

**FIELD TRIALS WITH CERTAIN ENTOMOPATHOGENS
AND INSECTICIDES AGAINST BERSEEM
GRASSHOPPER *Euprepocnemis plorans* (CHARP.)
(ORTOPTERA: ACRIDIDAE)**



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ABSTRACT

Three field trials were conducted to evaluate three entomopathogens (*Metarhizium acridum*, *Beauveria bassiana* and *Nosema locustae*) and two chemical insecticides (Indoxacarb and Fipronil) against berseem grasshoppers *Euprepocnemis plorans* (Charp.). Results from the first trial indicated that both *M. acridum* and *N. locustae* are successful pathogens against grasshoppers in the field but their effect was slow, while both Indoxacarb and Fipronil are effective insecticides against *E. plorans* in the field when used as spray or bait techniques. Moreover, results from second trial showed that mixtures of *M. acridum* with low dose (10% of recommended dose) of Indoxacarb and Fipronil caused acceleration in grasshopper mortality when used as spray technique. In the third trial the obtained results showed that *N. locustae* mixtures with Indoxacarb and Fipronil also caused acceleration in grasshopper's mortality. It could be concluded that using *M. acridum* and *N. locustae* in combination with low dose (10% of recommended dose) of Indoxacarb and Fipronil could achieve environmentally safe control operation, moreover Indoxacarb and Fipronil could be used in grasshoppers control as new group of insecticides in replacement of classical groups of insecticides.

Keywords: *Metarhizium acridum*, *Beauveria bassiana*, *Nosema locustae*, locusts, grasshoppers, Biological Control, IPM

INTRODUCTION

Among the species of grasshoppers known in Egypt only two species of grasshoppers cause a serious damage to cultivated crops especially in the new reclaimed lands. These are *Euprepocnemis plorans* (Charp.) and *Heteracris annulosa* (Walker) (Orthoptera : Acrididae) Nakhla, (1957). Recently, survey studies of Plant Protection Institute, showed clearly that high population of grasshopper *E. plorans* was found infesting cultivated clover *Trifolium alexandrinum* at Atmeda village, Dakahlia Governorate.

Interest in using entomopathogens as biological control agents has grown since the last major locust plague of the 1980s. Millions liters of chemical pesticides were used to combat this plague, which led to an outcry among environmental groups Greathead, (1992 a). Locust and grasshoppers like most of insects are host to numerous microorganisms, the most extensively studied entomopathogens were the microsporidian, *Nosema locustae* and the deuteromycete fungi *Metarhizium acridum* (= *M. anisopliae* var. *acridum*) and *Beauveria bassiana* Greathead, (1992 b) and Lobo Lima *et al.*, (1992). All fungi isolates found highly virulent to locust and grasshoppers are in the genus *Metarhizium* and have originated from Orthoptera. The

isolate that was adopted as a standard and used in all assays for comparison is *M. acridum* IMI330189 originated from the grasshopper *Ornithacris cavroisi* (Finot) collected in Niger. No high virulent isolates to locust have yet been found originating in non orthopteran hosts Prior, (1992). *M. acridum* proved to be successful biocontrol agent against desert locust and grasshoppers under Egyptian conditions but it's relative slow of action might be disadvantage of this fungi when making control operation (Abdelatef, 2005), this conclusion could be apply also for most entomopathogens.

Highly efficacious insecticides with novel modes of action (*i.e.* indoxacarb) are becoming increasingly important in agriculture as components of integrated pest management to replace older classes of compounds which are perceived to carry higher environmental risk (Wing *et al.*, 2000). Indoxacarb is newly developed insecticide, it is highly active broad spectrum insecticide when directly administered topically or orally to pest insects (Harder *et al.*, 1997). Fipronil is a phenylpyrazole insecticide with novel mode of action that targets the GABA-gated chloride channel in nerve-cell membranes and is highly toxic to arthropods. Unlike many modern pesticides, fipronil and its derivatives are moderately persistent in the environment (Chandler *et al.*, 2004). Fipronil at low dosage provides long-term protection against major lepidopterous and orthopterous pests on crops (Colliot *et al.*, 1992 and Burris *et al.*, 1994).

