Effect of Different Fertilization Rates on Control of *Bemisia tabaci* (Genn.) by *Verticillium lecanii* and *Beauveria bassiana* in Potato Crop

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ABSTRACT

Pathogencity of the two entomopathogenic fungi isolates, *Verticillium lecanii* and *Beauveria bassiana* to the whitefly, *Bemisia tabaci* (Genn.) under laboratory conditions and also effect of different fertilization rates on its control by the two fungi in potato crop at El-Behira Governorate, Egypt for the two successive potato seasons 2006 and 2007 were studied. Three concentrations were used (2.5 x10⁵, 2.5 x 10⁶ and 2.5 x 10⁷ conidia/ ml.). Under laboratory conditions, results showed that *V. lecanii* and *B. bassiana* had induced death after the 4th day from treatment. The maximum percent of mortality (100 %) occurred after the 7th day post treatment with the third concentration (2.5 x 10⁷ conidia/ ml.) in both isolates. The third concentration was the highly toxic to the adults of *B. tabaci* compared with the other two concentrations. Under field conditions, the third concentration (2.5 x 10⁷) was also the most effective concentration against the whitefly after the third treatment by both *V. lecanii* and *B. bassiana*. Percent of reduction ranged between 55.8 and 100% in all concentrations. *V. lecanii* was slightly more effective than *B. bassiana* against *B. tabaci*. There were no direct or indirect effects of fertilization rates on the percent of infestation by *B. tabaci* and also its control by *V. lecanii* and *B. bassiana* in both seasons. These results confirmed that *V. lecanii* and *B. bassiana* isolates are promising agents for whitefly control in the field.

Key words: Entomopathogenic fungi, Bemisia tabaci, potato, fertilization rates, control, Egypt.

INTRODUCTION

The sweet potato whitefly, Bemisia tabaci Aleyrodidae) (Genn.) (Homoptera: causes significant damage to potato as direct feeding pest and vector of viruses (Bellows et al., 1994). B. tabaci is one of the most severe pests of crops in subtropical and tropical climates (Galina et. al., 1997). The widespread distribution of B. tabaci is attributed to their exceptionally wide host range and short generation time. The cause of its populations increase is unknown but it may be due to the extended use of synthetic organic insecticides and subsequent augmented resistance to pesticides, changing climatic conditions and international movement of plant materials in the nursery and horticultural trade (Wang et al., 2007).

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin has a high activity against whitefly (Al-Deghairi, 2008).

Dietary nitrogen and carbohydrates impact survival, growth and reproduction of insects (White, 1984). Bi et al. (2001) found that increasing plant nitrogen also enhanced cotton foliar photosynthetic rates and stomatal conductance, and altered concentrations of glucose, fructose and sucrose in cotton petioles. Petiole glucose levels were significantly correlated with numbers of whitefly adults on leaves during their peak populations. Significant correlations between whitefly numbers and other cotton physiological parameters occurred on only a few sampling dates. Seruwag et al. (2003)

investigated the influence of NPK fertilizer on the symptoms and spread of cassava mosaic virus disease (CMD) and on population of the whitefly vector (*Bemisia tabaci*) in Uganda using three cassava varieties.

The present work aims to use biocontrol agents to control the pest to avoid conventional insecticides pollution in potato tubers and environment as well as to reduce the costs of control. Besides, this work aims to test, if fertilization rates could affect the control of *B. tabaci* by *V. lecanii* and *B. bassiana*.

MATERIALS AND METHODS

Cultures of fungi

Fungi were isolated from different stages of Cassida vittata and Scrobipalpa ocellatella by collecting these insects from the field and transferred them to the laboratory. After few days, when infected insects, covered with fungal mycelium occurred, the insects' cadavers were placed on a wetted filter paper in a Petri-dish and incubated at 26±1 °C for 7 days. A potato dextrose agar (PDA) media was prepared (1 Kg potato, 100g dextrose, 80g agar, and 4L distilled water). This media was autoclaved at 120°C for 20 min, and poured in Petri- dishes (10cm diameter x 1.5 cm). The new fungal generation was isolated from the insect surface cadavers and cultured on PDA medium. Fungal culture purified weekly until pure cultures were obtained. Then the fungal cultures were incubated at 25±1°C and 92±5% RH.

