International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.12, pp 378-385, 2017

Entomopathogenic Fungi as A biological Control Agents on green peach aphid, *Myzus persicae* in Potatoes Crop.

Lamya Ahmed Al-Keridis

Department of Biology, Faculty of Sciences, Princess Nourah Bint Abdulrahman University, 11474 Riyadh, Saudi Arabia

Abstract : This study was carried out during two successive potatoes seasons (2016-2017), to study of entomopathogenic fungi on green peach aphid, *Myzus persicae* (Sulzer). The aphid Populations was evaluated in the field early in the season in December 2016 ,which began to appear on potatoes plants. Thereafter number of aphids increased gradually to reach a peak of abundance during to the first of December 2016 and to the first of January 2017. In laboratory experiment, the mortality of aphids was recorded daily. *Verticillium lecanii* and *Beauveria bassiana* caused 100 % mortality at the 8th day after inoculation. In field experiment, the first spray of the fungi (*Verticillium lecanii* or *Beauveria bassiana*) with concentrations 1×10^{4}, 1×10^{5} and 1×10^{6} spores /ml. was took place in December 2016 as well as 2017. The aphid populations were declined in the second week of spray to 2.67, 2 and 1.66 when treated with *Verticillium lecanii* at tested concentrations, respectively. Also, aphid populations were declined to 3.33, 7 and 2.67 when treated with *Beauveria bassiana* at the same concentrations, respectively. While, in the third week the populations were declined to 0.33 when treated with *Verticillium lecanii* at concentration 1×10^{6} spores /ml.

Keywords: *Verticillium lecanii, Beauveria bassiana, Myzus persicae*, Control

1. Introduction

The Potatoes is cool season crop it grown through the winter months and harvested in early spring. Autumn potatoes production usually results in poor plant stands and low production. Potatoes grown best in fertile, well-drained sandy loam soils.

It cultivated from mid August until mid February. Once emerged, Potatoes are susceptible to cut-worms, flea beetles, leaf hoppers, white fly and Aphids. Entomopathogenic fungi are considered by some entomologists to be the best candidates for the biological control of aphids [1-7],and numerous accounts of cereal aphids killed by entomopathogenic fungi have been documented in Europe [8-12]. Also, several species of entomopathogenic fungi can cause fatal disease in aphids, including *Conidiobolus obscures* (Hall& Dunn) Remaudiere & Keller, *Erynia neoaphidis* Remaudiere& Hennebert, *Verticilium lecanii* (Zimmerman) Viegas, various species of *Beauveria*, and *Paecilomyces farinosus* [13-17].

In Egypt, some studies revealed the effect of entomopathogenic fungi on the population dynamics of some pests such as: *Aphis craccivora* [18,19]. Cereal aphids [20-23], *Bemisia tabaci* [4], and *Spodoptera littoralis*, *S. exigua* and nymphs of *Aphis craccivora* [24-28].

Material and Methods

Fungi culture:

Fungi were used in this study; *Verticillium lecanii* and *Beauveria bassiana* were grown on Potato dextrose agar (PDA) (1 Kg potatoes, 80 g Agar, 100 gr. Dextrose and 4 lit. distilled water). The media was autoclaved at 120 ºc for 20 minutes, and poured in Petri- dishes (10 cm diameter x 1.5 cm h.). Then, the fungi were incubated at 25 ±1 ˚c and 92± 5 % RH. The fungal isolates were re-cultured every 14 – 30 days and kept at 4 ˚c.

Preparing of the concentrations:

Spores of fungal isolates were harvested by rising with sterilized 0.5 % Tween 80 from 14 day old culture (PDA) media. The suspensions were filtered through cheese cloth in order to get rid of mycelium clumping. The spores were counted in the suspension using a Haemocytometer (0.1 mm x 0.0025 mm^2).The concentrations were used 1 x 10^4, 1 x $10^5$and 1 x 10^6 spores / ml.

Laboratory inoculation:

The aphids were transferred to the Laboratory from the field and put it in Petri- Dishes with leaf disk potatoes under laboratory conditions 22 ± 2 ˚c and 65 ±5 % RH. Five individuals / dish, and replicated twenty-five /concentration. The fungi were applied in a suspension containing 1 x 10^4(C1) 1 x 10^5 (C2) and 1 x 10^6 (C3) spores / ml. In the control treatment 1 ml. of sterilized water was added to the leaves disks. The mortality of aphids was observed and recorded daily.

Field application:

Fungal suspensions of *V. lecanii* and *B. bassiana* were applied in potato field in December in both 2016 and 2017. Three concentrations of conidia spores of *V. lecanii* and *B. bassiana* were tested 1 x 10^4, 1 x 10^5 and 1 x 10^6 spores /ml.

