 to 61.4 ± 2 and from 165.6 ± 2.2 to 62.4 ±3.5 in *B. bassiana*, *V. lecanii* and *M. anisopliae* plots, respectively. As presented in Fig. (1), these results are equivalent to percentage reductions of 53.4, 54.5 and 65%, respectively. Mean numbers of *B. tabaci* continued to decrease from one application to the other in all the tested fungi plots till almost no whitefly individuals were found by the fourth application, i.e. 0.4 ± 1, 0.08 ± 0.05 and 0.1 ± 0.06, respectively. Control plot showed the highest numbers of *B. tabaci* throughout the 4 experimental weeks, as presented in Table (2). Statistical analysis proved there were significant differences between control and all the other treatments and also between *M. anisopliae, B. bassiana* and *V. lecanii.*

Table 2. *B. tabaci* mean numbers before and after fungi application in four successive weeks' in the first season

Fungi	*B. tabaci* Mean Number ± SE			
Sprays	*B. bassiana*	*V. lecanii*	*M. anisopliae*	Control
Pre-application	126 ± 2.8	130 ± 3.8	165.6 ± 2.2	117.2 ± 3.8
App. 1	62.8 ±3.3	61.4 ± 2	62.4 ±3.5	122.4 ± 3.5
App. 2	30.2 ± 2.3	20 ± 2	18.8 ± 0.7	115 ± 2.6
App. 3	7.2 ± 0.4	4.4 ±0.7	3 ± 0.3	83 ±1.5
App.4	0.4 ± 1	0.08 ± 0.05	0.1 ± 0.06	18 ± 1
General mean	$45.3^c \pm 4.2$	$43.2^c \pm .5$	$50^b \pm 5.6$	$91^a.1 \pm 3.7$

Both *V. lecanii* and *M. anisopliae,* cleared that, percentage reductions higher than *B. bassiana*, during the 4 week treatments which was respectively: 54.6, 65, 74 and 94% and 65, 66, 77 and 84.25%, whereas *B. bassiana* caused reduction did not exceed 75% by the 4^{th} spray, as illustrated in Fig. (1). Results also revealed that, during the first three applications, *M. anisopliae* effect was higher than *V. lecanii*, but by the 4th week the opposite occurred as *V. lecanii* exceeded the mortality % induced by *M. anisopliae* (about 10% more) keeping its virulence. Furthermore, the study results confirmed that repeating applications for four successive times increased *B. tabaci* percentage reductions gradually.

2. Season two

Surprisingly, *B. tabaci* mean numbers in the second season of the study was dramatically lower than the first season before using any fungi products as pre-application count of whiteflies registered almost half the numbers that was recorded in the first season (Table 3). Mean numbers were 70 ± 2, 60 ± 3.4, 61 ± 2.8 and 70 ± 3.2, for *B. bassiana*, *V. lecanii*, *M. anisopliae* and control, respectively. Yet, results followed the same trend of season one as the three entomopathogenic fungi reduced pest's population. *B. bassiana* gave mean numbers of 30.4 ±3, 10.4 ± 1, 2.8 ± 0.5 and 1 ± 0.1, *V. lecanii* gave mean numbers of 17.4 ± 2, 5.8 ± 0.5, 0.8 ±0.1 and 0.04 ± 0.04, and *M. anisopliae* mean numbers were 27.4 ± 2.2, 10 ± 1, 3 ± 0.4 and 0.4 ± 0.1 for the four sprays, respectively. Control plots showed that, fluctuations in whiteflies mean numbers as it ranged between 70 ± 3.2 to 98 ± 2.6. General mean numbers showed significant differences between treatments as recorded in Table (3). Control results were significantly different from all the other treatments. *B. bassiana* mean numbers were also significantly different from *V. lecanii* and control but not from *M. anisopliae*, still, no significant differences were noticed between both *V. lecanii* and *M. anisopliae*.

