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Entomopathogenic microbes naturally infecting whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) on vegetable plants

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Abstract
Twelve fungal species naturally infected *B. tabaci* in tomato, cucumber and cabbage fields were recorded from October (2021) to September (2022). Seven genera showed safety to plants. Five of them showed good epizootic and ability to establish themselves in the environment. Theses genera were: *Beauveria bassiana*, *Metarhizium anisopliae, Cladosporium cladosporioides*, *Trichoderma longibrachiatum* and *Verticillium lecanii*. Also, two species of *Bacillus* were recorded. They were identified according to 16S rRNA gene sequencing as *Bacillus thuringiensis* and *B. velezensis*. They were bioassayed for contact toxicity to both adults and the 3rd instar nymphs, and both fungal and bacterial isolates showed significant virulence qualifying them to be used as eco-friendly microbial control agents.

Keywords: *Bemisia tabaci*, fungi, bacteria, identification, and contact toxicity

1. Introduction
Bemisia tabaci (Gennadius) (Homoptera: Sternorrhyncha: Aleyrodidae) is a major serious pest infesting over 600 plants belong to ornamentals and horticulture crops, in both open fields and greenhouses all over the world [1]. It causes authentic damage and economic losses to the susceptible crops [2]. Host plant damage may be happened directly by adults and nymphs feeding on phloem sap [3], and indirectly by honeydew secretion which promotes the growth of sooty mold that impairs photosynthesis [4] and adversely affecting on crop productivity [5, 6]. In addition, *B. tabaci* is a transporter of more than 300 plant virus species [7, 8] including Begomovirus, Crinivirus, Carlavirus, Ipomovirus, and Torradovirus [9-12]. In some crops, the plant viral infections lead to plant growth suppression or total crop loss [13]. Excessive use of chemical insecticides adversely affected on humans, non-target organisms and environment. Also, it develop the insect resistance and subsequently population outbreaks [14, 15]. Therefore, searching for secure alternatives is a must. One of the most significant alternatives is using microbial control agents to control the insect pests. The aim of the present study was, therefore, to survey, identify and test the pathogenicity of the microbes naturally associated with both adults and nymphs of *B. tabaci* in order to employ and integrate them in programs of *B. tabaci* control.

2. Materials and methods
2.1 Insect Collection
B. tabaci adults and nymphs were collected from tomato, *Solanum lycopersicum*; cabbage, *Brassica oleracea* var. capitate, and cucumber, *Cucumis sativus* fields in Dakahlia governorate, Egypt from October 2021 to September 2022. Alive whiteflies without and/or with any infection symptoms were collected then transferred to the laboratory and were quickly surface sterilized in 70% ethanol, in 5% sodium hypochlorite solution followed by three washes in sterile water [16].

2.2. Isolation and Purification of the microbes
Each *B. tabaci* cadaver was crushed and homogenized in 1ml of sterilized water, then one drop of this homogenate was incubated in Autoclaved Sabouraud dextrose yeast extract agar

(SADYA) media [40 g/l dextrose, 10 g/l yeast extract, 10 g/l peptone, and 20 g/l agar] at 25± 2 ℃ and 70±5% RH until further growth. Then, cultures were purified using single spore or hyphal tip technique for fungi and using streak plate method for bacteria.

2.3. Identification of the surveyed microbes

2.3.1. Identification of the fungal isolates

The fungal isolates were identified based on the morphotaxonomic characteristics of spores according to the keys of Humber (1997) [17].

2.3.2. Identification of the bacterial isolates

2.3.2.1. DNA isolation and Polymerase Chain Reaction (PCR): Genomic DNA was extracted from the tested bacteria using QIAamp DNA Micro Kit (QIAGEN, Germany) according to the manufacturer's instructions. The PCR amplification was performed in a total volume of 50 µl, containing 25 µl Master Mix (sigma), 3 µl of each primer (10 pcmol/ µl) (Table 1), and 3 µl template DNA (10ng/ µl) and 16 µl dH_2O. PCR amplification was conducted in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to perform an initial denaturation cycle for 5 min at 94 ℃, then fulfilled 40 cycles each cycle consisted of a denaturation step at 94 ℃ for 30 sec., an annealing step at 45 ℃ for 30 sec. and an elongation step at 72 ℃ for 1 min. In the final cycle, the primer extension segment was extended to 7 min at 72 ℃.

Table 1: Primers employed in the current study.