An area (one feddan) was divided into four parts; General agriculture practices were performed three plots were treated with three concentrations of *V. lecanii* and three with *B. bassiana* and the 7^{th} one used as control which treated by water only). The fungal suspensions were sprayed three times (one week interval) early in the morning. The live insects of *Myzus persicae* per leaf/ replicate were counted before as well as after all treatments.

Results

Three concentrations of two isolates *V. lecanii* and *B. bassiana* were evaluated against *Myzus persicae* under laboratory and field conditions.

1- Laboratory experiments:

The effect of *V. lecanii* and *B. bassiana* on *Myzus persicae* was tested under laboratory conditions. The data in Table (1) indicated that the both of *V. lecanii* and *B. bassiana* have not effect on *Myzus persicae* after two days from treatment.

Table (1): Effect of *Verticillium lecanii* and *Beauveria bassiana* on green peach aphid, *Myzus persicae* survival under laboratory conditions.

Treatments	Concentration	% Mortality after days						
		2nd	3rd	4th	5th	6th	7th	8th
Verticillium lecanii	*C1	0.0	5	33	55	80	87	100
	C2	0.0	8	47	70	82	97	100
	C3	0.0	10	67	88	97	100	100
Beauveria bassiana	C1	0.0	3	28	50	70	77	80
	C2	0.0	4	33	65	77	85	87
	C3	0.0	7	45	76	85	87	100
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0

***C_1** = 1 x 10^4 spores/ml. **C_2** =1x 10^5 spores/ ml. **C_3** = 1 x 10^6 spores/ ml.

Mortalities are occurred in the 3rd day. % mortalities are increased gradually and reached to the maximum in the 8th day from treatment. % mortalities ranged between 87 to 100 and 77 to 87 % with *V. lecanii* and *B. bassiana*, respectively, in the 7th day after treatment. This mean that *V. lecanii* isolation is more effective than *B. bassiana*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *V. lecanii* isolation at 6th day were 80, 82 and 97 %, respectively. The corresponding results with *B. bassiana* isolation were 70, 77 and 85 %, respectively.

2 – Field experiments:

A. Effect of different fertilization rates on control of *Myzus persicae* by *V. lecanii* and *B. bassiana* during season (2016):

Data in (Table 2) indicated that there were significant difference among all plots (without fertilization, 50NPK, 75 NPK and 125 NPK) which treated with *V. lecanii* after the first application.

After the second application the number of *Myzus persicae* / leaf were decreased compared with control. The mean numbers by *V. lecanii* after the third applications in the first plot (without fertilization) were 0.60, 0.30 and 0.28 with C_1, C_2 and C_3, respectively. The corresponding results with the second part (with 50 NPK) were1.30, 0.63 and 0.0, respectively, when 50 units of NPK were used. While, 75 NPK units were used the percent of the mean numbers were 2.0, 1.2 and 0.0, respectively. The same trends approximately were found when 125 NPK units were used.

The reduction of infestation ranged from 3.36 % in C_1 when treated with 75 NPK to 100 % when treated with 50, 75 and 125 NPK. These results cleared that the percent of infestation by *Myzus persicae* / leaf were affected by fertilization.

Table (2): Effect of *V. lecanii* on *Myzus persicae* in potato field at different fertilization rates (season 2016):

Fertilization rates	Fungi spores concentration	Mean aphid population / leaf and % reduction in infestation after spores applications					
		1st		2nd		3rd	
		** M. no.	*** %R.	M. no.	%R.	M. no.	%R.
0.0 NPK	C1*	5.66	48.2	2.65	75.40	0.60	55.32
	C2	9.0	75.0	2.0	97.47	0.30	37.93
	C3	6.68	72.01	1.66	80.95	0.28	42.10
	control	9.1	--	12.3	--	7.67	--
50 NPK	C1	10.1	64.12	4.30	73.30	1.30	26.30
	C2	9.69	67.30	3.60	92.0	0.63	84.1
	C3	27.0	75.9	8.1	100	0.0	100
	control	9.30	--	8.61	--	9.66	--
75 NPK	C1	16.2	3.30	6.7	9.3	2.0	44.80
	C2	17.1	29.3	5.1	17.3	1.2	21.2
	C3	16.7	91.1	0.7	100	0.0	100
	control	15.3	---	15.7	--	16.4	--
125 NPK	C1	18.4	12.5	4.4	51.3	0.5	100
	C2	19.3	21.2	4.1	74.1	0.2	100
	C3	19.0	33.4	3.3	100	0.0	100
	control	18.2	--	19.3	--	19.1	--

***C_1** = 1 x 10^4 spores/ml. **C_2** =1x 10^5 spores/ ml. **C_3** = 1x 10^6 spores/ ml.
**M. no. = Mean number.
***% R = Percent reduction in infestation.

Data in (Table 3) indicated that there was significant difference among all plots (without fertilization, 50 NPK, 75 NPK and 125 NPK) which treated with *B. bassiana* after the first application.