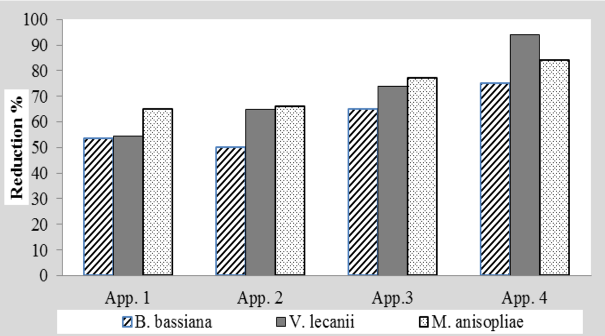


Fig. 1: Reduction % of *B. tabaci* treated with the three fungi during 4 successive weeks in season 1

Table 3. *B. tabaci* mean numbers before and after fungi application in four successive weeks' in the second season

Fungi Sprays	*B. tabaci* Mean Number ± SE			
	B. bassiana	***V. lecanii***	***M. anisopliae***	**Control**
Pre-application	70 ± 2	60± 3.4	61± 2.8	70± 3.2
App. 1	30.4±3	17.4± 2	27.4±2.2	76.6± 2.5
App. 2	10.4± 1	5.8± 0.5	10± 1	90± 3.5
App. 3	2.8± 0.5	0.8±0.1	3± 0.4	77± 2
App.4	1± 0.1	0.04± 0.04	0.4± 0.1	98± 2.6
General mean	22.9^b± 2.4	16.8^c± 2.1	22^{bc}±2.3	82.3^a± 1.6

As illustrated in Fig. (2), percentage reductions in *B. tabaci* population confirmed season one result. The highest percentage occurred in the plots treated with *V. lecanii* (94%) followed by *M. anisopliae* (89%) and *B. bassiana* (73.5%) after the 4 successive applications. Repeating spraying increased effectiveness in all the treatments as occurred in season one.

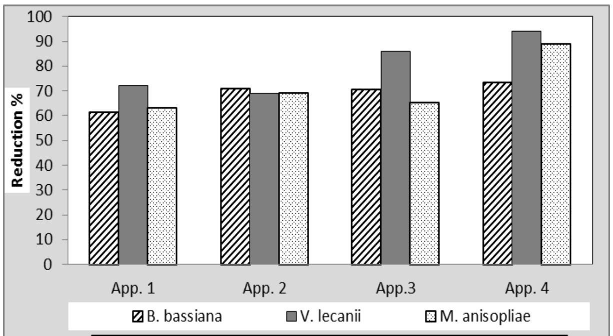


Fig. 2: Reduction % of *B. tabaci* treated with the three fungi during 4 successive weeks in season 2

By comparing the general mean numbers of *B. tabaci* in the two seasons after the four weeks treatments, it is clear that using entomopathogenic fungi reduced pest's population to a high extent as illustrated in Fig. (3). Control mean numbers in seasons 1 and 2 was 91 and 82.3 individuals, respectively, which is much higher than those in the treated plots. The least mean number was recorded in case of *V. lecanii* which was 43.2 and 17 individuals, in the two seasons, respectively.

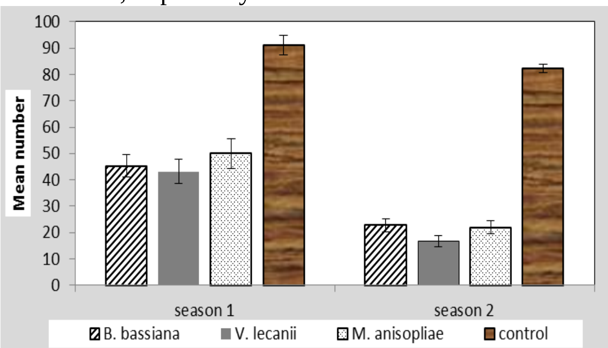


Fig. 3: General mean number of *B. tabaci* treated with the three fungi in two seasons

