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Combined Effect of Entomopathogenic Fungi, *Beauveria bassiana* and Certain Insecticides for the Control of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under Semi-Field Conditions

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ABSTRACT

This study was carried out at Quesna district, Menoufia governorate during 2017 growing season under semi field conditions against 2nd and 4th instar larva of *Spodoptera littoralis* (Boisd.) to evaluate the efficiency of combining *Beauveria bassiana* with certain insecticides (emamectin benzoate, methomyl and *Bacillus thuringiensis*) in tomato field. In general, data revealed, clearly, that methomyl had the highest efficacy against 2nd larval instar, where, the percentages corrected mortality were 98.28, 62.68 and 27.09% at concentrations RC,1/2RC and 1/4RC respectively. While, when 4th larval instar treated by emamectin benzoate, the observed total percentage corrected mortality was the highest value (90.54%) at RC followed by methomyl (85.79%) at RC, whereas, when treated 2nd and 4th larval instar with fungi had the lowest values of percentage corrected mortality at all concentrations compared with remaining treatments. When treated larvae with mixture of fungi (*B. bassiana*) and insecticides percentages mortalities of second and fourth larval instar of emamectin benzoate + *B. bassiana* and methomyl + *B. bassiana* had the highest values after 24hrs. then decreasing gradually, but the highest values of percentage mortality of second and fourth larval instar when mixtures of *Bacillus thuringiensis* + *B. bassiana* were after fifth days at concentrate of insecticide 1/2RC and after third day at concentrate of insecticide 1/4RC. So mixing increases the efficiency of their insecticides.

INTRODUCTION

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is one of the most notorious chewing insect pests that causes heavy losses in cotton, thus, deprives the farmers from getting high yield. Insecticides of synthetic origin have been used to manage insect pests for more than 50 years (Charnley and Collins, 2007). However, due to adverse effects of insecticides on environment, their rational use is being advocated. Development of an effective control method against the cotton leaf worm has been urgently needed since it does serious damage to many important agricultural crops in Egypt. There is a serious interest in the use of microbial insecticides for biological control of insect pests, as alternatives to chemical control, since they neither leave toxic chemical residues in the environment nor do they induce resistance in their insect hosts (Evans, 1999). And hence, the public awareness and concern for environmental quality, has led to more focused attention on research aiming at developing biological agents (Hidalgo *et al*., 1998, Ibrahim, A. Amira 1974 and Shairra, 2010). A promising strategy with good potential to control insect pests and at the same time, to minimize adverse effects of chemical insecticides is the use of entomopathogenic microbial agents such as nematodes and fungi.

Microbial control agents can be attractive alternatives to chemical pesticides as the natural enemies of the pest population devastate pests with no hazard effects on human health and environment. As microbial control agents have complex mode of action, it's very difficult for a pest to develop resistance against these agents. The most common microbial agents are viruses, bacteria, nematodes, and fungi and they are used throughout the world with great advantage and success. But fungal biocontrol agents are the most important among all of them due to easy delivery, improving formulation, vast number of pathogenic strains known, and easy engineering techniques (Butt *et al*., 2001; Wraight *et al*., 2001; Butt, 2002 and Goettel *et al.*, 2010). Ibrahim *et al.,* 2017 found that, the utilization of combination of *Bacillus thuringiensis* (Dipel 2X), emamectin benzoate (Absolute) and methomyl (Jito) with *B. bassiana* is feasible for the control of polyphagous insect, *S. littralis* especially the second larval instar.

This study amid to evaluate the efficiency of certain insecticides alone and mixture with *B. bassiana* to try reducing dose of insecticides in the field.

MATERIALS AND METHODS

Rearing of insect:

Spodoptera littoralis was obtained from Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. Eggs hatching and larval rearing took place at 27 ± 1°C, 65 ± 5% R.H. and a photoperiod of LD 14:10h according to (Hegazi *et al*., 1977). Emerged adult moths were maintained together on 80% bee-honey solution until egg laying. Newly molted 2nd & 4th instar larvae were utilized in all experiments.

Tested compounds:

1- Biovar 10% WP produced by Plant Protection Research Institute, Egypt.

Common name: *Beauveria bassiana*

Recommended rate: 200g / 100L

2- Jito 90% SP, distributed by Kafr El Zayat Pesticides & Chemicals Co. Egypt

Common name: Methomyl

Recommended rate: 300g / feddan.

