

## BIOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF *VERTICILLIUM LECANII* AGAINST *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) ON EGGPLANT

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### ABSTRACT

Four strains of *Verticillium lecanii* (Zimmermann) Viegas (V20, V26, V07 and V17) were tested for the biological characteristics and pathogenicity against *Bemisia tabaci*. The four strains were morphologically compared at three different artificial media (SDAY, PDA and CZAPECK-DOX) under laboratory conditions at  $25 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  RH and 16:8h (L: D). The four isolates were significantly different from each other. The isolate V20 showed the greatest potential in mycelium growth rate than other three strains. The highest ( $0.37\text{cm.day}^{-1}$ ) and the lowest ( $0.161\text{cm.day}^{-1}$ ) colony growth rates were associated with V20 and V17 respectively cultured on SDAY and CZAPEK respectively. SDAY is the best media for the sporulation of four strains. The isolate V20 showed the highest sporulation ( $32.75 \times 10^7$  conidia  $\text{mL}^{-1}$ ) and the isolate V17 showed lowest sporulation ( $3.45 \times 10^7$  conidia  $\text{mL}^{-1}$ ) during the present investigation. Pathogenicity test at saturated humidity showed that the third instar is very susceptible to fungal infection. The virulence is significantly different. The  $\text{LC}_{50}$  values for third instars were  $1.65 \times 10^7$ ,  $1.87 \times 10^7$ ,  $2.2 \times 10^7$  and  $2.58 \times 10^7$  conidia/ml for isolate V20, V26, V07 and V17 respectively. The least and highest  $\text{LT}_{50}$  values were 2.909 and 3.534 noted for isolate V20 and V17 respectively. The isolate V20 is more virulent on third instar of *Bemisia tabaci* compare with other isolates.

**Key words:** *Verticillium lecanii*, *Bemisia tabaci*, growth rate, sporulation, pathogenicity

### INTRODUCTION

*Bemisia tabaci* (Gennadius) has become a serious world-wide pest since the early 80s (Gerling, 1990). Since it first recorded by Chou (1949) in china *B. tabaci* was considered as a non-severe pest until mid 1990s (Ren *et al.*, 2001) when sporadic outbreak occurred affecting a wide range of host plants. The outbreak of the pest in china 1990s is thought to have originated from the invasion of the new B-biotype on ornamentals crops (Qiu *et al.*, 2003), it attacks 176 plant species and is considered as a severe pest on vegetables, field crops and ornamental plant and fruits( Ren *et al.*, 2001). Direct damage occurs as a result of sucking plant saps from phloem leading to physiological disorders such as a wilting, stunted growth and irregular ripening of fruits (Oliviera *et al.*, 2001). As an alternative to chemical control, the use of natural enemies which include a wide range of predators, parasitoids and pathogens, is considered an appropriate and plausible measure to control *B. tabaci*.

To control arthropods and more than 750 fungi from 90 species have described as entomopathogenic against insects species up to date. *Verticillium lecanii* is a very common fungal species, which was firstly described by Zimmermann (Zimmermann, 1898), in coffee scale insects in Java. It was capable of infecting a wide range of insect hosts from board geographical and climatic locations. Like all microorganisms, entomopathogenic fungi have specific biological characteristics that influence their activity in the environment (Parker *et al.*, 2003). To select fungal pathogen for controlling whiteflies it is necessary to study simple basic characteristics that are required to kill the target insects in both field and greenhouse conditions. According to Moore and Prior (1993) relevant characteristics were identified as good mass production features such as high sporulation on artificial media, high virulence against the target organisms and the ability to

withstand the environment in which the pest is occurring. Fungal isolates with rapid germination and hyphal growth rates have an advantage as biological control agents because host infection can potentially occur much more quickly (Hadjeck and Leger, 1994; Varela and Morales, 1996). Mor *et al* (1996) compared 35 strains of *V. lecanii* from different hosts of geographical location against *B. tabaci* and found that the virulence on larvae of *B. tabaci* within these isolates ranged from 0 to 83%. Further study of these strains of *V. lecanii* against *B. argentifolii* (biotype B of *B. tabaci*) classified the 35 *V. lecanii* strains for degree of the virulence. Pathogenicity of *V. lecanii* involves adhesion of spores to the insect cuticle, germination, penetration and internal colonization culminating in host death (Gindin *et al.*, 1994). Insect mortality is effected when the fungus gain entry into the haemoceol by penetrating the host cuticle by combination of hydrolytic enzymes and mechanical force. The morphology of the insect stage may somehow influence fungal infection (Wriaght *et al.*, 2000).