Primer Code	Sequence	Product Size
27F	5′- AGAGTTTGATCCTGGCTAG -3′	1500 bp
1492R	5′- GGTTACCTTGTTACGACTT -3′	

The amplification products were subjected to electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was considered as a molecular size standard. PCR products were visualized by UV light and photographed using a Gel Documentation System (BIO-RAD 2000). Purification of PCR products were conducted by using EZ-10 spin column PCR products purification. PCR reaction mixture was transferred to 1.5 ml microfuge tube and three volumes of binding buffer 1 was added. Then, the mixture solution was transferred to the EZ-10 column and let it stand for 2 minutes at room temperature then centrifuge. 750 ul of wash solution was added to the column and centrifuge at 10.000 rpm for two minutes, repeated washing, and complete centrifuge at 10.000 rpm for an additional minute to get rid of any residual wash solution. The column was transferred into a clean 1.5 ml microfuge tube and adds 50 ul of elution buffer, incubated for 2 minutes at room temperature and when store purified DNA at -20 °C.

2.3.2.2. 16s sequencing analysis

Automatic sequencer ABI PRISM 3730XL Analyzer using Big Dye TM Terminator Cycle Sequencing Kits was used for sequencing the product PCR following the manufacturer's protocols. Single-pass sequencing was performed on each template using Rbcl Forward primer. Purification of the fluorescent-labeled fragments from unincorporated terminators was conducted with an ethanol precipitation protocol. The samples were homogenated in distilled water and analyzed by electrophoresis in an ABI 3730xl sequencer (Microgen Company). BLAST program was used for analyzing the sequences [18] and Align Sequences Nucleotide BLAST for sequences alignment.

2.4. Rearing of whitefly *B. tabaci*

A pure strain of *B. tabaci* biotype B was acquired from tomato plants under glass greenhouse at Faculty of Agriculture, Mansoura Univ. The whitefly strain was kept on tomato seedlings planted in small pots inside plastic cages under laboratorial conditions of 27±2 ℃, 75±5 RH and photoperiod of 14:10 Light: Dark.

2.5. Preparation of microbial suspensions

Isolates from SADYA slant was transferred into a fresh SADY broth and incubated at 27 °C for complete growth/sporulation. Spores from 14 days old fungal cultures were harvested and suspended in sterilized 0.05% aquoes Tween 80, then, filtered through nylon mesh to get rid of mycelia. Also, bacterial cells were harvested from 10 days old culture broth. Then the bacterial cells and/or spores were counted using a haemocytometer Neeubauer. Series of concentrations of each tested isolate were prepared.

2.5. Virulence of the tested microbes against *B. tabaci*

2.5.1. Contact toxicity on the third instar stage nymphs

Each 50 Adults of *B. tabaci* were released on a tomato seedling with 5 leaves, planted in small cubs kept in plastic cage with fine netting side panels for ventilation and allowed to oviposit for 48 hrs on leaves. Then, adults were removed, and whitefly eggs were allowed to develop into the third instar stage nymphs for assessment. Thirty individuals of the third instar stage nymphs were counted and marked with a water proof colored pen under dissecting microscope to represent a replicate. Three replicates in addition to control (sprayed only with water and 0.05% Tween 80) were used per treatment.

2.5.2. Contact toxicity on adults

A sterilized tomato seedling with 3 leaves was sprayed with the tested concentration and kept in a cage. Then, after an hour of treatment, thirty adult individuals of *B. tabaci* were released inside the cage. Each cage represented a replicate, where each concentration represented by three ones, in addition to three replicates sprayed only with water and 0.05% Tween 80 to serve as control. The dead individuals were recorded daily.

Contact toxicity experiments for both nymphs and adults were conducted in stable conditions of 27±2 ℃, 70±5 RH and 14:10 L: D. Mortality percentage was recorded daily along the seven days of the experiment.

2.6. Statistical analysis

The average of mortality percentages of *B. tabaci* adults and /or the third instar stage nymphs were calculated and corrected using Abbott's formula (1925) [19]. The LC_{50}, LC_{90} and slope values were estimated [20]. Also, the efficiency of different tested entomopathogens was measured and compared with the most effective one [21].

3. Results and Discussions

3.1 Identification of the isolated microbes associated with *B. tabaci*

3.1.1 Identification of the fungal associates

The present study recorded 12 fungal species infected *B.*

tabaci in tomato, cucumber and cabbage fields from October (2021) to September (2022) as shown in Table 2. They belong to phylum Ascomycota (11 hyphomycetes* and one Mucoromycota (Mucorales**). Seven genera showed safety to plants. Five genera of them showed good epizootic and proved their capacity to establish their selves in the environment. This was seemed clear through their coexistence with the pest in different weather conditions almost year-round. Theses genera were: *B. bassiana*, *M. anisopliae, C. cladosporioides*, *T. longibrachiatum* and *V. lecanii*.