3- Absolute 5% ME, distributed by Agrogroup Company, Egypt

Common name: Emamectin benzoate

Recommended rate: 75ml / feddan.

4- Dipel 2X 6.4% WP, distributed by My Trade Company, Egypt

Common name: *Bacillus thuringiensis*

Dipel 2X; a selective bacterial insecticide containing 32000 IU of *Bacillus thuringiensis* subsp. *kurstaki* per mg of product.

Recommended rate: 200g / feddan.

The experiment was carried out at Quesna district, Menoufia governorate during February 2017 growing season under semi field conditions to evaluate the efficiency of the insecticides; emamectin benzoate, methomyl and *Bacillus thuringiensis* (at their

recommended rate (RC), half recommended rate (1/2RC) and quarter recommended rate (1/4RC)). Also, evaluation the mixture of *B. bassiana* with 1/2RC and 1/4RC of other compounds (emamectin benzoate, methomyl and *Bacillus thuringiensis*) against second and fourth larval instars of *S. littoralis*.

An area of about quarter feddan with Tomato was chosen for each treatment and control. Each area was divided into four experimental plots as replicates. Untreated belt (42x7m) was left between each two treatments as a border. By using a knapsack sprayer (20 liters) the compounds were sprayed.

After spraying treatments, hand picking of tomato leaves then put in clear paper package then move to laboratory.

In laboratory, freshly moulted 25 second and fourth instar larvae were, gently, placed in each jar with four replications including controls. Mortality percentages were calculated after 1day, 3days, 5days, 7days and 9days then percentages of mortalities were corrected according to Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

Efficacy of the tested compounds alone:

Results in (Table 1) showed that, the efficacies of the insecticides against the 2nd larval instar of *S. littoralis.* In general, data revealed, clearly, that methomyl had the highest efficacy against 2nd larval instars, with 98.28, 62.68 and 27.09% at concentrations RC,1/2RC and 1/4RC respectively, while fungi had the lowest efficacy where, total percentage corrected mortality were 50.42, 28.53 and 10.08% under the same concentrations. Treatments with emamectin benzoate and methomyl had high values of percentage corrected mortality after 24hrs. (values were 54.24, 30.51 and 16.95% of emamectin benzoate and were 77.97, 45.76 and 18.64%, at concentrations RC, 1/2RC and 1/4RC respectively) then gradually decreasing until the ninth day reaches the lowest values, whereas at case treated with *B. thuringiensis* the highest values of percentage corrected mortality after the seventh day and when treated with fungi the highest values of percentage corrected mortality after the fifth day.

Table 1. Percentage corrected mortality of second larval instar of cotton leaf worm after treatment with emamectin benzoate, methomyl, *Bacillus thuringiensis* and fungi.

Treatments	Concentrate	Percentage corrected mortality after					Total
		1day	3days	5days	7days	9days	
Emamectin benzoate	RC	54.24	16.95	13.33	10.00	1.67	96.19
	1/2RC	30.51	13.56	11.67	1.67	3.33	60.73
	1/4RC	16.95	3.39	1.67	1.67	0.00	23.67
Methomyl	RC	77.97	18.64	1.67	0.00	0.00	98.28
	1/2RC	45.76	15.25	0.00	1.67	0.00	62.68
	1/4RC	18.64	6.78	1.67	0.00	0.00	27.09
Bacillus thuringiensis	RC	11.86	8.47	18.33	20.00	15.00	73.67
	1/2RC	5.08	5.08	11.67	13.33	3.33	38.50
	1/4RC	1.69	5.08	8.33	10.00	0.00	25.11
Fungi	RC	11.86	13.56	16.67	6.67	1.67	50.42
	1/2RC	5.08	6.78	10.00	6.67	0.00	28.53
	1/4RC	3.39	1.69	3.33	1.67	0.00	10.08

RC=Recommended concentrate, 1/2RC=half of the Recommended concentrate and 1/4RC=fourth of the Recommended concentrate.

The obtained data in Table (2), cleared that, the *S. littoralis* 4th larval instar treatments by emamectin benzoate, the observed total percentage corrected mortality showed that, the highest value (90.54%) at RC followed by methomyl (85.79%) at RC, whereas, total percentage corrected mortality had the lowest values when treated larvae with fungi at all concentrations compared with remaining treatments.

