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Bioassay Evaluation of the Entomopathogenic Fungi, *Beauveria bassaina* Vuellemin Against Eggs and Nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae)

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Abstract: This study was carried out to determine the lethal effect of the entomopathogenic fungi, *Beauveria bassaina* Vuell. on eggs, young and old nymphs of the whitefly, *Bemisia tabaci* Genn. Mortality percentage was significantly differed based on stage of *B. tabaci* and conidial concentrations of *B. bassina*. Average of the infection level to insect was very low particularly in eggs with only 4.49%, even with higher conidial concentrations (6×10^6 conidia mL^{-1}). Whereas, it was higher with 1st and 2nd instars (42.045%) and 3rd and 4th instars (35.93%). Three parameters was assessed with *B. tabaci* eggs, namely; egg infection, egg hatchability and crawlers emergence. Egg mortality percentages averaged 1.2, 4.27 and 8.0% with fungal concentration 2×10^6, 4×10^6 and 6×10^6 conidia mL^{-1}, respectively. Daily infection percentages were varied depend upon the conidial concentration where the highest infection rate of eggs was occurred with 6×10^6, followed by 4×10^6 conidia mL^{-1}. Egg hatch was very high, while the mortality among the emerged crawlers was neglectable compared with the check. Efficiency of *B. bassaina* on whitefly nymphs also was varied based on the insect instar and fungal concentration. Mortality percentages were obviously higher to young nymphs (1st and 2nd instars) than to older ones (3rd and 4th instars). The results indicated that nymphs were highly susceptible to fungal treatment compared with eggs. Additionally, pathogenicity and virulence of *B. bassaina* against *B. tabaci* immatures was not indicated by LC_{50} only, but also, by the time in days (LT_{50}) required to achieve 50% mortality of an insect.

Key words: Whitefly, *Bemisia tabaci*, entomopathogenic fungi, *Beauveria bassaina*, biological control

INTRODUCTION

The whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), is one of the most cosmopolitan, intractable and damaging insect pests in tropical and subtropical areas within an agricultural and horticultural production systems (Brown and Bird, 1992; Brown, 1994; Perring, 2001; Carabali *et al*., 2005). Since late 1980's, the insect has risen from relative obscurity to become one of the primary insect pests of agriculture worldwide (Castle, 2006; Lin *et al*., 2007). At least 24 biotypes of this pest have been identified around the world (Peering, 2001; Dong *et al*., 2007), which suggest that *B. tabaci* is a species complex (Brown *et al*., 1995). Therefore, it is considered one of the worst world's top 100 invasive species (International Union for the Conservation of Nature and Natural Resources (IUCN) list (http://www.issg.org)). It has been found as a pest of tobacco in Greece 100 years ago (Gennadius, 1889). Regardless of habitat change, *B. tabaci* has a long history as a serious pest of field and greenhouse crops (Byrne and Bellows, 1991).

Bemisia tabaci species complex are multivoltine and highly polyphagous pests. A major reason for its expansion in importance and geographical range appears to be the replacement of native races or biotypes by ones of greater economic significance that are capable of: i) invading new cropping systems (Peering, 2001), ii) developing on a broad range of cultivated and non-crop species (over 900 different plant species, belonging to 74 families (Summers *et al*., 1995; Secker *et al*., 1998), iii) inducing physiological disorders through feeding (Schuster *et al*., 1995), iv) vectoring of over 100 plant viruses (Jones, 2003) and v) rapidly evolving resistance to chemical control agents (Ma *et al*., 2007; Erdogan *et al*., 2008). *B. tabaci* estimated annual economic losses ranged from several hundred millions to billions of dollars worldwide (Oliveira *et al*., 2001).

Chemical control provides only short-term solutions. Moreover, the overuse of pesticides, in controlling whiteflies, has provoked the development of resistance to insecticides (Denholm, 1988; Dittritch *et al*., 1990; Damásio *et al*., 2007). Environmental residues and human health safety are also a concern, supported by consumers

demand of pesticide-free food. Thus, many countries are trying to reduce their use of pesticides (van Lenteren, 2000; Carvalho, 2006) by developing an alternative safety control methods. Since myco-insecticides considered of the most effective alternative control method (Latge and Papierok, 1988; Hajek and St-Leger, 1994), therefore, the success of pest control program depends on conidia survival in the field environment (De La Rosa *et al*., 2000). Conidia survival may be affected either by environmental factors, host nutritional status and/or control products used to protect crop plants (Monzon *et al*., 2008).