3. Entomopathogenic fungi relative efficiency:

Data arranged in Table (4) showed that, the general reduction, control index, and potency level of each entomopathogenic fungi used during the two seasons of the study. According to Khidr *et al*. (2004) the control index is an approach to compare relative efficiency of the tested materials. Thus, we chose *V. lecanii* to be the standard control method as it gave an index control value of 100 units (the most pathogenic). The efficiency of the other two control methods, i.e. *B. bassiana* and *M. anisopliae* attained (79.84 and 93.86 units) during the first season and (68.28 and 89.53 units) during the second season, respectively. On the other side, the relative potency level can be used in comparing the degree of different control methods efficiency as well. The relative potency level is expressed as the number of folds. As shown in Table (4), *V. lecanii* was 1.25 times more effective for controlling the whiteflies than *B. bassiana* (1) and *M. anisopliae* (1.17) during the first season. In the second season, *V. lecanii* was 1.1 times more effective than the efficacy of *B. bassiana* (1) and *M. anisopliae* (1.04).

4.Eggplant production rate/ treatment

Starting from week eight post cultivation and after one week of the first entomopathogenic fungi application, eggplant fruits were weekly harvested and production rate per feddan was calculated. This part of the investigation was carried out to study the effect of using entomopathogenic fungi on eggplant's production rate and its relation to whiteflies' population reduction.

Table 4. Relative comparison between the efficiency of the three tested entomopathogenic fungi in controlling *B. tabaci*.

Treatments used	% General reduction		Control index		Potency level	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
B. bassiana	62.078	69.12	79.84	86.28	1.00	1.00
V. lecani	77.75	80.11	100	100	1.25	1.1
M. anisopliae	72.98	71.72	93.86	89.53	1.17	1.04

Data results in Table (5), revealed that, in the first season, the highest production after each application during the 4 weeks of the experiment occurred in case of *V. lecanii* (2448, 2480, 2540 and 2640 Kg/feddan, respectively). The second highest production rate was achieved when *M. anisopliae* was applied (2164, 2220, 2240 and 2340 Kg/feddan during the 4 applications, respectively) followed by *B. bassiana* (1788, 1976, 2040 and 2320 Kg/feddan) as shown in the same table. Again season two results followed the same trend of season one as shown in Table (5). Control plot in the two seasons showed the least production rates. Statistical analysis for each season proved there were significant differences between the four treatments, i.e. the three tested fungi and control ($P < 0.001$). Besides, repeating fungi applications from one week to the other till the 4th week raised production rates in all treatments except the control in which production rates decreased from one week to the next till in reached its minimum rate on the 4th week of the study, i.e. 1480 and 1380 Kg/feddan, for the two seasons, respectively. ANOVA test proved there were significant differences between numbers of sprays in the two seasons of the study ($P < 0.001$).

Table 5. Production rates of eggplant (Kg/feddan) during 4 weeks in entompathogenic fungi treated plots and control treatment in two successive seasons

sprays	Season one (Kg/Feddan)				Season two (Kg/Feddan)			
	B. bassiana	*V. lecanii*	*M. anisopliae*	Control	*B. bassiana*	*V. lecanii*	*M. anisopliae*	Control
App. 1	1788	2448	2164	1912	1880	2340	2000	1700
App. 2	1976	2480	2220	1732	1980	2360	2180	1420
App. 3	2040	2540	2240	1680	1996	2400	2220	1440
App. 4	2320	2640	2340	1480	2040	2440	2260	1380

Figure (4) illustrated the total production rate in each season after four sprays in all the treatments. Results of season one and season two, respectively, reveal *V. lecanii* achieved the highest rank among all treatments as the eggplant sprayed with this fungus produced 10108 and 9540 Kg fruits/ feddan, followed by *M. anisopliae* (8964 and 8660 Kg/ feddan), *B. bassiana* (8124 and 7896 Kg/feddan) and finally comes the control plot giving the least yield, i.e. 6804 and 5940 Kg/ feddan). The statistical analysis proved there was no significant difference between the production rates of the two seasons ($P=1$).