Our studies were aimed at developing a laboratory bioassay system for efficient assessment of fungal biocontrol agent against *B. tabaci*. The four strains of *V. lecanii* derived from same geographical location and originated from the same host pest (*Trialeurodes* sp.) were compared morphologically by culturing them on different media. These strains were also compared for their pathogenicity against third instar of *B. tabaci* to search for highly ovicidal isolate for further study for microbial control of whitefly pests.

**Table 1. Composition of different media used to study biological characteristics of *V.lecanii***

Media	Composition	Carbon source	Status
<b>PDA</b>	200g potatoes (infusion)	Dextrose	Original formulation
	20g dextrose 20g Agar		
<b>SDA</b>	3g yeast extract	Glucose	Original formulation
	10g Glucose		
	10g Peptone 20g Agar		
<b>CZAPEK</b>	30g Sucrose	Sucrose	Original formulation
	30g Sodium nitrate		
	1g dipotassium phosphate		
	0.5g Magnesium Sulfate		
	0.5g Potassium Chloride		
	0.01g Ferrous Sulfate 20g Agar		

## MATERIALS AND METHODS

### Plant materials

Eggplant *Solanum melongena* var. dafeng L. were grown individually in plastic pots (16cm diameter). The pots were placed inside cages (60×60×60 cm) under laboratory condition having  $25 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  R.H and 16:8 (L: D) light period. Water and fertilizer were supplied as per requirement of the plants. The plants were used for the experiments at 4-6 leaf stage.

### Fungal strains, insect and media

Four strains of *V. lecanii*(V07, V17, V20 and V26), used during this study, were obtained from Fujian Agricultural and Forestry University, China. These strains were isolated from *Trialeurodes* sp.

*B. tabaci* used in this experiment were obtained from laboratory of Insect Ecology South China Agricultural University (SCAU), Guangzhou China, reared in greenhouse under non-insecticidal and non infective conditions. The adults were collected with aspirator and released onto eggplant placed inside a cage (60×60×60 cm) containing four eggplants having  $25 \pm 2^\circ\text{C}$  temperature,  $65 \pm 5\%$  R.H. and 16:8 (L: D) light period. After 24 h all adults were expelled out from the cages. The eggs were cultured until the development of third instar.

Three different growth media (SDAY, PDA and Czapek-Dox), were used to study the biological characteristics of four *V. lecanii* isolates. (Table 1)

### Biological characteristics of *Verticillium lecanii*

6 ml of each medium was poured into Petri dishes (7.5 cm in Ø). The concentration of suspension used was approximately  $1 \times 10^6$  spores/ml. Then, 1 µl of suspension was inoculated in the centre of the three different media plates. The plates were incubated at  $25 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  RH and 16:8h (L: D). There were four plates in each treatment. The measurements of colony diameter were taken after 5, 10, 15 and 20 days of incubation. The diameter of every colony was the means of long and short diameter. Spore production was investigated after 20 days of culturing. Conidia from the four plates in each treatment were dislodged by gently scarping with a sterile blade into 500 mL 0.01% Tween 80 and agitated for 10 min. Then, the number of conidiospores was determined using a hemocytometer under microscope. Thereupon, the spores production in 1 ml medium was evaluated. The data were subjected to F-test and LSD test by means of SAS 8.1 software (SAS, 2000).