Table 2: Fungal species naturally infecting *B. tabaci* in tomato, cucumber and cabbage fields from October (2021) to September (2022) in Dakahlia governorate

Fungi	Safety to plants	Host-Plant	Period
B. bassiana (Ascomycota).	Safe	Tomato	All year-round except January and August
		Cucumber	March, April, May and October
		Cabbage	February, March, April and May
M. anisopliae (Ascomycota).	Safe	Tomato	February, March, April, May, October and November
		Cucumber	February, March, April and November
		Cabbage	February, March, April and October
C. cladosporioides (Ascomycota).	Safe	Tomato	All year-round except July and August
		Cucumber	All year-round except August
		Cabbage	March, April, May, June, October and November
T. longibrachiatum (Ascomycota).	Safe	Tomato	March, April, May, June and November
		Cucumber	February, March, April, May, June, November
		Cabbage	March, April, May and November
V. lecanii (Ascomycota).	Safe	Tomato	March, April, May and November
		Cucumber	March, April, May, November and December
		Cabbage	February, March, April, May, June and November
Epicoccum sp. (Ascomycota).	Safe	Tomato	February and March
		Cucumber	March, April and May
		Cabbage	February, March and November
Paecilomyces fumosoroseus	Safe	Tomato	February, March, April
		Cucumber	February, March
		Cabbage	January, February, March and December
Aspergillus niger (Ascomycota).	Not safe	Tomato	May, June, July, August and September
		Cucumber	June, July, August and September
		Cabbage	June, July and August
A. Flavus (Ascomycota).	Not safe	Tomato	April, May, June, July, August and September
		Cucumber	May, June, July, August and September
		Cabbage	May, June and July
Fusarium oxysporum (Ascomycota).	Not safe	Tomato	All year-round except January and July
		Cucumber	All year-round except January and February
		Cabbage	--------
F. solani (Ascomycota).	Not safe	Tomato	March, April, May, October and November
		Cucumber	March, April, May, September and October
		Cabbage	April
Mucor sp. (Mucoromycota, Mucorales).	Not safe	Tomato	June and July
		Cucumber	July and October
		Cabbage	-------

B. bassiana

It formed wide white circular colonies with smooth cottony surface texture turned to powdery type with age. It showed a flask-like shape in a conidiogenous cell, a branched conidiophore. Hyaline, smooth walled conidia were spherical to sub-globose in shape, Fig. 1. Earlier, the nature infection of *B. tabaci* by this fungus was recorded [22-25].

M. anisopliae

It formed circular colonies and a milky mycelium with cottony texture. Hyaline cylindrical conidia, with round edges and olive-green color with an average diameter of 4.5 µm, Fig. 2. In previous studies, it was already recorded as natural microbial agent infecting whiteflies in tomato fields [26].

C. cladosporioides

Colonies are mostly olivaceous-green to olivaceous- brown. Branched conidiophores bearing many conidial chains arising below septa without swellings or sympodial elongations. Ellipsoidal, ovoid smooth-walled conidia are mostly olivaceous-brown, Fig. 3. The natural infection of *Cladosporium* spp of *B. tabaci* nymphs was 87.8% in Dakahlia Governorate [27]. Also, It has been reported to infect many species of insects naturally such as aphids and whiteflies [28], *Aphis craccivora* [29] and *Brevicoryne brassicae* [30].

T. longibrachiatum

Hyaline branched phialides are flask –shape, appeared solitary or in clusters, and are attached to hyaline conidiophores which branched constructing a pyramidal arrangement. Conidia are round or ellipsoidal, smooth walled or rough, green colored with an average diameter of 3 µm. It produce characteristic greenish-yellow pigments in the reverse of cultures, Fig. 4. It was recorded for the first time in all over the world as an entomopathogenic agent when isolated from *A. craccivora* [29].