Aim of this work is to evaluate the efficacy of two entomopathogenic fungi *M. acridum* and *B. bassiana* and the microsporidian, *N. locustae* and two insecticides fipronil and indoxacarb against *E. plorans*, also the combination between entomopathogens and insecticides, to achieve integrated pest management program for *E. plorans*.

MATERIALS AND METHODS

Entomopathogens

The fungi *M. acridum* strain IMI330189 (kindly provided by Basf South Africa under commercial name Green Muscle). It was used at rate of 25 g. spores/ha.

The spores of *B. bassiana* were originally isolated from adult cadavers of the red palm weevil *Rhynchophorus ferrugineus* Oliv. collected from El-Kassasien area, El-Esmaaliala Governorate Heikal, (2001) and passaged in grasshoppers and desert locust for several passages in laboratory. It was used at rate of 25 g. spores/ha.

The microsporidian *N. locustae* (obtained from Rangeland insect Laboratory, Bozeman Montana State USA), used at rate of 1.4×10^9 spores/100 g of wheat bran/m² according to Henry, (1971).

Insecticides

Indoxacarb (15 % EC) under the commercial name Avaunt produced by DuPont, used at rate of 238 ml/ha

Fipronil (20 % SC) under the commercial name Regent produced by Basf, used at rate of 238 ml/ha.

Treatments

Three trials were done at Atmeda village, Dakahlia Governorate,

against grasshopper *E. plorans plorans*, the grasshopper population were consist of 4th and 5th nymphal instars and adults. All treatments were applied to heavily infested areas with grasshoppers and planted with clover *T. alexandrinum* (which left to produce seeds), each plot varied in their size, ranged between 500-1000 M². Two types of application were done, spray for fungi and the 2 insecticides and baits for the protozoa and the 2 insecticides. The spray solutions were consist of the applied treatment and 0.05 % Tween 80 and applied using Cifarelli knapsack mist blower sprayer. While baits were, consist of the applied treatments + wheat bran + 5% mollas and applied manually at the edges of treated plots. After each treatment, samples of treated insects were collected and kept in cages under the field conditions, each cage was feed same plants of same treated area, while in bait treatment some of same bait was kept in cages for 3 days, the mortalities were recorded daily. In addition, grasshopper's populations were counted in field by counting number of grasshoppers in four virtual square meters, by walking a transect and making quadrate counts of the number of living grasshoppers in 1 m² within the target area and the average number was calculated. An imaginary 1 m² quadrate can be estimated by spreading the feet apart to form a base almost 1 m wide (Dobson, 2001).

First trial

The following treatments were done at the recommended doses : 1- *M. acridum* (spray) 2- *B. bassiana* (spray) 3- *N. locustae* (bait) 4- Indoxacarb (spray) 5- Fipronil (spray) 6- Indoxacarb (bait) 7- Fipronil (bait).

Second trial

In this trial the following treatments were done as previously mentioned: 1- *M. acridum* + Indoxacarb (10 % of recommended dose) 2- Indoxacarb (10% of recommended dose). 3- *M. acridum* + Fipronil (10 % of recommended dose) 4-Fipronil (10% of recommended dose).

Third trial

While in third trial the following treatments were done: 1- *N. locustae*+ Indoxacarb (10 % of recommended dose) 2-Indoxacarb (10% of recommended dose). 3- *N. locustae* + Fipronil (10 % of recommended dose) 4-Fipronil (10% of recommended dose).