Methomyl had the highest values of percentage corrected mortality after 24hrs. (values were 47.46, 20.00 and 13.56% at concentrations RC, 1/2RC and 1/4RC respectively) then gradually decreasing until the ninth day reaches the lowest values. Likewise, also emamectin benzoate except at 1/2RC where the highest values of percentage corrected mortality after fifth day (16.67%).

Table 2. Percentage corrected mortality of fourth larval instar of cotton leaf worm after treatment with emamectin benzoate, methomyl, *Bacillus thuringiensis* and fungi.

Treatments	Concentrate	Percentage corrected mortality after					Total
		1day	3days	5days	7days	9days	
Emamectin benzoate	RC	32.20	31.67	20.00	6.67	0.00	90.54
	1/2RC	13.56	15.00	16.67	11.67	0.00	56.89
	1/4RC	8.47	8.33	6.67	3.33	0.00	26.81
Methomyl	RC	47.46	21.67	11.67	5.00	0.00	85.79
	1/2RC	20.00	18.33	16.95	5.00	0.00	60.28
	1/4RC	13.56	8.33	3.33	0.00	0.00	25.23
Bacillus thuringiensis	RC	16.95	20.00	16.67	11.67	1.67	66.95
	1/2RC	10.17	10.00	10.00	0.00	3.33	33.50
	1/4RC	10.17	10.00	3.33	1.67	0.00	25.17
Fungi	RC	10.00	13.56	8.33	5.00	0.00	36.89
	1/2RC	6.78	8.33	6.67	1.67	0.00	23.45
	1/4RC	0.00	5.00	3.33	0.00	0.00	8.33

RC=Recommended concentrate, 1/2RC=half of the Recommended concentrate and 1/4RC=fourth of the Recommended concentrate.

Efficacy of combining *Beauveria bassiana* with certain pesticides:

The effects of mixtures of the fungi formulation with 1/2RC and 1/4RC of other insecticides (emamectin benzoate, methomyl and *Bacillus thuringiensis*) against second and fourth larval instars of *S. littoralis* are presented in (Table 3 & 4).

Results revealed that, when mixtures of the fungi formulation with 1/2RC and 1/4RC of methomyl, total percentage corrected mortality of second instar larval of *S. littoralis* were 77.49 and 62.34% and they were the highest values than mixtures of emamectin benzoate plus fungi (values were 75.85 and 57.29% at concentrations of insecticide 1/2RC and 1/4RC, respectively) and lastly mixtures of *B. thuringiensis* plus fungi values were 63.90 and 43.67% at concentrations of insecticide 1/2RC and 1/4RC, respectively).

Also, mixtures of emamectin benzoate plus fungi were more effect on fourth larval instar followed by mixtures of methomyl plus fungi then mixtures of *Bacillus thuringiensis* plus fungi (Table 4).

Percentage mortality of second and fourth larval instars when treated with emamectin benzoate + fungi and methomyl + fungi had the highest values after 24hrs. then decreasing gradually, but the highest values of percentage mortality of second and fourth larval instars when mixtures of *B. thuringiensis* + fungi were after fifth days at concentrate of insecticide 1/2RC and after third day at concentrate of insecticide 1/4RC (Table 3& 4).

From (Table 1) and (Table 3) when compared between insecticides alone at concentrate 1/2RC and 1/4RC and mixtures of the fungi formulation with 1/2RC and 1/4RC of other insecticides (emamectin benzoate, methomyl and *B. thuringiensis*) against second larval instar of *S. littoralis.* Data revealed that, in case of treated with emamectin benzoate alone total percentage corrected mortality were 75.85 and 57.29% while, increased to 60.73 and 23.67 % when treated with mixes fungi formulation at concentrations 1/2RC and 1/4RC, respectively. increase when mixtures emamectin benzoate + fungi. Likewise, also methomyl and *B. thuringiensis.*

By treatment of the fourth instar larvae, data revealed also that total percentage corrected mortality increased when mixtures compared to insecticides alone especially at concentrate 1/4RC of all insecticides (Table 2 &4). So mixing increases the efficiency of their insecticides.

Data agree with Dayakar *et al.* (2000) where, found that the combination of insecticides with *B. bassiana* and *M. anisopliae* showed 1.05–1.24 and 1.19–1.42 fold increase in virulence over the sole treatment, respectively. In another study, Purwar and Sachan (2004) also found similar results with *Lipaphis erysimi*. Thus, the combination of insecticide and entomogenous fungi was more deleterious to the insect than application of insecticides or entomogenous fungi alone.