Beauveria bassiana Vuill (Moniliales: Deuteromycetes) is considered to be the most promising candidate entomopathogenic fungi against whiteflies (Olson and Oetting, 1999; Faria and Wriaght, 2007). Also, the fungus has potentiality to infect a wide host range of insects within different orders including, Homoptera, Hymenoptera, Lepidoptera, Coleoptera and etc., most of which are agricultural pests (Padmaja and Kaur, 2001).

Laboratory and field bioassay have revealed that *B. bassaina* to be an effective pathogen against whiteflies when applied directly as a concentrated conidial suspension (Wraight *et al*., 2000). Nevertheless, acceptance of the myco-insecticides has been limited among farmers compared with conventional chemical control products. This may be due to low performance and rapid lose of effectiveness (St. Legar and Screen, 2001; Quesada-Moraga *et al*., 2006). In contrary, the advantages of the use of myco-insecticides include, no-resistant could be earned among insect pests and it is also more safer to non-target insects (Castrillo *et al*., 2008; Monzón *et al*., 2008).

Beauveria bassaina, which disease insect pests, is characterized by rapid sporulation and germination, with high virulence and active discharge of conidia, all attractive traits in a fungal pathogen (Hall and Papierok, 1982; Hall, 1985; Fransen, 1990). Moreover, *B. bassaina* is cheap to mass produce, easy to store and effective over a wide range of temperatures and humidity levels. It also provides a rapid kill at economical doses and, recently, the fungus has commercially been widely developed as a microbial insecticide agent for pest management, particularly against whiteflies and aphids (Wright, 1992; Faria and Wraight, 2007).

Consequently, this study aimed to evaluate, *in vitro*, the effect of *B. bassaina* on eggs and nymphs of *Bemisia tabaci* species complex.

MATERIALS AND METHODS

Insects: In order to obtain *B. tabaci* immature stages that are free of entomopathogenic fungi infection, whitefly adults were collected, in spring of 2007, by aspirator from squash plants grown in greenhouse at the Research station of Qassim University. Insects were then mass-reared in large screen cages (40×40×30 cm) fed on white kidney beans (*Phaseolus vulgaris* L.) planted in small pots. The insects and plants were maintained at 26±2°C, 65% RH with a 14:10 (L:D) photoperiod. To produce plants with heavy infestation of whitefly eggs and nymphs, newly white kidney pots were infested with whitefly adults and allowed to lay eggs for three days, resulting in at least 50-100 eggs leaf^{-1}.

Whitefly adults were removed and plants infested by eggs were transferred to another cages and been divided to two groups. The first group were directly used for *B. bassaina* bioassay against whitefly eggs while the second one was kept until nymphs emergence to evaluate the efficacy of the fungus against nymphal stages of *B. tabaci*.

Fungal culture: *Beauveria bassiana* strain, used in this study obtained from the fungal culture, isolated from naturally infected whiteflies according to Abdel-Baky *et al*. (1998) and kept in slant Agar media at 5°C. The fungal spores were harvested from two weeks old cultures on autoclaved PDA media at 28±1°C by rinsing with sterilized distilled water.

To maintain its virulence, the fungus was passed on to *Bemisia* spp. and re-isolated before each experiment. For laboratory tests, the fungus was subsequently cultured for 10-15 days on PDA media at 28±1°C and a photophase of 12 h.

Fungal preparations: To produce fungal inocula, slant culture of *B. bassiana* was subcultured by mixed conidial transfer to PDA media in petri-dishes that were always placed for 15 days at 25°C in darkness. Petri-dishes were sealed with Parafilm and freshly collected conidia from 15-day-old cultures were used for each experiment with replicated run to each. Conidial suspensions were prepared by scraping conidia from petri-dishes into distilled water. The conidial suspension was filtered through several layers of cheesecloth to remove mycelial mats. Viability of conidia was assessed before preparation of suspensions by germinating tests in liquid Czapek-Dox broth plus 1% (w/v) yeast extract medium (Quesada-Moraga *et al*., 2006). In all experiments, germination rates were higher than 95% after 24 h at 28°C. The concentration of conidia in the final suspension was determined using a Neubauer hemocytometer. The conidial suspension used for the bioassays was adjusted by diluting conidia with distilled water. Virulence bioassay used three concentrations of conidia of 2×10^6, 4×10^6 and 6×10^6 conidia mL^{-1}. In all cases, replicate dilution series for inoculation of replicate leaf disks were prepared.