Statistics

To calculate the efficacy of the tested control agents, the mortality of caged grasshoppers were subjected to Schneider-Orelli formula as following (Püntener 1981):

The efficacy % = (Mortality % in treatment - Mortality % in control)/(100 - Mortality % in control) X 100

While those data from field counting were subjected to Analyses of Variance using costat 6.4 software from CoHort Software, 2 way completely randomized procedure was utilized.

RESULTS AND DISCUSSION

First trial:

Table (1) and Fig. (1) show corrected mortality of first trial treatment of caged insects, it's clear that both used insecticides treatments as spray or bait caused 100 % mortality within four to seven days after treatment, also

bait treatments take about 1-2 days longer than spray treatments to reach 100% mortality. While the used entomopathogens act slowly where *M. acridum*, *B. bassiana* and *N. locustae* caused 84.08, 41.49, 77.66 % mortality by the 14th day post treatment.

While data in Table (2) and Fig (2) show number of grasshoppers per square meter in the treated plots as well as control treatment. These data clearly rival that Indoxacarb as spray and reagent as spray reached to 100 % reduction (0 grasshoppers/ m²) by day 2 and 3 respectively, while in bait treatments it were 5 and 6 days post treatment. But in *M. acridum*, *B. bassiana* and *N. locustae* by the 14th day post treatment number of grasshoppers/m² were decreased to 1, 3.75, and 2.

Second trial:

In this trial, the efficacy of *M. acridum* mixed with 10% of recommended dose of both used insecticides was evaluated. Obviously data in Table (3) and Fig. (3), show that mixing *M. acridum* with low dose of both used insecticides caused acceleration in grasshopper mortality in compare with the application of used insecticides at low dose and *M. acridum* alone. The mortality in *M. acridum* + Indoxacarb and *M. acridum* + Fipronil reached to 88 and 89 % by the 3rd day post treatment, respectively, while the mortality reached to 100 % by the 8th and 7th day post treatment. While in case of Indoxacarb and Fipronil alone the mortality reached to 75 and 69.79 % by the 11th and 10th day post treatment, respectively.

Table (4) and Fig (4) show number of grasshoppers per square meter, it is clear that mixtures of *M. acridum* with both used insecticides (Indoxacarb and Fipronil) at low doses caused 100 % reduction by the 7th and 6th day post treatment. While in case of application of Indoxacarb and Fipronil at low doses alone reached to 100 % reduction by the 9th day post treatment.

Third trial

The third trial was conducted to test the efficacy of *N. locustae* mixtures with Indoxacarb and Fipronil at low doses as baits application. Table (5) and Fig. (5) Show the corrected mortality of *N. locustae* mixtures and both used insecticides. It is clear that, *N. locustae* and Indoxacarb mixture caused 77.55 % mortality after 5 days and *N. locustae* and Fipronil mixture caused 79.38 % mortality after 7 days, however in Indoxacarb and Fipronil sole treatments at low dose reached 75 and 72.92 % by day 10 post treatment, respectively.

Table (6) and Fig. (6) show the mean number of grasshoppers per square meter, it is clear that in *N. locustae* + Indoxacarb and *N. locustae* + Fipronil treatment the reduction in grasshoppers numbers reached to 100% after 8 and 7 days post treatment, respectively, while in case of Indoxacarb and Fipronil sole treatments 100% reduction occurred at day 12 post treatment.

M. acridum (applied as spray technique) was the most virulent entomopathogen against *E. plorans* in the field followed by *N. locustae* (applied as bait technique), several virulence screening tests and trials using *M. acridum* IMI 330189 revealed that 94 % of 17 different orthopteran species were susceptible, and 95 % - all within acrididae- highly susceptible (Prior, 1997). Many authors reported field efficacy of *M. acridum* against grasshoppers species and confirmed the results of the present study for

example against Variegated grasshopper *Zonocerus variegates* in Benin (Lomer *et al.*, 1993 and Douro-Kpindou *et al.*, 1995), against Wingless grasshopper *Phaulacridum vittatum* in Australia (Baker *et al.*, 1994 and Milner *et al.*, 1994), against grasshoppers particularly Rice grasshopper *Hieroglyphus daganensis*, in northern Benin (Lomer *et al.*, 1997) and against Sahelian grasshoppers (Kooyman *et al.*, 1997).