### Pathogenicity against *Bemisia tabaci*

After the passage of the fungus through *B. tabaci*, the *V. lecanii* strains were cultured on SDAY media. Application of *V. lecanii* was made with aqueous suspension of 15 days old conidia. The series of suspension tested was  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  spores/ml. The blastospores were quantified using a standard hemacytometer at 40x magnification. The viability of fungi was determined by using standard techniques (Goettel and Inglis, 1997). Spores viability was determined by culturing 0.1ml aliquot of the stock suspension onto three potato dextrose agar (PDA) plates amended with 0.005% benomyl. The plates were incubated at  $25^\circ\text{C}$  for 24h. 300 spores were examined and scored for viability (Goettel and Inglis, 1997).

Leaves with *B. tabaci* (>100 nymph/leaf) were used for the test. The leaves were immersed into 20ml spore suspension for 10 s, while control leaves were immersed into 0.01% Tween 80 for the same period. After air-dryness, the treated leaves were cultured at  $25 \pm 2^\circ\text{C}$ ,  $90 \pm 5\%$  RH and 16:8h (L: D) and sealed for 2 days into bags to maintain the humidity around the leaves. There were four leaves in each treatment. Mortality of *B. tabaci* was surveyed under a binocular microscope every day from the 2nd day after inoculation. In order to identify if infected by

*V. lecanii*, the dead whiteflies were transferred to wet filters paper. For colony growth and sporulation the scales were cultured on SDYA media, if mycelia and conidia of *V. lecanii* were observed the insect was decided to be died from infection. The mortality data was subjected to probit analysis to calculate  $\text{LC}_{50}$  and  $\text{LT}_{50}$  values (Gindin *et al.*, 2000).

The data were analyzed with the model of time-dose-mortality (TDM) to calculate  $\text{LC}_{50}$  and  $\text{LT}_{50}$  values. The DPS (Data Processing System) software (Tang and Feng 2002) was employed.

## RESULTS

### Effect of culture media on colony size of *V. lecanii*

The results of mycelial growth on different media incubated at 5, 10, 15 and 20 days have been presented in Table-2. The highest and least mycelia diameters after 5 days were recorded for isolates V20 and V17 on media of SDAY and Czapek-Dox with values of 1.75 and 1.3 cm respectively. At 20 days incubation period, the highest and least values were associated with isolates V20 and V17 on the SDAY, PDA and Czapek-Dox with the values 7.23, 6.2, 5.43, 5.28, 5.13 and 4.92 cm respectively. Results were subjected to analysis of variance tests at  $P < 0.05$  to determine any effect of growth media on the mycelium growth for the four incubation periods. No significant difference was observed among the five days incubational period for isolates cultured on the three media. However when the incubation period was increased to 20 days, a significant difference was observed between isolate V20 and V17. This difference was also observed on the different media at different incubational periods. The four strains (V20, V26, V17 and V07) showed a significant difference for growth rate on different media ( $F=141.62$ ;  $df=3$ ;  $P=0.001$ ). The four strains differed for growth rate depending on culture media. The colony diameter was larger on SDAY and PDA than Czapek-Dox (Table 2). The strains V20 and V26 had the best development on SDAY with growth rate 1.75 and 1.65cm/day respectively after 5days and then V07 did not show a big difference in growth rate with an average value of 1.47 cm/day. The V17 had the lowest growth rate with value 1.36 cm/day. These results also showed that Czapek-Dox medium allowed slower growth when compared to the growth reported on SDAY and PDA. Slower growth rate of V20, V26 on Czapek-Dox attaining

an average 5.43, 5.26 cm and V17, V07 were 4.43, 4.92 cm respectively under laboratory condition of  $25 \pm 2^\circ\text{C}$ . The strains differed significantly in growth depending on culture media ( $F=91.91$ ;  $df=2$ ;

$P=0.002$ ). For all the isolates SDAY was the best media for the growth and the strain V20 showed the highest growth rate than V17 that shown the lowest.