Infection of *B. tabaci* eggs and nymphs in the laboratory: Three stages of *B. tabaci* were involved *in vitro* bioassay studies, namely; eggs, young nymphs (1st and 2nd instars) and old nymphs (3rd and 4th instars). Three conidial concentrations (2×10^6, 4×10^6 and 6×10^6 conidia mL^{-1}) were used with each *B. tabaci* life stage mentioned before. For each fungal bioassay test, a kidney bean leaf contained 50 uninfected eggs was selected, labeled and treated by the fungus. A total of 750 individuals of eggs were immersed in 1 mL of conidial suspension for 10 min in the laboratory experiments, the assays were repeated 15 times. The same procedure was repeated with young whitefly nymphs and also with old nymphs. The same number of insects treated with distilled water was used as a check. All experiments were held in a climate chamber at 28±1°C, 60±5% humidity and 14:10 (L:D) photoperiod. Egg infection, hatchability, eclosion, mortality and infection of nymphs were assess and recorded daily for 15 days.

Data analysis: Two-way analysis of variance was conducted to demonstrate variability among conidial concentrations and check treatment. Treatments mean was compared at 0.05% probability level using Least Significant Difference (LSD). All statistical analysis were preformed by using CoStat Software Program (1990). Further, obtained data were corrected using Abott's formula on the basis of check treatment and subjected to probit analysis (Finney, 1971) generating a concentration-mortality relationship for estimates of LC_{50} in Confidence Intervals (CI) 95% for each conidial concentration. Moreover, the relationship between time and mortality (lethal time-LT_{50}) was also calculated.

RESULTS

Susceptibility of *Bemisia tabaci* eggs and nymphs to *Beauveria bassiaua*: Mortality percentage, as a result of *B. bassina* treatment, was significantly different ($p = 0.05$) among *B. tabaci* eggs and nymphs. Infection levels were generally higher with the 1st and 2nd instars, followed by the 3rd and 4th instars. Meanwhile, infection level of *B. tabaci* eggs was very low and was more tolerant to *B. bassaina* infection. The mean mortality percentages of eggs averaged only 4.49%. Whereas, this value was 42.045 and 35.93% for young and old nymphs, respectively. This means that *Bemisia* nymphs were highly susceptible to fungal treatment compared with eggs infection.

Efficiency of *Beauveria bassiana* against *Bemisia tabaci*

***Bemisia tabaci* eggs:** *Beauveria bassaina* had a low lethal impact on *B. tabaci* eggs. The egg mortality percentages significantly differed among all conidial concentrations and check treatment, except between the moderate (4×10^6) and higher conidial (6×10^6) concentrations. Egg mortality percentages averaged 1.2, 4.27 and 8.0% with fungal concentration 2×10^6, 4×10^6 and 6×10^6 conidia mL^{-1}, respectively (Table 1). No mortality was observed with the check treatment. A high conidial concentration (6×10^6 conidia mL^{-1}) caused significantly higher mortalities than low concentration (2×10^6 conidia mL^{-1}). Thus, different fungal concentrations varied in ability to infect *B. tabaci* eggs where *B. bassina* impacts on the egg mortalities largely based upon on its conidial concentrations applied. In general, *B. tabaci* eggs showed little susceptibility to all fungal concentrations used.

Egg infection process by the fungal spores were slow compared with the nymphs. Within the four days of treatment, the eggs that became subsequently infected by the fungal had little changes in color but appeared slightly shrunk when observed under microscope. One week after treatment, most of the unhatched eggs became conspicuously shrunk and had less fungal outgrowths on the surface. In all fungal concentrations, infection symptoms on the eggs were observed on the 3rd day of inoculation and onwards, which slowly increased till the 6th day (Fig. 1). After that, egg infection sharply increased till the end of treatment. A significant variation was observed in infection process (in days) among the three tested fungal concentrations. The daily infection percentages were obviously varied depend upon the conidial concentration. A higher daily percentage of egg infection was observed with 6×10^6, followed by 4×10^6 conidia mL^{-1}.