Preparing the concentrations

Conidia of fungal isolates were harvested by rising with sterilized 0.5 % Tween 80 at 14th day old culture media. The conidia suspensions were filtered through cheese cloth to reduce mycelium clumps. Conidia were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm2). To restore the virulence of the isolates they were passed through their natural host or through the wax moth larvae Galleria mellonella.

Laboratory inoculation

Adults of the whitefly, *B. tabaci* were transferred to the laboratory from the field into Petri-dishes with potato leaf disk and incubated in 21±2°C and 75 ±5 % RH. Twenty five adults /concentration (five adults /replicate) were used in all treatments. The entomopathogenic fungi were sprayed using a manual sprayer in a suspension containing 2.5 x 10⁵, 2.5 x 10⁶ and 2.5 x 10⁷ conidia / ml; while sterilized water was sprayed to the leaves disks as blank control. The mortality of whitefly was observed daily for seven days.

Field applications

To obtain a large number of conidia for the field experiments, both V. lecanii and B. bassiana isolates were propagated on wetted rice. Two kilograms wetted rice were washed in boiled water for 10 min. and placed into thermal bags. These bags were autoclaved at 120°C for 20 min., then infected by both isolates and incubated at 26 ± 1 °C for 15 days. The conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added (Lacey 1997). The conidia were counted in the suspension using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm ²). The suspension was placed in plastic bottles (20 liter). The concentrations used were 2.5 x 10⁵, 2.5 x 10^6 and 2.5×10^7 conidia /ml. The fungal suspensions of V. lecanii and B. bassiana were applied in a field at El-Reiad region. El-Behira Governorate, Egypt during December of 2006 and 2007 seasons.

An area of one feddan (= 4200 m²) was divided into four plots; each was divided into seven subplots (three sub-plots were treated with three concentrations of *V. lecanii* and three with *B. bassiana* and the seventh one was the control, treated only with water). Every sub-plot was divided into three replicates. General agriculture practices were performed without any fertilization treatments in the first sub-plot. The second sub-plot was treated with 100 % NPK (Nitrogen: Phosphate: Potassium), the third sub-plot with 125 % NPK, and the fourth sub-plot treated with 150 % NPK. The suspensions were sprayed early in the morning by knapsack

sprayer (driven by a 0.5 -horsepower gasoline engine). Spraying rate was 5 liter/ sub-plot. The suspensions were sprayed three times (one week interval). The live insects of *B. tabaci* per leaf/ replicate were counted after each treatment. Percentages of reduction were calculated according to Henderson and Tilton formula (1955).

Statistical analysis

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

RESULTS AND DISCUSSION

Three concentrations from each of the two isolates of *V. lecanii* and *B. bassiana* were evaluated against *B. tabaci* under laboratory and field conditions.

1- Effect of V. lecanii and B. bassiana on B. tabaci under laboratory conditions.

As mentioned in Table (1), there was no effect of V. lecanii and B. bassiana on B. tabaci, three days post treatment. Mortalities occurred in the 4th day. This means that the entomopathogenic fungi, weather V. lecanii or B. bassiana had proliferated its mycotoxins on the 4th day post treatment. Percent of mortalities increased gradually and reached the maximum on the 7th day from treatment. With all concentrations, the percents of mortalities increased with increase concentrations. They ranged between 67.6 and 100 and from 62.7 to 100%, with V. lecanii and B. bassiana, respectively, on the 7th day after treatment. There were slight differences between effect of V. lecanii and B. bassiana on B. tabaci. The percent of mortalities with all concentrations $(C_1, C_2 \text{ and } C_3)$ for V. lecanii were 67.6, 88.94 and 100%, respectively. The corresponding results with B. bassiana were 62.7, 87.64 and 100%, respectively. Statistical analysis showed significant differences among all concentrations in both fungi. The less significant difference (L.S.D) increased gradually and reached 9.03 on the 7th day. This result compatible with Maniania (1991) who found that both of B. bassiana and V. lecanii caused mortalities of up to 97 and 100% in Chilo partellus, respectively. Zaki (1998) reported that B. bassiana showed high efficacy on the aphid, Aphis craccivora and the whitefly, B. tabaci infesting cucumber.

Gindin et al. (2000) reported that V. lecanii caused high virulence in the early stages of whitefly that became with older instars. B. bassiana had a

Table (1): Effect of V. lecanii and B. bassiana on B. tabaci under laboratory conditions.