V. lecanii

Colonies are velvet-like. Hyaline conidiophores are erect,

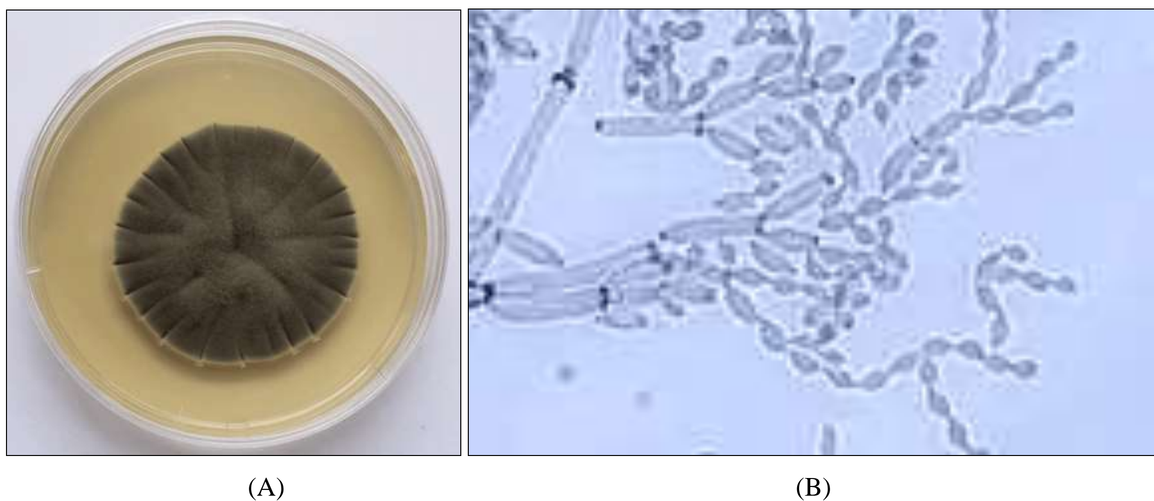
septate, simples or branched. phialides bears clusters of cylindrical, ellipsoid spores, Fig. 5. The nature infection of this fungus was recorded in Aleyrodidae sp. [31].

(A) (B)

Fig 1: (A) Culture of *B. bassiana* on SADYA media, (B) Light micrograph showing branched conidiophores bearing numerous spherical to sub-globose conidia.

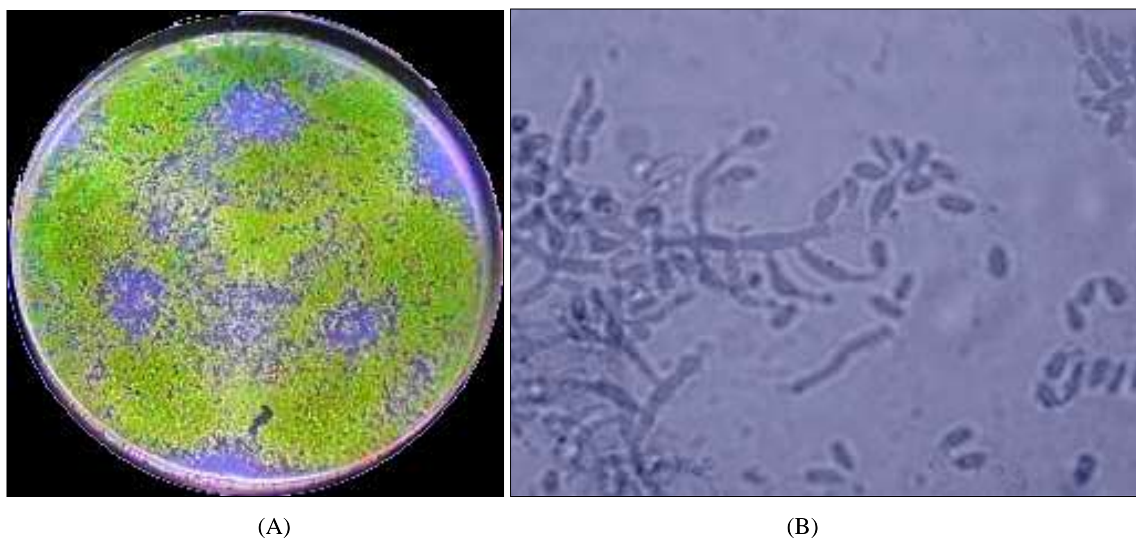
(A) (B)

Fig 2: (A) Culture of *M. anisopliae* on SADYA media, (B) Light micrograph showing branched conidiophores bearing numerous spherical to sub-globose conidia.