**Table 2. Effects of media on mycelial growth of different *V. lecanii* strains**

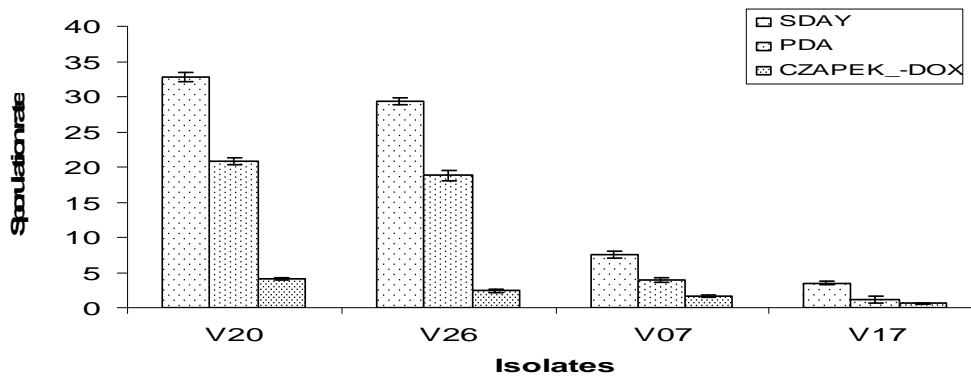
Strains	Media	Diameters of colonies (cm)			
		5 d	10 d	15 d	20 d
V20	SDAY	1.75±0.04a	3.53±0.11a	5.38±0.02a	7.23±0.06a
	PDA	1.61±0.06bc	3.30±0.10bc	4.85±0.06bc	6.20±0.09bc
	Czapek-Dox	1.46±0.07d	3.08±0.05de	4.23±0.05d	5.43±0.03d
V26	SDAY	1.65±0.04d	3.41±0.08ab	4.95±0.17b	6.25±0.20b
	PDA	1.57±0.03c	3.21±0.09cd	4.68±0.12c	5.94±0.05c
	Czapek-Dox	1.43±0.05de	3.03±0.05e	4.16±0.25d	5.26±0.25de
V07	SDAY	1.36±0.06fg	3.10±0.14de	3.48±0.04e	5.02±0.03ef
	PDA	1.33±0.03fg	2.88±0.06f	3.31±0.17ef	4.93±0.02f
	Czapek-Dox	1.32±0.04g	2.25±0.05h	2.93±0.48g	4.43±0.12g
V17	SDAY	1.47±0.09d	3.30±0.11bc	4.25±0.05d	5.27±0.12de
	PDA	1.43±0.03de	3.13±0.05de	4.25±0.05d	5.13±0.02ef
	Czapek-Dox	1.39±0.03ef	2.68±0.16g	3.21±0.10f	4.92±0.5f

\*Means in the same column followed by the same letter are not significantly different ( $P < 0.05$ ) by LSD test.

**Effect of culture media on Sporulation of *V. lecanii***

Results indicated that four strains of *V. lecanii* can produce abundant spores in solid state media culture. All the media provided sporulation  $1 \times 10^7$  conidia  $\text{ml}^{-1}$  when 0.03% tween 80 was used to dislodge the spores from twenty days old colonies (Figure 1). In this trail an initial spore concentration of  $1 \times 10^6$  conidia  $\text{ml}^{-1}$  was cultured on three culture media. The sporulation rate was significantly influenced by culture media ( $F=220.38$ ;  $df= 2$ ;  $p= 0.024$ ). Significant difference was also observed for isolates ( $F= 174.71$ ;  $df= 3$ ;

$p=0.004$ ). The means values of sporulation after 20 days are given in table-3. The highest sporulation was observed for isolate V20 cultured on SDAY with value  $32.75 \times 10^7$  conidia  $\text{ml}^{-1}$ , SDAY media was noticed as the best media for sporulation of all the isolate. Lowest value was recorded on Czapek-Dox with a value of  $4.12 \times 10^7$  conidia  $\text{ml}^{-1}$  for V20. Generally the sporulation was observed to be higher for the four isolate on SDAY and PDA and low on Czapek-Dox. Least value was noted for the isolate V17 on SDAY and Czapek-Dox media with  $3.45 \times 10^7$  and  $0.60 \times 10^7$  conidia  $\text{ml}^{-1}$  respectively.