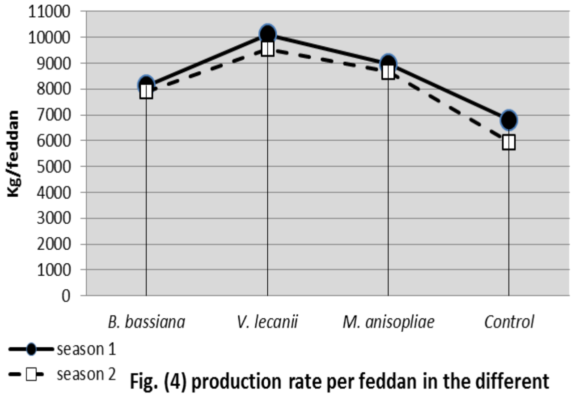


Fig. (4) production rate per feddan in the different treatements in two seasons

Discussion

Agricultural scientists face a main challenge when setting a sustainable strategy aiming to improve crop protection (Berg, 2009). As intensive use of chemical insecticides killed non-target species. In addition, their accumulated residues in environment and food, and built resistance in target pests has all led to the disruption of ecosystems (FAO, 1989). Therefore, bio-pesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are promising plant protection agents against several insects.

Recent advances in fungal production, formulation, stabilization, and implementation have led the way toward commercialization of a large number of new fungus-based bio-pesticide products (Wraight *et al*., 2001).

The current field study revealed that all three used entomopathogenic fungi, i.e. *V. lecanii, B. bassiana* and *M. anisopliae* were effective and succeeded to control whiteflies in eggplant field to a high extent. Abdel-Baky *et al.* (2005) supported this result as they mention entomopathogenic fungi caused good mortality to whitefly. Numbers of whiteflies in the treated plots were always less than those in control plot. *V. lecanii* gave the highest control percentage compared to the other two fungi, i.e. *M. anisopliae* and *B. bassiana.* This finding was confirmed by calculated control index and potency level which was higher in case of *V. lecanii*. The results of Naglaa (2017) matches what we have found as she tested the same three entomopathogenic fungi against whiteflies on tomato crop and proved *V. lecanii* gave the greatest effect and highest mortality percentage. Boopathi *et al*. (2015) found similar results when they tested *V. lecanii* against *A. disperses* (Hemiptera, Aleyrodidae) and highlighted that *V. lecanii* exhibited promising levels of control (> 70% mortality) among the other tested fungi which were *B. bassiana* and *M. anisopliae*. However, our results were not in accordance with Javed *et al.* (2019) who reported *B. bassiana* was more effective than *V. lecanii* against whiteflies under laboratory conditions. This contradiction might either due to the difference in experimental conditions in both studies, as our work was carried out in the open field which might influence *B. bassian* virulence. Or other explanation could be the difference in used host plant as they tested the fungus on whiteflies reared on tomato potted plants but we tested it on eggplant. This explanation is supported by the findings of Ollek *et al*. (2014), who stated that susceptibility of *B. tabaci* to *B. bassiana* is significantly affected by host plants.

Biological control of the inoculative type expects that the agent will multiply, spread, and provide extended control of an insect pest, but only for a limited period of time. However, this requires the ability for reproduction and horizontal transmission (Pell *et al*., 2009). Repeating fungi application for several times could help overcoming this issue through supporting fungal persistence for longer periods and consequently increasing pest's control level. This point of view was confirmed through our results as we find that spraying for four successive weeks increased fungi effectiveness and caused ascending control levels of *B. tabaci*, whereas the contrary occurred in case of control plot during the two experimental seasons as number of the pest increased gradually.