Days after application	Percent of mortalities %							
	Control	C_1		C ₂		C ₃		L.S.D
		V. lecanii	B. bassiana	V. lecanii	B. bassiana	V. lecanii	B. bassiana	-
2 nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 th	0.0^{a}	22.1 ± 2.63^{b}	22.0 ± 1.73^{b}	25.8 ± 3.4^{b}	25.07 ± 1.1^{b}	41.8±6.25°	42.22±3.15°	6.33
5 th	0.0^{a}	45.56± 4.1°	33.7 ± 4.3^{b}	55.64 ± 4.9^d	$52.32 {\pm~} 3.2^{\textrm{d}}$	67.9 ± 3.7^{e}	65.55±2.4 ^e	7.0
6 th	4.0± 0.04°	54.4±2.3 ^b	48.06 ± 6.9^{b}	77.61 ±2.3°	68.66± 2.7 ^b	89.8± 4.01 °	85.69 ±1.8°	8.3
7 th	4.0 ± 0.04^{a}	67.6± 3.3 ^b	62.70± 2.7 ^b	88.94± 4.3°	87.64± 5.6°	100± 7.4 ^d	100 ± 5.5^{d}	9.03

Table (2): Effect of *V. lecanii* and *B. bassiana* on *B. tabaci* in potato field at El-Behira Governorate, Egypt under different fertilization rates during season 2006.

Applications -	No. of alive adult / leave							
	Control	C_1		C_2		C ₃		L.S.D
		V. lecanii	B. bassiana	V. lecanii	B. bassiana	V. lecanii	B. bassiana	-
			with	out NPK treatn	nents			
1 st	7 ± 1.7^{a}	6 ± 2.1^{a}	8 ±1 a	8 ±1.7 ^a	6 ±1.5 a	9 ±2 a	10 ± 2.6^{a}	3.06
2 nd	9 ±1.7 a	5 ±2 ^{bc}	6 ± 1 b	3 ±1 ^{bc}	4 ±1.7	2 ±1°	5 ±1 bc	2.5
3 rd	8 ±2 ^a	3 ±1.7 ^{bc}	4 ±1 ^b	1 ±0.0 ^{bc}	2 ±1.7 ^{bc}	0°	0°	2.4
% Reduction		56.25	56.25	89.06	70.8	100	100	
			With	100 NPK treat	ments			
1 st	11 ± 1.7^{a}	11 ± 1.7^{a}	12 ± 2^{a}	12 ± 2.6^{a}	11 ± 5.2^{a}	12 ± 3.4^{a}	13 ±2.6 ^a	5.99
2 nd	11 ± 3.5^{a}	5 ±1 ⁶	5 ±1 ^b	4 ±1 ^b	5 ±2 ^b	3 ±1 ^b	4 ±1 ^b	2.99
3 rd	14 ±4 ^a	3±2 ^{bc}	5 ±2 ^b	1 ±1 ^{bc}	3 ±1 ^{bc}	0°	2 ±1 ^{bc}	3.80
% Reduction		78.6	67.5	93.5	78.7	100	88	
			With	125 NPK treat	ments			
1 st	17 ±1.7 ^a	16 ± 3.5^{a}	17 ± 4.6^{a}	18 ± 3.5^{a}	16 ± 3.6^{a}	15 ±6 ^a	15 ±2 ^a	7.2
2 nd	16 ±2 ^a	10 ± 2.6^{ab}	11 ± 1.7^{ab}	8 ±1 ^b	11 ± 3.6^{ab}	10 ± 4.6^{ab}	9 ±1 ^{ab}	4.98
3 rd	18 ± 3.5^{a}	5 ±2 ^{bc}	8 ± 3.6^{b}	2 ±2°	3 ±1 ^{bc}	0°	2 ±2°	4.23
% Reduction		70.5	55.8	89.6	82.4	100	87.5	
			With	150 NPK treat	ments			
1 st	9 ±2ª	11 ± 2.6^{a}	13 ±3 ^a	9 ±2ª	11 ±1 ^a	12 ±3 ^a	10 ± 2.6^{a}	4.1
2 nd	13 ±1 ^a	7 ± 2.6^{b}	9 ±2 ^b	5 ±1 ^b	7 ±2 ^b	6 ±3 ^b	5 ± 2.6^{b}	3.8
3 rd	11 ±1.7 ^a	4 ±2.6 ^{bc}	5 ±3.5 ⁶	2 ±1.7 ^{bc}	3- ±2 ^{bc}	0°	1 ±1.7°	3.03
% Reduction		70.2	68.8	82	77.9	100	91.9	

Tables (3): Effect of *V. lecanii* and *B. bassiana* on *B. tabaci* in potato field at El-Behira Governorate, Egypt under different fertilization rates during season 2007.