After the second application the number of *Myzus persicae* / leaf were decreased compared with control. The mean numbers by *B. bassiana* after the third application in the first plot (without fertilization) were 1.30, 1.2 and 1.0 with C_1, C_2 and C_3, respectively.

Table (3): Effect of *B. bassiana* on *Myzus persicae* in potato field at different fertilization rates (season 2016):

Fertilization rates	Fungi spores concentration	Mean aphid population / leaf and % reduction in infestation after spores applications					
		1st		2nd		3rd	
		** M. no.	*** %R.	M. no.	%R.	M. no.	%R.
0.0 NPK	*C1	7.2	46.2	3.2	55.1	1.30	34.3
	C2	13.0	40.1	7.2	80.2	1.2	35.4
	C3	6.30	51.2	2.3	63.3	1.0	53.3
	control	9.1	--	12.2	--	7.3	--
50 NPK	C1	9.2	57.2	5.1	65.2	2.3	19.1
	C2	10.1	63.2	4.2	77.2	1.0	12.2
	C3	9.2	62.3	4.4	68.4	1.3	39.2
	control	9.30	--	8.60	--	9.60	--
75NPK	C1	17.2	3.2	6.1	7. 2	2.2	24.3
	C2	15.6	8.2	6.0	14.92	2.0	24.22
	C3	16.0	6.02	6.3	34.22	1.2	100
	control	15.3	--	15.4	--	16.2	--
125 NPK	C1	18.0	7.2	4.4	39.3	1.2	7.4
	C2	19.3	1.0	5.5	79.69	0.3	100
	C3	21.0	41.2	3.2	27.3	0.5	100
	control	18.1	--	19.4	--	19.0	--

$*C_1$ = 1 x 10^4 spores/ml. **C_2** =1x 10^5 spores/ ml**.** **C_3** = 1x 10^6 spores/ ml**.**
**M. no. = Mean number.
***% R = Percent reduction in infestation**.**

The corresponding results with the second part (with 50 NPK) were 2.3, 1.0 and 1.3, respectively, when 50 units of NPK were used. While, 75 NPK units were used the percent of the mean numbers were 3.2, 2.0 and 1.2, respectively. The same trends approximately were found when 125 NPK units were used. These results cleared that the percent of infestation by *Myzus persicae* / leaf were affected by fertilization.

B. Effect of different fertilization rates on control of *Myzus persicae* by *V. lecanii* and *B. bassiana* during season (2017):

During 2017 season (Table 4) showed that there are no significant difference between 1^{st} (C_1) or 2^{nd} (C_2) spores concentrations and control after the first application in all parts, the differences appear gradually after the second and third application. On the other hand the third concentration (C_3) in *V. lecanii* was the best concentration against *Myzus persicae* followed by the third concentration in *B. bassiana* isolation at all fertilization rates, First (without fertilization), second (with 50 NPK), third (with 75 NPK) and fourth plots (with 125 NPK).

As mentioned before in both tables (2 & 3) after the third application, there are significant differences between all concentrations tested and the third one (C_3) in *V. lecanii* isolate. This means that the third concentration in *V. lecanii* was the best concentrations against *Myzus persicae.*

After the second application the number of *Myzus persicae* / leaf were decreased compared with control. The mean numbers by *V. lecanii* after the third application in the first plot (without fertilization) were 1.0, 0.2 and 0.0 with C_1, C_2 and C_3, respectively. The corresponding results with the second part (with 50 NPK) were 0.5, 0.2 and 0.0, respectively, when 75 units of NPK were used. While, 125 NPK units were used the percent of the mean numbers were 1.2, 1.0 and 0, respectively. The same results approximately were found when 125 NPK units were used.

The reduction of infestation ranged from 10.3 % in (C_1) when treated with 75 NPK to 100 % when treated with 50, 75 and 125 NPK.

Table (4): Effect of *V. lecanii* on *Myzus persicae* in potato field at different fertilization rates (season 2017):

Fertilization rates	Fungi spores concentration	Mean aphid population / leaf and % reduction in infestation after spores applications					
		1st		2nd		3rd	
		** M. no	*** %R	M. no	%R	M. no	%R
0.0 NPK	*C1	6.1	82.1	2.2	83.2	1.0	55.3
	C2	5.5	84.2	2.0	92.3	0.2	100
	C3	6.5	88.2	2.5	100	0.0	100
	control	6.3	--	6.5	--	6.2	--
50 NPK	C1	3.5	91.4	2.7	95.2	0.5	95.2
	C2	3.2	93.2	1.2	96.2	0.2	100
	C3	2.6	80.0	1.0	100	0.0	100
	control	2.3	--	3.2	--	3.0	--
75 NPK	C1	9.2	50.3	4.2	67.4	1.2	10.3
	C2	9.3	59.3	3.3	74.2	1.0	97.1
	C3	10.0	64.2	3.0	100	0.0	100
	control	10.0	--	10.2	--	9.6	--
125 NPK	C1	18.0	3.5	7.0	14.2	1.2	45.7
	C2	16.2	33.0	5.0	24.0	1.0	97.1
	C3	14.2	35.3	3.4	100	0.0	100
	control	13.2	--	16.2	--	17.2	--

***C_1** = 1 x 10^4 spores/ml. **C_2** =1x 10^5 spores/ ml. **C_3** = 1x 10^6 spores/ ml.
**M. no. = Mean number.
***% R = Percent reduction in infestation.