Table 1: Efficiency of *Beauveria bassaina* on *Bemisia tabaci* eggs and crawlers, at 28±1°C, 60±5% RH and 14:10 (L:D)

	B. tabaci eggs				Crawlers (1st instar)			
Fungal concentrations	Egg mortality		Egg hatchability		Crawlers emergence		Crawlers mortality	
conidia mL^{-1}	%	Mean±SE	%	Mean±SE	%	Mean±SE	%	Mean±SE
Control (Untreated)	0.00	0.00±0.00c	96.13	48.06±0.71a	95.60	47.8±2.70a	0.96	0.46±0.002a
2×10^6	1.20	0.60±0.05b	94.93	47.47±0.79a	89.20	44.6±2.55b	6.44	2.87±0.045b
4×10^6	4.27	2.13±0.52a	91.20	45.60±0.88b	77.07	41.2±2.34c	10.68	4.40±0.76c
6×10^6	8.00	4.00±0.74a	85.20	42.60±0.69c	71.47	38.4±2.12d	10.94	4.20±0.73d
LSD		0.613		1.488		1.197		0.76

[a]The numbers followed by the same letter(s) within a column are not significantly different at 5% level (Duncan Multiple Rang Test, Duncan, 1951)

Table 2: LC_{50} values of *Bemisia tabaci* immature after treatment with *Beauveria bassaina*, at 28±1°C, 60±5% RH and 14:10 (L:D)

	Probit analysis characters						
B. tabaci immature	LC_{50} (conidia mL^{-1})	Y =	Upper	Lower	Slope	χ^2	p
Eggs	3.61×10^{7}	-13.51+1.79x	5.54×10^{6}	2.44×10^{6}	1.79	0.63	0.99
1st and 2nd instars	4.60×10^{6}	-14.83+2.23x	5.41×10^{6}	3.87×10^{6}	3.23	0.70	0.06
3rd and 4th instars	5.45×10^{7}	-15.37+2.28x	6.61×10^{6}	4.49×10^{6}	3.23	0.27	0.33

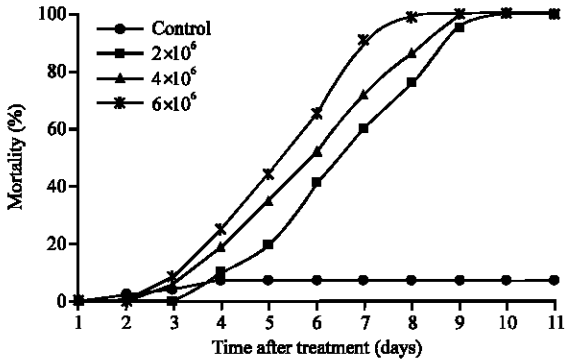

Fig. 1: Daily mortality percentage of *Bemisia tabaci* eggs treated by three conidial concentrations of *Beauveria bassaina* at Lab. conditions

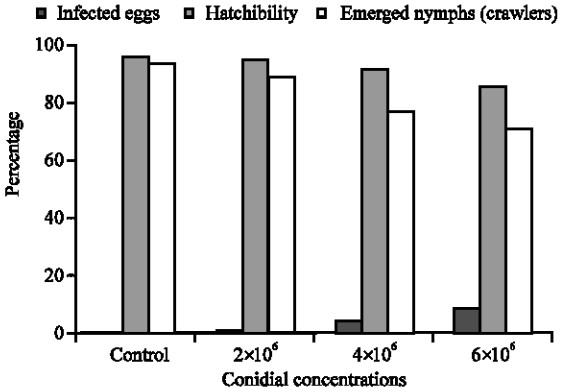

Fig. 2: Percentages of the infected eggs, hatchability and crawlers emergence of *Bemisia tabaci* eggs treated by three conidial concentrations of *Beauveria bassaina* at Lab. conditions

Probit analysis test showed that the medium lethal conidial concentration (LC_{50}) for *B. tabaci* eggs was 3.61×10^{7} conidia mL^{-1} (r = 1.79; p = 0.99). Low fungal concentration (2×10^{6} conidia mL^{-1}) showed relatively low mortality which the mortality percentage never pass 1.2% 11 days post treatment, while the higher concentration (6×10^{6} conidia mL^{-1}) gave its highest mortality percentage (8.0%) after 9 days (Fig. 1).

The lethal time (LT_{50}) of treated eggs was calculated for three conidial concentrations varied according to the conidial concentration used (Table 3). LT_{50} of 2×10^{6} conidia mL^{-1} gave a value of 7.55 (7.37-7.74 day) (Slope = 11.45; χ^2 = 9.67; p = 0.169). The LT_{50} for 4×10^{6} conidia mL^{-1} was 6.74 (5.67-8.02 day) (Slope = 0.237; χ^2 = 6.70; p = 0.152) and 6.10 (5.92-6.28 day) (Slope = 9.67; χ^2 = 17.92; p = 0.141) with the higher conidial concentration used (4×10^{6} conidia mL^{-1}) (Table 3).