**Fig-1: Effect of different media on sporulation rate ( $1 \times 10^7$  conidia /  $\text{ml}^{-1}$ ) of different isolate of *V. lecanii***

### Pathogenicity against third instars nymphs of *B. tabaci*

The mortality caused by different isolates of *V. lecanii* on the third instar is presented in Table-3. *B. tabaci* mortality was significantly different between the different isolates varying from 87% to 56.19% (F= 12.21, df=3 and P=0.024), however the four strains were pathogenic against third instar at different concentrations. The isolate V20 was more virulent than other strains, whereas V26 isolate displayed similar level of virulence but statistically differed from V20. The lowest mortality was observed for V17 and V07. These results revealed that strains of *V. lecanii* derived from the same pest and same geographical location have different virulence against third instar of *B. tabaci*.

The insect mortality started to increase 6 days after the inoculation and cumulative mortality was highest after 8 days. 5 days post treatment the lethal time required for 50% mortality varied for among the

isolates. The isolate V20 had shortest LT<sub>50</sub> value of 5.69d at the concentration  $1 \times 10^8$  and 6.22 at  $10^7$  while isolate V17 showed the highest LT<sub>50</sub> value of 8days. The LT<sub>50</sub> for V26 was 6.11 days at  $10^8$  and 6.69 days at  $1 \times 10^7$  conidia/ml whereas the LT<sub>50</sub> for V07 was 6.65 at  $10^8$  and was 7.47 at  $10^7$ . The LC<sub>50</sub> values were  $1.07 \times 10^6$  (Pearson chi-square Test;  $\chi^2=8.83862$ , df=23, P=0.99792, Hosmer & Lemeshow Test,  $\chi^2=4.4573$ , df=9, P=0.87883),  $1.19 \times 10^7$  (Pearson chi-square Test;  $\chi^2=10.7846$ , df=23, P=0.9905, Hosmer & Lemeshow Test,  $\chi^2=4.8780$ , df=9, P=0.8448),  $1.30 \times 10^8$  (Pearson chi-square Test;  $\chi^2=5.69818$ , df=19, P=0.99925, Hosmer & Lemeshow Test,  $\chi^2=1.0223$ , df=8, P=0.99810) and  $5.08 \times 10^8$  (Pearson chi-square Test;  $\chi^2=3.35081$ , df=19, P=0.99999, Hosmer & Lemeshow Test,  $\chi^2=1.0476$ , df=8, P=0.99793) for V20, V26, V07 and V17 respectively (Table-4). LC<sub>90</sub> Values for V20 and V17 were  $3.67 \times 10^9$  and  $7.69 \times 10^{10}$  conidia/ml respectively where as LC<sub>90</sub> value for V26 was  $1.41 \times 10^{10}$  conidia/ml.

**Table 3. Cumulative mortality of *B. tabaci* after 8 days caused by different isolates at different concentrations of *V. lecanii* on eggplant**

Isolates	<i>V. lecanii</i> concentrations (conidia/ml)				
	$10^4$	$10^5$	$10^6$	$10^7$	$10^8$
V20	60.66±5.11 cA	62.63b±1.15 cA	64.64±3.73 b cA	72.00±9.23 abA	87±1.73 aA
V26	42.00±1.15 bA	49.92±1.44 bA	51.34±9.11 bAB	56.54±11.75 abA	78.57±6.96 aAB
V07	28.28±3.72 bB	34.59±7.24 bB	38.79±11.36 bB	51.93±9.40 abA	74.30±1.66 aB
V17	23.84±1.48 cB	32.11B±3.38 bC	35.15±1.81 abcB	42.06±12.01 abA	56.19±1.04 aC
df	3	3	3	3	3
F	14.04	12.06	2.61	1.36	12.21
P	0.0015	0.0024	0.1232	0.3218	0.024

\*Mean in the same row with same small letters are not significantly different from each other (DMRT, P<0.05). Mean in the same column with same capital letters are not significantly different from each other (DMRT, P<0.05)