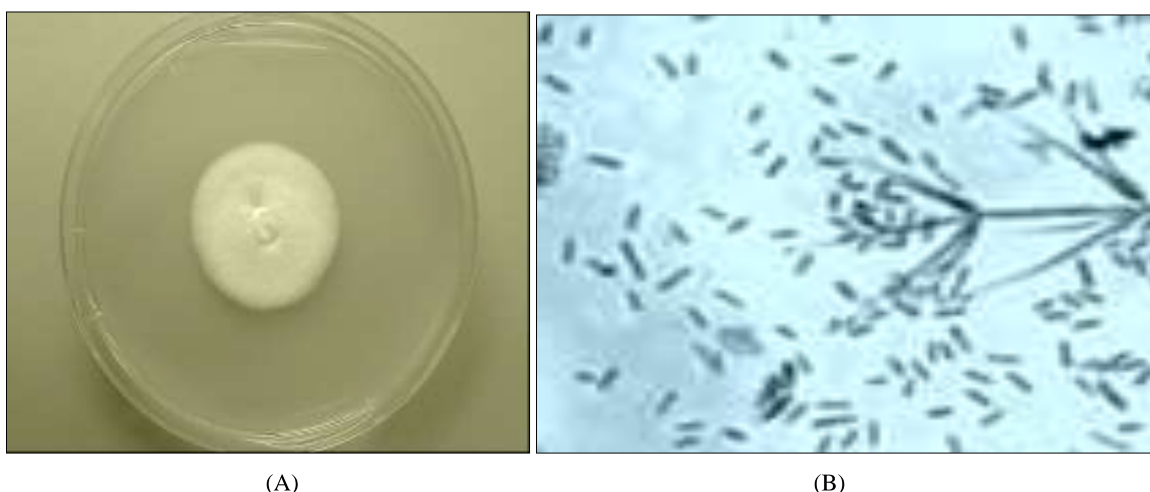
(A) (B)

Fig 3: (A) Culture of *C. cladosporioides* on SADYA media, (B) Light micrograph showing branched conidiophores bearing chains of ellipsoidal conidia.



(A) (B)

Fig 4: (A) Culture of *T. longibrachiatum* on SADYA media, (B) Light micrograph showing flask –shape branched phialides. Conidia are round or ellipsoidal.



(A) (B)

Fig 5: (A) Culture of *V. lecanii* on SADYA media, (B) Light micrograph showing phialides bears numerous cylindrical, ellipsoid spores.

3.1.2 Dentification of the bacterial isolates

16Sr DNA BLASTx analyses revealed two bacterial isolates associated with *B. tabaci*. These bacterial isolates were identified as *B. thuringiensis* strain FDAARGOS_793 and *B. velezensis* strain IICE-1. The phylogenetic tree of the bacterial isolates were constructed as shown in Fig. 6&7. *Bacillus* species are one of the most important biocontrol agents for controlling plant pathogens [32]; act as insect pathogens [33, 34], and biofertilizers or biostimulators [35].

B. thuringiensis is a rode Gram-positive, endospore-forming bacterium. It produces indigenous crystals during sporulation. These crystals are mostly composed of one or more proteins (Cry and Cyt toxins) [36], which is specific for insect orders causing gut paralysis in some insect orders, leading to feeding-inability, starvation then death [37].

B. velezensis is a widely distributed Gram-positive aerobic, endospore-forming bacterium. It showed plant growth promotion and suppression of many pathogenic fungi growth, including *A. flavus* [38], *F. oxysporum* [39], bacteria, and nematodes by biosynthesis of secondary metabolites, such as lipopeptide antibiotics and β-1,3-1,4-glucanase [40, 41].