Table 3. Efficacy of various combinations (full dose of Fungi + 1/2RC or 1/4RC of other insecticides) on the second larval instar of cotton leafworm.

Treatments	Conc. of insecticide	Percentage corrected mortality after					Total
		1day	3days	5days	7days	9days	
Emamectin benzoate + Fungi	1/2RC	32.20	21.67	18.64	1.67	1.67	75.85
	1/4RC	22.03	20.00	15.25	0.00	0.00	57.29
Methomyl + Fungi	1/2RC	45.76	25.00	3.39	1.67	1.67	77.49
	1/4RC	35.59	18.33	5.08	3.33	0.00	62.34
Bacillus thuringiensis + Fungi	1/2RC	11.86	15.00	22.03	11.67	3.33	63.90
	1/4RC	8.47	13.33	11.86	6.67	3.33	43.67

Table 4. Efficacy of various combinations (full dose of Fungi + 1/2RC or 1/4RC of other insecticides) on the fourth larval instar of cotton leafworm.

Treatments	Conc. of insecticide	Percentage corrected mortality after					Total
		1day	3days	5days	7days	9days	
Emamectin benzoate + Fungi	1/2RC	42.37	13.33	13.33	0.00	0.00	69.04
	1/4RC	28.81	16.67	10.00	0.00	0.00	55.48
Methomyl + Fungi	1/2RC	35.59	25.00	5.00	0.00	0.00	65.59
	1/4RC	30.51	16.67	8.33	0.00	0.00	55.51
Bacillus thuringiensis + Fungi	1/2RC	13.33	11.67	23.73	1.67	1.67	52.06
	1/4RC	0.00	18.64	16.67	0.00	0.00	35.31

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الاستخدام المشترك لفطر *Beauveria bassiana* وبعض المبيدات الحشرية لمكافحة دودة ورق القطن *Spodoptera littoralis* (Boisd.) تحت ظروف شبه حقلية

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تم إجراء هذه الدراسة في منطقة قويسنا بمحافظة المنوفية خلال موسم الزراعة لعام 2017تحت ظروف شبه حقلية ضد يرقات العمر الثاني والرابع لحشرة دودة ورق القطن *Spodoptera littoralis* (Boisd.) لتقييم كفاءة emamectin benzoate, methomyl and *Bacillus thuringiensis* منفردة وخلطا مع الفطر *Beauveria bassiana* وذلك في حقل طماطم . وقد كشفت النتائج أن الميثوميل كان أعلى فعالية ضد العمر اليرقي الثاني حيث كانت نسب الموت المصححة 98,28 و 62,68 و 27,9 ٪ في تركيزات RC ، 1/2RC و 1/4RC على التوالي . في حين أن يرقات العمر الرابع التي تم تغذيتها علي أوراق طماطم تم معاملتها بمبيد إيمامكتين بنزوات كانت النسبة المئوية لموت اليرقات قد سجلت اعلي قيمة (90,54 ٪) عند تركيز RC متبوعة بالميثوميل (85,79 ٪) عند نفس التركيز (RC) ، في حين أن يرقات العمر الثاني والرابع التي تم تغذيتها علي أوراق معاملة بالمبيد الفطري قد اعطي أدنى نسبة مئوية من معدل الوفيات المصححة في جميع التركيزات مقارنة مع المعاملات المتبقية. عندما تمت تغذية اليرقات علي أوراق طماطم تم معاملتها بمخلوط المبيد الفطري (*B. bassiana*) مع المبيدات الأخرى ، كانت النسب المئوية للموت المصححة للاعمار اليرقة الثاني والرابع أعطت اعلي قيم بعد 24 ساعة عند المعاملة بمخلوط *B. bassiana* + emamectin benzoate وmethomyl + *B. bassiana* ثم تتناقص تدريجيا ، ولكن عند المعاملة بمخلوط *B. thuringiensis* + *B. bassiana* فقد كانت أعلى قيم للنسبة المئوية للوفيات في العمرين الثاني والرابع بعد اليوم الخامس عند تركيز المبيد الحشري 1/2RC وبعد اليوم الثالث في تركيز المبيد الحشري1/4RC . لذلك فإن الخلط يزيد من كفاءة مبيدات الحشرات .