**Table 4. LC<sub>50</sub> and LC<sub>90</sub> (conidia/ml) values for different isolates of *V. lecanii***

Isolates	LC <sub>50</sub>	LC <sub>90</sub>	Slope± SE
V20	$1.07 \times 10^6$ ( $1.75 \times 10^6$ - $6.38 \times 10^5$ )	$3.67 \times 10^9$ ( $4.78 \times 10^9$ - $2.8 \times 10^9$ )	0.23±4.38
V26	$1.19 \times 10^7$ ( $1.64 \times 10^7$ - $8.58 \times 10^6$ )	$1.41 \times 10^{10}$ ( $2.05 \times 10^{10}$ - $9.57 \times 10^9$ )	0.30±5.64
V07	$1.30 \times 10^8$ ( $1.50 \times 10^8$ - $1.14 \times 10^8$ )	$1.33 \times 10^{10}$ ( $1.69 \times 10^{10}$ - $1.04 \times 10^{10}$ )	0.38±7.44
V17	$5.08 \times 10^8$ ( $5.84 \times 10^8$ - $4.41 \times 10^8$ )	$7.69 \times 10^{10}$ ( $1.04 \times 10^{11}$ - $5.66 \times 10^{10}$ )	0.23±4.92

## DISCUSSION

Morphological characteristics, commonly evaluated, include colony appearance, shape and size of conidia. Such characteristics may not be important in the separation of isolates (Hadjek and Leger, 1994). Several studies have found morphological characteristics of little use in the selection of entomogenous fungi for biocontrol (Hadjek and Leger, 1994; Varela and Morales, 1996). However, morphological characteristics could be associated with physiological and pathogenic characteristics (Hall, 1984; Jackson *et al.*, 1985). Surface culture is used for the routine maintenance of isolates and for production of conidia. One of the most commonly used media by insect pathologists is Sabourauds Dextrose Agar supplemented with yeast extract (SDA); however many other media such as cornmeal, Czapeck-Dox, malt extract, potato dextrose agar (PDA), and Sabourauds maltose agar have also been used oftenly. Some researchers advocate more complex media such as mixed cereal agar claiming that there is less chance of loss of vigor when such media are used. However, this media has not been substantiated and this media has not been widely accepted in insect pathology (Lacey, 1994). Gottel *et al* (2000) mentioned that the addition of potato extract to a culture medium supplies nutrient such as starch, minerals mainly Ca, K, Ca, P, Mg Na, S, Zn, N, crude proteins and vitamins needed by the fungus.

Many of the classical and cosmopolitan entomopathogenic fungi, *Beauveria bassiana*, *M. anisoplaie* and *N. rileyi* are most easy to grow on standard agar media and can be commercially produced as mycoinsecticides. For small scale inoculums productions where economics are not a primary concern, relatively expensive media such as Sabouraud Dextrose Agar and Potato Dextrose Agar have been used successfully to induce sporulation and obtain inoculums of several entomopathogenic fungi. Then the common technique for cultivation of fungal spores is either a surface culture with a solid substrate (moistened wheat bran, millet or rice), or a submerged culture with a liquid medium (Feng *et al.*, 2000). Most facultative entomogenous fungi will grow on one or more defined or semi-defined gar-based medium or on natural substrates. Specialist fungi are usually fastidious on artificial media and are usually best maintained on their respective hosts.

A few can be grown in vitro but require a complex media. For example, *Lagenidium giganteum* can be cultivated on simple medium but requires sterols to induce oosrogenesis (Navon and Ascher, 2000). However in case of *V. lecanii* conidiation occurs without starvation of the mycelium and factors contributing to the onset of conidiation in *V. lecanii* have not been elucidated the ability of *M. anisoplaie*, *B. bassiana* and *V. lecanii* to grow and produce conidia on artificial media is one of the main advantages in the commercial development of these fungi. These organisms are also amicable to molecular and biochemical laboratory investigations (Bidochka *et al.*, 2000). *A. aleyrodis* isolates are mostly cultivated on different kinds of media ranging from solid grain media to water agar (Liu *et al.*, 2002). *V. lecanii* can produce abundant spores in solid media state culture (Feng *et al.*, 2000).