Efficient pest management toward maximizing actual yields on existing farmland is crucial. The idea of "heavy pest attack may result in total crop failure and devastate production" was confirmed by many authors. Crop yield depends, to a high extent, on the pests' pressures farmers face and on provided control treatments (Ronald *et al*., 2020; Waterfield and Zilberman, 2012). This opinion was confirmed through the current study. Production rates were in line with percentage reductions of *B. tabaci*. The largest yield of eggplant was produced in the plot treated with *V. lecanii* that caused the highest control level compared to the other two fungi (*B. bassiana* and *M. anisopliae*) and the control which gave the least production rate. Production rates augmented in the fungal treated plots to reach their maximum levels by the end of the 4th week, whereas the opposite occurred in case of control plot, which was treated with water only, to reach its least level by the end of the 4th week. This might be attributed to percentage reduction of *B. tabaci* in the treated plots and their enlargement in the control.

CONCLUSION

Entomopathogenic fungi are promising candidates as they have major power to invade *B. tabaci* by contact, cause high percentage reduction under field condition, and lead to high production rates in eggplant fruits after four weeks of treatment. The most promising fungus of the tested ones was *V. lecanii,* followed by *M. anisopliae* and finally comes *B. bassiana.* High production leads to enhanced farmers' income and consequently livelihoods. Accordingly, *V. lecanii,* is recommended to be integrated in whiteflies control strategies in Egypt. For further investigations, attention must be given to the compatibility of entomopathogenic fungi with other bio-control agents to study their effectiveness on whitefly and on each other. Insights benefits of such studies might lead to the effective use of these promising organisms as an integral part of Egyptian agricultural systems.

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التأثير المقارن لثلاث فطريات ممرضة للحشرات ضد الذبابة البيضاء *Bemisia tabaci* التي تصيب الباذنجان تحت الظروف الحقلية في محافظة كفر الشيخ ـ مصر

مها صلاح ندا[1] ، هاله عادل[2]* ، سعد جعفر[2] * و أحمد طمان[1]

[1] معهد بحوث وقاية النباتات – مركز البحوث الزراعية – دقى ـ جيزة

[2] المعمل المركزي للزراعة العضوية ، مركز البحوث الزراعية ، مصر

يعتبر استخدام الفطريات الممرضة للحشرات ، كعامل قوي للمكافحة الحيوية ، أحد الأساليب الواعدة في برامج المكافحة المتكاملة للآفات. يهدف البحث الحالي إلى اختبار كفاءة *Metarhizium anisopliae* و *Verticillium lecanii* و *Beauveria bassiana* ضد الذبابة البيضاء على الباذنجان (صنف Anann) في محافظة كفر الشيخ ، مصر. نفذت التجربة بتصميم القطاعات العشوائية الكاملة في مساحة فدان، مقسمة إلى 4 قطع (5 مكررات لكل قطعة). امتدت الدراسة من يوليو حتى سبتمبر خلال موسمين متتاليين (2019 و 2020) وتم رش كل منتج فطري أربع مرات (رشة واحدة / أسبوع). تراوحت درجة الحرارة من 29 إلى 35 درجة مئوية وتراوحت الرطوبة النسبية من 49 إلى 52٪. أشارت النتائج إلى أن استخدام الفطريات الممرضة للحشرات أدى إلى انخفاض معنوي في الذبابة البيضاء *B. tabaci*. كان الفطر *V. lecanii* أكثر الفطريات تأثيراً، حيث تسبب في أعلى نسبة مكافحة مقارنة بالفطريتين الأخريين والكونترول. أدى تكرار المعاملة خلال 4 أسابيع متتالية إلى زيادة فعالية الفطريات، وتسبب في ارتفاع الوفيات في تعداد *B. tabaci* . كانت معدلات الإنتاج متوافقة مع النسبة المئوية لنقص الذبابة البيضاء. وتم إنتاج أكبر محصول من ثمار الباذنجان في القطعة المعاملة بـ *V. lecanii*. وعليه ، توصي الدراسة باستخدام *V. lecanii* في استراتيجيات مكافحة الذبابة البيضاء في مصر. علاوة على ذلك ، هناك حاجة إلى مزيد من الأبحاث لدراسة مدي توافق *V. lecani* مع العوامل الحيوية الأخرى لتجنب عدم الاتساق عند استخدامهما معًا في برامج إدارة الآفات.