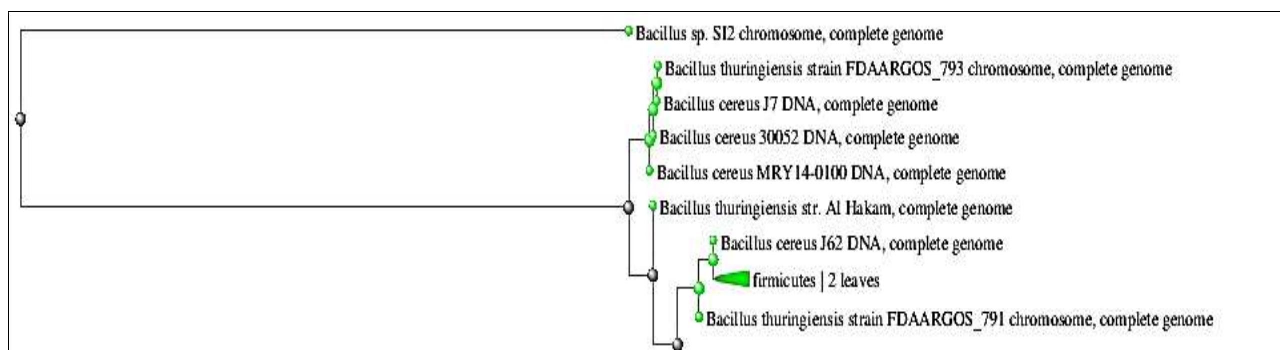


Fig 6: Phylogenetic tree of *B. thuringiensis* isolate

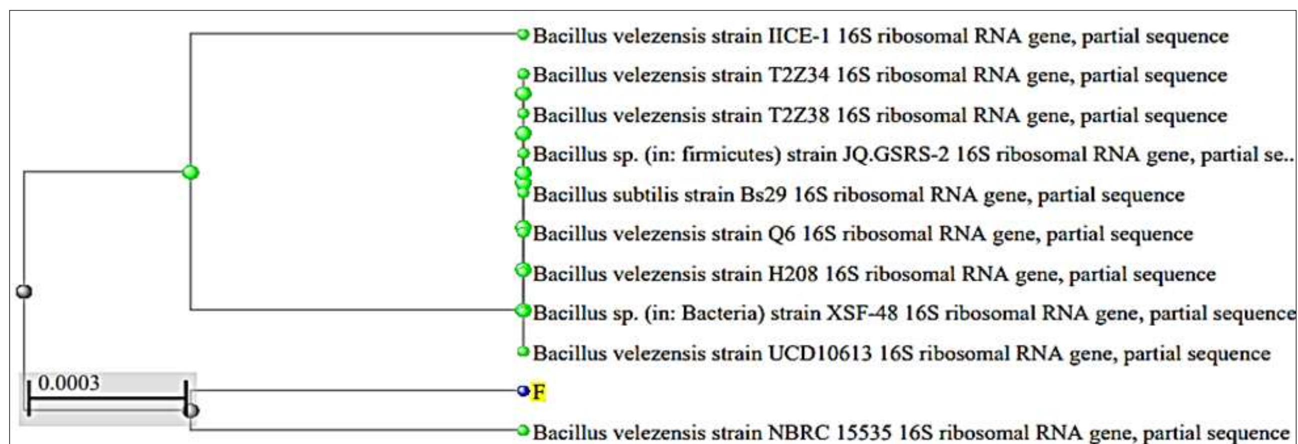


Fig 7: Phylogenetic tree of *B. velezensis* isolate

3.2 Virulence of the tested microbes against *B. tabaci*

Potentiality of the five best fungal isolates: *B. bassiana*, *M. anisopliae, C. cladosporioides*, *T. longibrachiatum* and *V. lecanii* in addition to the two bacterial isolates, *B. thuringiensis* and *B. velezensis* were evaluated against both of the 3rd instar stage nymphs and adults. Data in Table 3 & 4 show that mortality percent of *B. tabaci* is directly proportional to the time elapsed after treatments with different tested microbes. In spite of delayed effect of the tested fungi, they revealed significant potentiality against both nymphs and adults after the 3rd day of treatment. This slow action was due to the nature mechanism of fungi depending on the time intervals for tissues invasion, depletion of nutrient resources and accumulation of toxins inside their cadavers. In contrast, bacterial isolates showed fast effect after 24 hrs reaching the top at the 5th day post treatment.

Regarding to the 3rd instar nymphs, *C. cladosporioides* was the most potential agent followed by *B. bassiana, V. lecanii*, *T. longibrachiatum*, *M. anisopliae*, then *B. thuringiensis* and *B. velezensis* with LC_{50}s: 15.2x 10^4, 20.3x10^4, 2.1 x10^5, 4.2x10^5, 5.3x 10^5, 6.1x 10^5, 15x 10^6 and toxicity indexes at LC_{50} of 100.00, 74.83, 72.774, 36.12, 28.90, 24.913 and 10.465%, respectively.