In contrast, *B. tabaci* eggs hatch significantly varied among the treatments, except between check treatment and low fungal concentration. The Hatchability percentages listed 96.13, 94.93, 91.2 and 85.2% for check, 2×10^{6}, 4×10^{6} and 6×10^{6}, respectively (Fig. 2). This means that, with low, moderate and high fungal concentrations, at least 47.80, 45.60 and 42.60 eggs of 50 subjected to *B. bassaina* were able to produce a new whitefly nymphs (Table 2).

In addition, emergence of *B. tabaci* crawlers obtained from the treated eggs lasted 46.8 (93.6%), 44.6 (89.2%), 41.2 (77.07%) and 38.4 (71.74%) for the check, 2×10^{6}, 4×10^{6} and 6×10^{6}, respectively (Table 1, Fig. 3). A highly significant variation among the treatment, in respect of the crawlers emergences, was observed.

In most cases, the crawlers failed to escape from the fungal impact, followed the indirect effects on eggs and/or a direct contact with the fungal mycelium when emerged from eggs. Differed mortalities by the fungus among the crawlers were also observed (Table 1). Crawlers mortality was neglectable with the check treatment, while it listed 6.44, 10.69 and 10.93% for 2×10^{6}, 4×10^{6} and 6×10^{6}, respectively (Table 1).

***Bemisia tabaci* nymphs:** All tested conidial concentrations were pathogenic and highly virulent against *B. tabaci* nymphs (Fig. 3). Moreover, young nymphs (1st and 2nd instars) were more susceptible to pathogen infection than older ones. The average mortality percentages of the young nymphs were 22.27, 41.47 and 62.40% with 2×10^{6}, 4×10^{6} and 6×10^{6}, respectively (Fig. 3). Whereas, with older nymphs, mortality averaged 16.76 (2×10^{6}), 36.0 (4×10^{6}) and 55.13 (6×10^{6}). This means that mortality among *B. tabaci* nymphs gradually increased depending on the fungal concentrations and satisfied control could be achieved and maximized with higher conidial concentrations. The statistical analysis revealed that nymphs mortality significantly varied among fungal concentrations and insect instars.

Table 3: LT_{50} values 95% fiducial limits of *Bemisia tabaci* eggs and nymphs treated by different conidial concentrations of *Beauveria bassaina*, at 28±1°C, 60±5% RH and 14:10 (L:D)

		Probit analysis characters							
Bemisia tabaci life stage	Fungal doses (Spores mL^{-1})	LT_{50}	LT_{95}	Y =	Upper	Lower	Slope	χ^2	p
Eggs	2×10^6	7.55	10.51	-10.10+11.45X	7.74	7.37	11.45	9.67	0.169
	4×10^6	6.74	10.29	-7.44+0.237X	8.02	5.67	0.273	6.70	0.152
	6×10^6	6.10	9.02	-7.59+9.67X	6.28	5.92	9.67	17.92	0.141
1st and 2nd instars	2×10^6	4.77	7.92	-5.06+0.262X	6.29	3.61	0.262	6.43	0.144
	4×10^6	3.84	6.44	-4.29+0.356X	4.03	3.67	0.365	5.43	0.183
	6×10^6	3.52	6.44	-3.42+6.26X	3.77	3.28	6.26	34.58	0.101
3rd and 4th instars	2×10^6	5.41	9.36	-5.08+6.92X	5.63	5.22	6.92	24.65	0.01
	4×10^6	4.58	8.00	-4.49+6.80X	8.41	2.48	6.80	17.13	0.002
	6×10^6	4.01	7.31	-3.80+6.30X	9.80	1.62	6.30	8.45	0.02

Table 4: Efficiency of *Beauveria bassaina* on the 1st and 2nd instars of *B. tabaci*, at 28±1°C, 60±5% RH and 14:10 (L:D)

	1st and 2nd instars of *Bemisia* spp.			
	Infected Nymphs		Non- infected Nymphs	
Fungal concentrations conidia mL^{-1}	%	Mean±SE	%	Mean±SE
Control (Untreated)	0.00	0.00±0.0d	100.00	50.00±5.32a
$2\times2\times10^6$	22.267	11.13±3.56c	72.67	38.87±3.67b
$4\times2\times10^6$	41.467	20.73±3.33b	58.53	29.27±3.11c
$6\times2\times10^6$	62.400	31.80±4.25a	36.40	18.20±2.87d
LSD			1.379	