			No. o	of alive adult /	leave			
Applications	Control	C_1		C ₂		C ₃		L.S.D
		V. lecanii	B. bassiana	V. lecanii	B. bassiana	V. lecanii	B. bassiana	
			without	NPK treatme	·			
1 st	12 ± 2.6^{a}	10 ± 2.6^{a}	9 ±2 ª	9 ±2 a	11 ± 1^a	12 ± 3^{a}	10 ± 2.6^{a}	4.08
2 nd	14 ± 2.6^{a}	8 ±1.7 ^b	6 ±2.6 ^b	3 ± 1.7^{b}	5 ±2.6 ⁶	5 ±1 ^b	5 ±1 ^b	3.84
3 rd	17 ±5°	5 ±1 ^b	5 ±1 ^b	2 ±1 ^b	4 ±1 ^b	1 ±1°	3 ±1 ^b	3.98
% Reduction		65	61.1	84.4	74.5	90	79	
			With 10	0 NPK treatm	ents			
1st	16 ± 2.6^{a}	15 ±4.3°	20 ±3.5 ^a	21 ± 3.6^{a}	18 ±3 ^a	19 ±5.3 ^a	17 ± 1^{a}	7.00
2nd	18 ±1.7°	10 ±2 ^b	18 ±2 ^a	15 ± 1.7^{a}	10 ± 2.6^{b}	7 ±2 ⁶	9 ±1.7 ^b	4.3
3rd	17 ±4.5°	5 ±2°	9 ±0.0 ^b	3 ±1°	5 ±1°	2 ±2°	3 ±1.7°	2.93
% Reduction		68.6	57.7	86.6	73.9	90.1	83.4	
	1.071		With 12	5 NPK treatm	ents			
1st	20 ±5 ^a	15 ±4.6°	16 ± 3.8^{a}	19 ±3 ^a	20 ± 3.6^{a}	20 ±5°	21 ±2 ^a	6.75
2nd	19 ± 2.6^{a}	13 ±2 ^b	12 ±4.6 ^b	10 ± 2.6^{b}	11 ±2 ^b	9 ±3.6 ^b	11 ±1.7 ^b	4.98
3rd	21 ± 1.7^{a}	7 ± 1.7^{b}	7 ± 2.6^{b}	5 ± 1.7^{bc}	6 ±1.5 ^{bc}	2 ±1°	4 ±1b ^c	3.02
% Reduction		55.6	58.4	75	71.5	90.5	81.9	
				0 NPK treatm	ents			
1st	15 ±4.6 ^{ab}	13 ±3.5 ^{ab}	14 ±4 ^{ab}	17 ± 2.6^{a}	16 ±2.6 ^a	19 ±1.7°	10 ±2.6 ^b	4.08
2nd	17 ±3.6a	10 ±2 ^b	9 ±1 bc	10 ±1 ^b	7 ±1.7 ^{bc}	6 ±1°	5 ±1.7°	2.65
3rd	20 ±2.5 ^a	6 ±1 ^{bc}	7 ±1 ^b	4 ±2.6 ^{bc}	5 ±1 ^{bc}	1 ±1.7°	3 ±1.7 ^{bc}	3.67
% Reduction		65.4	62.5	82.4	76.6	96.1	77.5	

high activity against whitefly (Al-Deghairi, 2008). Abdel-Baky *et al.* (2005) mentioned that entomopathogenic fungi caused good mortality to whitefly.

On the other hand, Wraight *et al.* (2000) described the control of the silver leaf whitefly *Bemisia argentifolii* on several plants (including cucurbits, broccoli, tomatoes, and cotton) using *B. bassiana* by only a few fungus-killed adults on the plants, did not exceed 1 %. Gindin *et al.* (2000) found that the maximum adult mortality caused by *V. lecanii* to *B. argentifolii* was between 34.1±5.1 and 52.6±3.8%.

2- Effect of different fertilization rates on control of *B. tabaci* by *V. lecanii* and *B. bassiana* during 2006 season.