As for the adults, *M. anisopliae* showed the most efficacy for suppressing adults followed by *C. cladosporioides*, *B. thuringiensis*, *B. bassiana, T. longibrachiatum*, *B. velezensis*, then *V. lecanii* with LC_{50}s: 1.9x 10^5, 2.1x 10^5, 2.5x 10^5, 3.2x 10^5, 3.6x 10^5, 4.6x 10^5, 7.6x 10^5 and toxicity indexes at LC_{50} of 100.00, 91.149, 78.66, 60.193, 53.315, 42.457 and 25.547%, respectively. From the above, it is clear that there were clear difference of adults and the 3rd instar nymphs susceptibility to the tested microbial isolates. This may be due to variation of different stage insect host habitat (settled or mobile) when feeding and its relation to the exposure rate to the tested microbes. Also, it may be due to different body topology of the insect stages and its relation of the attachment and consolidation of the pathogen within the host.

Present results agreed with previous studies proved efficacy of *B. bassiana* which caused 91.8% mortality on whiteflies 4th instar nymphs within 8 days on vegetable crops [42]. Also, it was demonstrated that *B. bassiana* reduced whitefly eggs by 65.3% and nymphs by 88.82% on cotton and tomato fields [43]. In another study, application of *B. bassiana* and *M. anisopliae* with synthetic insecticides goal mortality rate of whiteflies ranging from 62 to 84% [44].

Also, the pathogenicity of the entomopathogenic fungal isolates of *M. anisopliae* and *B. bassiana* was evaluated against adults and 2nd instar nymphs of whitefly, *Trialeurodes vaporariorum*. The tested isolates caused mortality of 45–93% in adults and 24–89% in the nymphs. *M. anisopliae* strains showed higher virulence to both developmental stages as compared to *B. bassiana* strains [45].

The efficacy of *V. lecanii* was previously proved Reducing whitefly population on tomato, cucumber and melon [46]. Also, previous study reported a high mortality, 71% of *B. tabaci* on eggplant leaves under laboratory conditions when treated with at 1x10^8 conidia/mL of *C. cladosporioides* [47].

Also, high Virulence of *B. thuringiensis* isolate was reported against first- instar nymphs and newly emerged adults of *B. tabaci* with LC_{50} values 17.7 and 18.21 CFU/ml respectively [48].

Table 3: Efficiency of the tested entomopathogenic microbes against 3rd instar nymphs of *B. Tabaci* under laboratory conditions of 27±2 °C, 70±5 RH and 14:10 L: D.

Treatment	Conc. (cell/ml)	Mortality % at indicated day after treatment. 3 day	5 day	7day	LC_{50} (cell/ml) and confidence limits at 95%		LC_{90} (cell/ml) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
B. bassiana	1x10^5	26.67	36.67	43.33	20.3x10^4		6.4x10^7		0.514 ± 0.122	0.538	74.83
	1x10^6	36.67	50.00	66.67							
	1x10^7	46.67	63.33	76.67	18.8x 10^3	67.9x 10^4	14.2x 10^6	21.2x 10^8			
	1x10^8	53.33	73.33	93.33							
M. anisopliae	1x10^5	13.33	26.67	36.67	5.3x10^5		29.5x 10^7		0.466 ± 0.114	0.144	28.90
	1x10^6	20.00	36.67	56.67							
	1x10^7	30.00	46.67	70.00	6.7 x10^4	17.4 x10^5	9.6 x10^7	35.1 x10^9			
	1x10^8	46.67	66.67	86.67							
C. cladosporioides	1x10^5	30.00	40.00	46.67	15.2 x10^4		52.4x 10^6		0.51 ± 0.123	0.143	100.00
	1x10^6	36.67	53.33	66.67							
	1x10^7	40.00	63.33	80.00	10.5 x10^3	54.2 x 10^4	11.8 x10^6	18.5 x10^8			
	1x10^8	53.33	76.67	93.33							

T. longibrachiatum	1x10^5	23.33	33.33	36.67	4.2x10^5		3.8x10^8		0.434 ± 0.113	0.308	36.12
	1x10^6	33.33	46.67	60.00							
	1x10^7	33.33	60.00	73.33	3.6 x10^4	15.3x 10^5	5 x10^7	10.6 x 10^{10}			
	1x10^8	40.00	70.00	83.33							
V. lecanii	1x10^5	20.00	30.00	43.33	2.1 x10^5		8.9x 10^7		0.488 ± 0.12	0.018	72.774
	1x10^6	26.67	46.67	63.33							
	1x10^7	36.67	63.33	80.00	1.6x 10^4	7.3 x 10^5	17.8x 10^6	4.6x 10^9			
	1x10^8	43.33	70.00	90.00							
B. thuringiensis	1x10^5	30.00	36.67	36.67	6.1x 10^5		2.9x 10^9		0.348 ± 0.108	0.277	24.913
	1x10^6	46.67	56.67	56.67							
	1x10^7	56.67	66.67	66.67	2.3x 10^4	2.9x 10^6	15.7x 10^7	2.2x 10^{14}			
	1x10^8	70.00	76.67	76.67							
B. velezensis	1x10^5	23.33	26.67	30.00	1.5x 10^6		19.6 x10^8		0.41 ± 0.11	0.134	10.465
	1x10^6	40.00	50.00	50.00							
	1x10^7	56.67	63.33	63.33	2.3x 10^5	5.7x 10^6	16.2x 10^7	33.6x 10^{11}			
	1x10^8	63.33	76.67	76.67							