During 2017 season (Table 5) showed that there are no significant difference between 1st (C_1) or 2nd (C_2) spores concentrations and control after the first application in all parts, the differences appear gradually after the second and third application. After the second application the number of *Myzus persicae* / leaf were decreased compared with control. The mean numbers *B. bassiana* after the third application in the first plot (without fertilization) were 2.1, 4.2 and 0.5 with C_1, C_2 and C_3, respectively. The corresponding results with the second part (with 50 NPK) were 1.5, 91.2 and 0.5, respectively, when 50 units of NPK were used. While, 75 NPK units were used the percent of the mean numbers were 3.2, 3.0 and 1.2, respectively. The same results approximately were found when 125 NPK units were used.

The reduction of infestation ranged from 92.2 % in (C_1) when treated with 50 NPK to 100 % when treated with 75 and 125 NPK.

Table (5): Effect of *B. bassiana* on *Myzus persicae* in potato field at different fertilization rates (season 2017).

Fertilization rates	Fungi spores concentration	Mean aphid population / leaf and % reduction in infestation after spores applications					
		1st		2nd		3rd	
		** M. no	*** %R	M. no	%R	M. no	%R
0.0 NPK	*C1	6.3	82.2	2.7	68.7	2.1	78.2
	C2	6.0	79.3	3.0	39.3	4.2	80.2
	C3	6.7	83.3	2.7	89.2	0.5	93.2
	control	6..2	--	6.7	--	6.3	--
50 NPK	C1	4.0	92.2	2.6	92.5	1.5	93.6
	C2	9.0	94.2	2.8	55.5	1.2	85.2
	C3	4.3	96.0	1.7	95.5	0.5	92.5
	control	2.3	--	3.3	--	3.0	--
75NPK	C1	11.2	44.3	5.8	45.4	3.2	35.5
	C2	10.1	57.4	4.1	22.9	3.0	77.2
	C3	9.5	55.2	4.1	33.4	1.2	100
	control	10.0	--	10.5	--	9.5	--
125 NPK	C1	19.5	2.32	7.7	23.5	4.3	33.3
	C2	15.6	21.2	5.7	24.5	3.0	40.2
	C3	16.3	28.3	5.0	45.7	2.7	100
	control	13.3	--	16.2	--	17.2	--

***C_1** = 1 x 10^4 spores/ml**.** **C_2** =1x 10^5 spores/ ml**. C_3** = 1x 10^6 spores/ ml**.**
**M. no. = Mean number.
***% R = Percent reduction in infestation**.**

Discussion

The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *V. lecanii* isolation at 6th day were 80, 82 and 97 %, respectively. The corresponding results with *B. bassiana* isolation were 70, 77 and 85 %, respectively. This result compatible with Maniania [29] who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. Zaki[30] reported that *B. bassiana* as an entomopathogenic fungi showed high effects on the aphid *Aphis craccivora* and the white fly *B. tabaci* infesting cucumber. Gindin [31]. reported that *V. lecanii* caused higher virulence in the early stages of whitefly and reduced with older instars. Abdel-Baky [32] mentioned that entomopathogenic fungi caused good mortality to whitefly.

During 2017 season (Table 4) showed that there are no significant difference between 1st (C_1) or 2nd (C_2) spores concentrations and control after the first application in all parts, the differences appear gradually after the second and third application. On the other hand the third concentration (C_3) in *V. lecanii* was the best concentration against *Myzus persicae* followed by the third concentration in *B. bassiana* isolation at all fertilization rates, First (without fertilization), second (with 50 NPK), third (with 75 NPK) and fourth plots (with 125 NPK). These results were in agreement with those found by Pineda, Ismail, et al., Abdel-Raheem, Abdel-Raheem, et al., [33-38], who stated that survival of Aphids nymphs decreased by 22 and 34% after the first and second fungal applications, respectively, in one trial, and by 72 and 81% in the other trial.

Conclusion

Finally, these data clear that the entomopathogenic fungi *V. lecanii* and *B. bassiana* can be used as a promising agent in pest control and integrated pest management programs instead of conventional pesticides to reduce the environmental pollution especially when the pests were under the economic threshold.

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