[a]The numbers followed by the same letter(s) within a column are not significantly different at 5% level (Duncan Multiple Rang Test, Duncan, 1951)

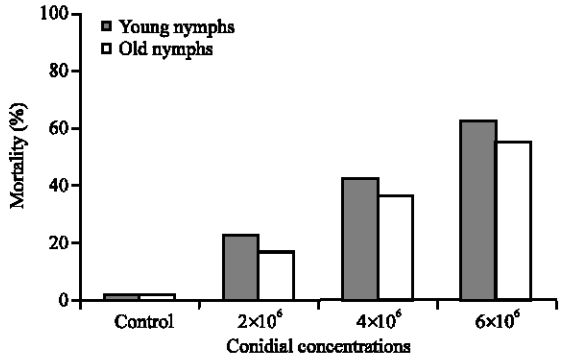

Fig. 3: Mortality percentage among *Bemisia tabaci* nymphs treated by three conidial concentration of *Beauveria bassaina* at Lab. conditions

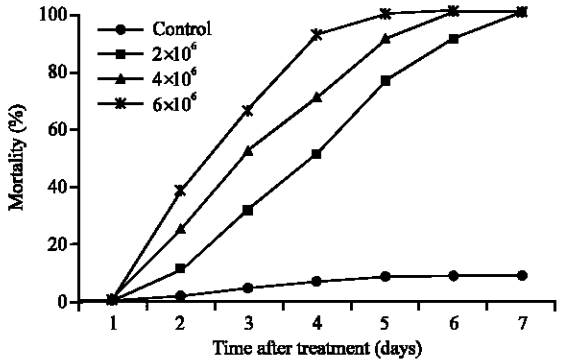

Fig. 4: Daily mortality percentage of *Bemisia tabaci* young nymphs treated by three conidial concentrations of *Beauveria bassaina* Lab. conditions

Young nymphs (1st and 2nd instar): A lethal effect on young nymphs (1st and 2nd instars) of *B. tabaci* was observed when they were treated by *B. bassaina*. Mortality percentages among young nymphs significantly varied within all conidial concentrations and check treatment. Mortality percentages averaged 22.27, 41.67 and 62.40% with fungal concentration 2×10^6, 4×10^6 and 6×10^6 conidia mL^{-1}, respectively (Table 4). No mortality was observed with the check treatment in which nymphs were treated with water only. A high conidial concentration (6×10^6) caused significant higher mortalities than lower concentrations and check treatment as well. It could be concluded that *B. bassina* varied in ability to infect young nymphs of *B. tabaci* based on conidial concentrations used.

Infection and development of fungus on the young nymphs was faster than other *B. tabaci* stages. Mortality within nymphs was observed by the 2nd day post-treatment and gradually increased onwards, but the infection symptoms were observed by the 3rd day. Five days latter, all dead nymphs were covered with the fungal mycelium. The fungus obviously killed and destroyed the nymphs completely within 6 days after treatment (Fig. 4). Significant variations in daily mortality percentages were observed among the conidial concentrations.

The results also showed that, LC_{50} required to kill 50% of young nymphs, was 4.6×10^6 conidia mL^{-1} ($r = 3.23$; $p = 0.06$), which its regression equation was $y = -14.83+2.23x$ (Table 2). Moreover, the LT_{50} of young

Table 5: Efficiency of *Beauveria bassaina* on the 3rd and 4th instars of *B. tabaci*, at 28±1°C, 60±5% RH and 14:10 (L:D)

	3rd and 4th instars of *Bemisia* spp.			
	Infected nymphs		Non- infected nymphs	
Fungal concentrations	%	Mean±SE	%	Mean±SE
Control (Untreated)	0.00	0.00±0.0d	100.00	50.00±4.11a
$2\times2\times10^6$ spores mL^{-1}	16.67	08.33±4.23c	83.30	41.67±4.05b
$4\times2\times10^6$ spores mL^{-1}	35.07	17.53±4.33b	65.07	32.53±3.96c
$6\times2\times10^6$ spores mL^{-1}	55.20	27.60±4.65a	44.93	22.47±3.90d
LSD		1.504		1.472

[a]The numbers followed by the same letter(s) within a column are not significantly different at 5% level (Duncan Multiple Rang Test, Duncan, 1951)

nymphs was calculated for three conidial concentrations and varied according to the conidial concentration used (Table 3). LT_{50} of 2×10^6 conidia mL^{-1} gave a value of 4.77 (3.61-6.29 day) (Slope = 0.262; $\chi^2 = 6.43$; p = 0.144). While LT_{50} of 4×10^6 conidia mL^{-1} was 3.84 (3.67-4.03 day) (Slope = 0.365; $\chi^2 = 5.43$; p = 0.183) and 3.52 (3.28-3.77 day) (Slope = 6.26; $\chi^2 = 34.58$; p = 0.101) with the higher conidial concentration used (4×10^6 conidia mL^{-1}) (Table 3).