Table 4: Efficiency of the tested entomopathogenic microbes against adults of *B. Tabaci* under laboratory conditions of 27±2 Cº, 70±5 RH and 14:10 L: D.

Treatment	Conc. (cell/ml)	Mortality % at indicated day after treatment.			LC$_{50}$ (cell/ml) and confidence limits at 95%		LC$_{90}$ (cell/ml) and confidence limits at 95%		Slope ± SE	X^2	Toxicity index
		3 day	5 day	7day							
B. bassiana	1x10^5	23.33	33.33	40.00	3.2x 10^5		1.1x 10^8		0.505 ± 0.12	0.016	60.193
	1x10^6	36.67	50.00	60.00							
	1x10^7	46.67	60.00	76.67	3.9x 10^4	10.3x 10^5	2.3x 10^7	4.9x 10^9			
	1x10^8	56.67	76.67	90.00							
M. anisopliae	1x10^5	20.00	36.67	46.67	1.9x 10^5		3.8x 10^8		0.389 ± 0.113	0.093	100.00
	1x10^6	23.33	46.67	60.00							
	1x10^7	36.67	56.67	73.33	4.2x 10^3	8.8x 10^5	4.2x 10^7	4.8x 10^{11}			
	1x10^8	53.33	70.00	86.67							
C. cladosporioides	1x10^5	26.67	36.67	46.67	2.1x 10^5		8.2x 10^7		0.495 ± 0.12	0.646	91.149
	1x10^6	33.33	46.67	60.00							
	1x10^7	40.00	60.00	76.67	1.8x 10^4	7.3x 10^5	1.7x 10^7	3.7x 10^9			
	1x10^8	56.67	80.00	93.33							
T. longibrachiatum	1x10^5	26.67	36.67	40.00	3.6x 10^5		20.2x 10^7		0.467 ± 0.115	0.086	53.315
	1x10^6	30.00	50.00	56.67							
	1x10^7	40.00	63.33	76.67	3.7x 10^4	1.2x 10^6	33.5x 10^6	20.1x 10^9			
	1x10^8	50.00	73.33	86.67							
V. lecanii	1x10^5	13.33	30.00	33.33	7.6x 10^5		4.4x 10^8		0.464 ± 0.113	0.028	25.547
	1x10^6	26.67	43.33	53.33							
	1x10^7	36.67	53.33	70.00	1.2x 10^5	2.5x 10^6	6.2x 10^7	64.8x 10^9			
	1x10^8	43.33	63.33	83.33							
B. thuringiensis	1x10^5	36.67	43.33	43.33	2.5x 10^5		5.9x 10^8		0.379 ± 0.112	0.026	78.66
	1x10^6	50.00	60.00	60.00							
	1x10^7	60.00	73.33	73.33	5.8x 10^3	11.1x 10^5	5.6x 10^7	1.7x 10^{12}			
	1x10^8	80.00	83.33	83.33							
B. velezensis	1x10^5	26.67	36.67	36.67	4.6x 10^5		5.1x 10^8		0.421 ± 0.112	0.33	42.457
	1x10^6	46.67	60.00	60.00							
	1x10^7	53.33	70.00	70.00	3.6x 10^4	1.7x 10^6	6x 10^7	2.5x 10^{11}			
	1x10^8	80.00	83.33	83.33							

4. Conclusion

The present study focused on the importance of identification of the microbes naturally associated with *B. tabaci* to enable us employing them as safe alternatives of traditional chemical insecticides for controlling *B. tabaci*. Our results indicated that there were variety of microbes infecting *B. tabaci* in different vegetable fields and greenhouses. They are belonging to fungi and bacteria and when evaluated for pathogenicity, they showed high contact toxicity to both of adults and the 3rd instar nymphs. This encouraging further studies for developing their application in wide scales as eco-friendly insecticides.

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