As mentioned in table (2), there was no difference among all sub-plots (without fertilization, 100 NPK, 125 NPK and 150 NPK) which were treated with *V. lecanii* and *B. bassiana* after the first application. After the second application, the number *B. tabaci* /leave decreased compared to the control. Percent of reduction by *V. lecanii* and *B. bassiana* after the third application in the first sub-plot (without fertilization) was 56.25, 89.06, and 100%; and 56.25, 70.8 and 100% with C₁, C₂ and C₃, respectively. Corresponding results with the second sub-plot (with 100 NPK) were 78.6, 93.5 and 100%; 67.578.7, and 88%, respectively, when 100 units of NPK were used.

When 125 NPK units were used, the percents of reduction were 70.5, 89.6 and 100%; 55.8, 82.4 and 87.5%, respectively. The same results were found when 150 NPK units were used. The percents of reduction after the third application was 70.2, 82 and 100%; 68.8, 77.9 and 91.9 in both isolates, respectively. These results cleared that the percent of infestation by *B. tabaci*/ leave was not affected by any of the fertilization rates tested.

Statistical analysis showed that there were no significant differences between the treatments and control after the first application in all sub-plots. After the second application, there were significant differences between all concentrations and control. After the third application highly significant differences appeared among all concentrations. The L.S.D values were 2.4, 3.8, 4.23 and 3.03 after the third application. The statistical analysis confirmed that the third concentration (2.5 x 10⁷) was the highly effective compared to the first and second concentrations in all treatment.

3- Effect of different fertilization rates on control of *B. tabaci* by *V. lecanii* and *B. bassiana* during 2007 season.

During 2007 season, as shown in table (3) the third concentration (2.5×10^7) in *V. lecanii* was the

most effective concentration against B. tabaci followed by the third concentration in B. bassiana isolation. The percent of reduction in both concentrations were 90, 79; 90.1, 83.4; 90.5, 81.9 and 96.1 and 77.5 in the first (without fertilization), second (with 100 NPK), third (with 125 NPK) and fourth sub-plots (with 150 NPK), respectively. Although there was no difference between all concentrations and control after the first application in all sub-plots, the differences occurred gradually after the second and third applications. The same results were found by Pineda et al. (2007) who found that survival of whitefly nymphs decreased by 19 and 28% after the first and second fungal applications, respectively, in one trial, and by 62 and 71% in the other trial.

Statistical analysis showed that there were no significant differences between the second and third concentrations, especially in *B. bassiana* isolates. As mentioned in table (2) and (3) after the third application, there were significant differences among all concentrations in *V. lecanii* isolate. This means that the third concentration in *V. lecanii* was the most effective concentration against *B. tabaci*. The L.S.D values after the third application were 3.98, 2.93, 3.02 and 3.03 in the first, second, third and fourth plots, respectively.

Dietary nitrogen and carbohydrates impact the survival, growth and reproduction of insects (White, 1984). Seruwag et al. (2003) investigated the influence of NPK fertilization on the symptoms and spread of cassava mosaic virus disease (CMD) and on populations of the whitefly vector (B. tabaci). Three varieties of cassava were used: Migyera (CMD-resistant). Nase 2 (tolerant) Ebwanatereka (highly susceptible) in 1995-96 and 1996-97 planting seasons. Adult populations per shoot were increased significantly (P<0.05) by NPK fertilizer on Nase 2 and Ebwanatereka in 1995-96 and on Ebwanatereka in 1996-97, although the increases were significantly different. Application of **NPK** fertilizers did not significantly influence the population of whiteflies on variety Migyera in either experiment.

On the other hand, Bi et al. (2001) found that increasing plant nitrogen also enhanced cotton foliar photosynthetic rates and stomatal conductance, and altered concentrations of glucose, fructose and sucrose in cotton petioles. Petiole glucose levels were significantly correlated with numbers of whitefly adults on leaves during their peak populations. Significant correlations between whitefly numbers and other cotton physiological parameters occurred on only a few sampling dates.

clear that obtained data Finally, entomopathogenic fungi V. lecanii and B. bassiana can be used as a promising agent in pest control programs instead of conventional pesticides to reduce the environmental pollution especially when the pests were under the economic threshold level. The results also cleared that there was no relation between fertilization and control of B. tabaci by V. lecanii and B. bassiana. Bi et al. (2003) reported that no significant correlations between levels of dietary N compounds in cotton petioles and numbers of whiteflies, either adults or immatures, on the cotton plants.

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