Old nymphs (3rd and 4th instar): Impact of *B. bassiana* on old nymphs (3rd and 4th instars) of *B. tabaci* was lower compared with young ones, but had a higher effect when compared with eggs. Significant variation within large nymphs mortalities percentages was recorded based on the conidial concentrations and check treatment (Table 5). Mortality percentages were 16.67%, with 2×10^6 conidia mL^{-1}, 35.07% with 4×10^6 conidia mL^{-1} and 55.20% with 6×10^6 conidia mL^{-1} (Table 5). No mortality observed with the check treatment (water only). A higher mortality percentages were obtained with the high conidial concentration (6×10^6) and caused significant mortality than low concentration and check treatment.

Infection and development of fungus on old nymphs was faster but not as with young nymphs. Mortality among large nymphs was observed on the 2nd day post-treatment and increased onwards. The infection symptoms was observed on the 3rd day. In the 5th day of treatment, dead nymphs was covered with the fungal mycelium. The fungus killed and destroyed the nymphs completely within 7-8 days of treatment (Fig. 5). Significant variations in daily mortality percentages were observed among the conidial concentrations.

Based on the Probit analysis, LC_{50} of large nymphs averaged 5.45×10^7 conidia mL^{-1} (r = 2.28; p = 0.328), which its regression equation y = -15.37+2.28x (Table 2). In addition, the LT_{50} varied according to the conidial concentration of *B. bassaina*. LT_{50} of 2×10^6 conidia mL^{-1} was 5.41 (5.22-5.63 day) (Slope = 6.92; $\chi^2 = 24.65$; p = 0.01), while LT_{50} of 4×10^6 conidia mL^{-1} was 4.58 (2.48-8.41 day) (Slope = 6.80; $\chi^2 = 17.13$; p = 0.002) and 4.01 (1.62-9.80 day) (Slope = 6.30; $\chi^2 = 8.45$; p = 0.02) with the higher conidial concentration (4×10^6 conidia mL^{-1}) as shown in Table 3.

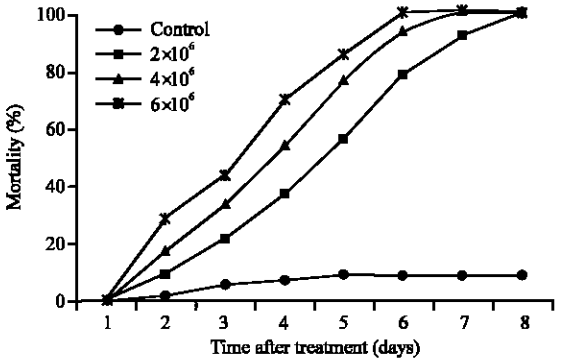

Fig. 5: Daily mortality % of *Bemisia tabaci* old nymphs treated by three conidial concentrations of *Beauveria bassaina* Lab. conditions

DISCUSSION

From the previous results, eggs of *B. tabaci* was more tolerant to *B. bassaina* infection and were not easily killed even by the highest conidial concentration of *B. bassaina*. In spite of that, there is a limited information on the lethal effect of entomopathogenic fungi with insect eggs when compared with other insect life stages. Generally, previous studies suggested that the egg stage of an insect is believed to be more resistant to infection than other stages. These results were in agreement with those conducted in Egypt on whitefly eggs, where higher conidial concentrations (10×10^6 conidia mL^{-1}) of *Cladosporium uredinicola* caused only 28% mortality among *B. argetifolii* eggs (Abdel-Baky *et al*., 1998; Abdel-Baky, 2002). Also, egg of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood couldn't be infected by *Ascheronia* species (Fransen *et al*., 1987). The authors attributed this phenomena to the egg chorion structure, which make a hard barrier against fungal spore invasion. This may be drawbacks the germ tubes of the fungal spores which need long time to germinate and penetrate the egg shell compared with the faster embryonic development. Additionally, fungal failure in egg infections may be attributed to antifungal compounds on the egg shell that hampered conidial germination