All the isolates tested proved to be pathogenic to the third instar of *B. tabaci* however several isolates of *V. lecanii* were virulent again *B. tabaci* (Gindin *et al.*, 2000; Wang, 2004). Jackson *et al.* (1989) tested 18 strains *V. lecanii* for virulence against the aphid *Macrosiphoniella sanborni* (Gillette) and a wide variation in virulence among strains was observed. The most virulent strain had an LT<sub>50</sub> value of 3d; whereas the least virulent strain was almost avirulent (10% kill in 14d) *Lecanicillium muscarium* (formally *V. lecanii*) was shown to have a high pathogenicity to all developmental stages of *B. argentifolii* (Gindin *et al.*, 2000). Compared to our results the best strain V20 had a LT<sub>50</sub> after value of 5 days. Wraight *et al.*, (2000) reported that multiple applications of four isolates of *B. bassiana* at rate 1-2.5x10<sup>3</sup> conidia/mm<sup>2</sup> at 4-5 days intervals provided more than 90% control of large (third and fourth instar) nymphs of *B. argentifolii* on cucumber and cantaloupe melon. The mortality response of *B. tabaci* nymphs from the infection with *V. lecanii* varied according to developmental stage (Landa *et al.*, 1994). The susceptibility of hosts belonging to different stages also varies in relation to broad spectrum fungi. Mead and Byrne (1991), found no significant differences in susceptibility to the fungus due to age or species of whitefly and reported infection of first to third nymphal instars of *B. tabaci* strain (*B. argentifolii*) and *Trialeurodes vaporariorum* on Poinsettia. The pathogenicity of *V. lecanii* has been previously reported against *B. tabaci* although all the isolates tested were

pathogenic the third instar of *B. tabaci*. Byrne (1991) reported that *V. lecanii* caused 96% mortality on third instars of *B. tabaci* and 95.6% on fourth instars of *T. vaporariorum* at 21±1°C and 89-95% R.H. There was considerable variation in virulence of these strains. The least pathogenic isolate of *V. lecanii* (V17) caused 50% mortality after 9 days. While the most virulent isolated caused 87% after 8 days. Similar intraspecific variation has been studied, previously reported with *M. anisoplaie* against *C. capitata* (Ekesi, 2002).

While some studies indicated that strains of entomopathogenic fungi more virulent to insect species from which they were isolated or from closely species. In our study, the four strains recovered from the same host and the same geographical location was significantly different for their virulence against *B. tabaci*. Direct immersion of the insects in a fungal suspension resulted in the highest percentage of mortality and spraying the broccoli florets produced the lowest levels of mortality among all of the indirect methods. Nymphal mortality increased from 22 to 54% when the immersion time was increased from 5 to 10S (Liu *et al.*, 2002). Mor *et al.* (1996) compared 35 strains of *V. lecanii* from different hosts of geographical location against *B. tabaci* and found that the virulence on larvae of *B. tabaci* within these isolates ranged from 0 to 83%. Further study of these strains of *V. lecanii* against *B. argentifolii* (biotype B of *B. tabaci*) classified the 35 *V. lecanii* strains for degree of the virulence.

The differences in the susceptibility of different larval stages to entomopathogens an increased adult mortality and decrease in the reproduction caused by *M. anisoplaie* (Malsam, 1999). The third instars of *Trialeurodes vaporariorum* were highly susceptible to infection by *Paecilomyces fumosoroseus* and *Beauveria bassiana* on cucumber plant. Detailed studies on the susceptibility of *T. vaporariorum* to infection by *A. aleyrodinis* revealed that eggs did not become infected but first instars nymphs hatching from those eggs were infected by the conidia surviving on the leaf surface. Over 90% of first and second instar nymphs became infected when treated with 2 ml of conidial suspension at a concentration of 4 × 10<sup>6</sup> conidia/ ml applied by means of a potter tower sprayer. The average percentage infection of treated third and fourth instars nymphs and so-called

pupae was 76, 28 and 12 respectively (Poprawski *et al.*, 2000).

The results of the present study suggest that *V. lecanii* could be considered for the microbial control of *B. tabaci*. Further studies are now required to determine pathogenicity and virulence of the isolates are independently from the original host and geographical location.

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