(Meeks *et al*., 2002). Consequentially, the neonate can escape from infection inside eggs, but may be contaminated with fungal spores during emergence. Deferred nymphal mortality was detected in 1st instar nymphs upon hatching from eggs contaminated by the fungus. Poprawski *et al.* (1985) reported that eggs of *Otiorhynchus sulcatus* F. and *Sitona lineatus* L. treated with different isolates of entomopathogenic hyphomycetes caused mortality of 0-68 and 26-88% in emerging larvae, respectively. Additionally, Gopalakrishnan and Narayanan (1989) reported that eggs of *Heliothes armigera* Hbn were not susceptible to *Metarhizium anisopliae* infection. The same trend was obtained with eggs of the Colorado potato beetle, *Leptinotarsa decemlineata* Say which were also not susceptible to infection by *B. bassiana* (Long *et al*., 1998). In contrary, when grasshoppers were exposed to *B. bassaina* during oviposition, the eggs and egg chorion were actively colonized by the fungus, but egg hatch was unaffected (Inglis *et al*., 1995).

On the other hand, Mamiania (1991) demonstrated that both of *B. bassiana* and *M. anisopliae* caused mortalities of up to 97 and 100% in *Chilo partellus* (Swinhoe) eggs, respectively. He explained that the precise mechanism surrounding the mode of egg infection is still unknown but they observed the fungal propagules inside eggs at 3-days post-inoculation when viewed under the microscope, suggesting the direct penetration of egg chorion by the fungal mycelium.

In case of *B. tabaci* nymphs, *B. bassaina* caused higher mortality percentages than in eggs. The results showed that the fungus was able to attach to nymph cuticle, germinate, penetrate the cuticle and cause a significant mortality among nymphs. Meanwhile, fungus ability was also varied among nymphs based on its age and cuticle hardening. The results obtained were in agreement with those of Ekbom (1979), Hall (1985), Masuda and Maeda (1989), Abdel-Baky *et al*. (1998) and Abdel-Baky (2002), who used different species of entomopathogenic fungi in controlling whiteflies. Germination and penetration of fungal spores on insect cuticle constitute an important step in the fungal infection process (Abdel-Baky *et al*., 2005). Mortality differences may be due to the difference in producing profuse amounts of cuticle lipids, especially long-chain wax esters (James, 2001). These lipids are produced in such a thick layer of insect cuticle that could inhibit fungal spores to penetrate the cuticle layers (Lecuona *et al*., 1997).

Lower mortality among *B. tabaci* immature by *B. bassiana* compared with *Aschersonia* spp. (Meeks *et al*., 2002) and *Verticillium lecanii* (Gindin *et al*., 2000) may be due to passing the fungal spores through whitefly before bioassay treatment. Brownbridge *et al.* (2001) reported that when *B. bassaina* invaded *Bemisia argentifolli* Bellows and Perring its virulence was enhanced and when repeated sub-culturing on artificial media virulence was decreased.

Statistically, the low regression coefficient obtained in Table 2 and 3 may be due to a certain degree of heterogeneity among *B. tabaci* immatures regarding their susceptibility to fungal infection, that lead to cause slower rise in mortality with a given increase in conidial concentration. Many authors, worked on whiteflies, found that the regression coefficient was not very high. Wraight *et al*. (1998) obtained regression coefficient, ranged from 0.5-2.0 for *Paecilomyces* spp. and *B. bassaina* when treated on the 3rd instars of *B. argentifolii*. Whereas, Vidal *et al*. (1997) obtained slopes varying from 0.62-1.13 for 2nd instar of *B. argentifolii* inoculated by *P. fumosoroseus*. The differences among regression slopes could be attributed to partially susceptibility among the stages of whitefly. This resulted was confirmed by Gindin *et al*. (2000) who reported that *V. lecanni* caused higher virulence in the early stages of whitefly (emerged nymphs) and reduced with older instars. As a result, the low slopes with whitefly eggs and large nymphs as being more resistant to fungal infections, usage of eggs and 4th instar nymphs for selection of fungal species and concentrations seems to guarantee the efficiency of the entomopathogeinc fungi against whiteflies. Finally, most of literature revealed that pathogenicity and virulence of any given fungi is not indicated by LC_{50} only, but also, by the time in days (LT_{50}) required to achieve 50% mortality of an insect pest.

In conclusion, the results suggest that *B. bassiana* alone may not achieved satisfactory control for *B. tabaci*. However, the fungus may be used within an integrated pest management program, or combined with other control tactics, that may enhance *B. bassiana* performance.

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