Unlike chemical pesticides pathogens are slow acting agent as discovered in the present work, Kooyman and Godonou, (1997), Bateman *et al.*, (1993) and Abdelatif, (2005) have pointed out how difficult it is to convincingly demonstrate field efficacy with slow acting agent against desert locust and other grasshoppers species using entomopathogens.

Fipronil is a relatively new insecticide that controls a broad spectrum of insects at low field application rates. It is a "new generation" insecticide because its mode of action, interference with the normal function of γ -aminobutyric acid (GABA)-gated channels, differs from the classical insecticides, such as organophosphates and carbamates (Gunasekara, *et al.*, 2007), it has been proposed as a replacement for dieldrin to control desert locusts, (Balanca and de Visscher, 1997). The present work proved also that fipronil affective against *E. plorans* in the field when applied as spray or bait, it could be used against grasshopper in replacement of old pesticides groups.

Indoxacarb is a promising new foliar insecticide with strong field activity against Lepidoptera, a key feature of this compound is its novel bioactivation, then block off the insect voltage-gated sodium channel by the N-decarbomethoxyllated metabolite (Wing *et al.*, 2000). Indoxacarb approved it is efficiency against *E. plorans* in the present work when used as spray or bait technique.

The results of the 2nd and 3rd trials strongly show that mixtures of *M. acridum* or *N. locustae* with indoxacarb or fipronil can be an effective and economic alternative to the use of particular control agents alone. Sub lethal doses of fipronil were reported by many authors to be affective against locusts and grasshoppers, (Balanca and de Visscher, 1997 and Lecoq and Balanca, 1997). While sub lethal doses of indoxacarb affected larval pupation and adult longevity and fecundity of *Spodoptera litura* (Wang *et al.*, 2009) also effect on the mortality of Formosan subterranean termite *Coptotermes formosanus* workers (Hu *et al.*, 2005). The sub lethal doses of chemicals give almost immediate crop protection in same time provide the entomopathogens more time to kill grasshoppers. Apparently the low rate insecticides stress the target or alter their behaviour in such a way as to make it more susceptible to the entomopathogens. The final conclusion of the present study is that, application of entomopathogens and sublethal doses of insecticides had the typical knockdown effect of a conventional insecticide that convinces farmers, while the biopesticide component maintained low population densities throughout the duration of the trial, and this even at 1/10 of the recommended dose.

Table (1) Accumulative corrected mortality percentages of caged insects from first trial

Treatments Days	<i>M. acridum</i>	<i>B. bassiana</i>	<i>N. locustae</i>	Indoxacarb (spray)	Fipronil (spray)	Indoxacarb (bait)	Fipronil (bait)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1.00	1.00	0.00	52.00	50.00	42.00	34.00
2	1.00	1.00	1.00	94.00	78.00	63.00	61.00
3	4.04	3.03	0.00	97.98	89.90	78.79	83.84
4	11.22	6.12	0.00	100.00	94.90	94.90	91.84
5	17.35	11.22	2.04		98.98	98.98	93.88
6	23.47	16.33	2.04		100.00	100.00	96.94
7	27.84	19.59	4.12				100.00
8	31.25	22.92	7.29				
9	38.54	26.04	17.71				
10	46.88	29.17	30.21				
11	58.33	32.29	41.67				
12	65.26	34.74	52.63				
13	72.63	38.95	66.32				
14	84.04	41.49	77.66				

Table (2) Mean number of grasshoppers per square meter from first trial

Treatments Days	Control	<i>M. acridum</i>	<i>B. bassiana</i>	<i>N. locustae</i>	Indoxacarb (spray)	Fipronil (spray)	Indoxacarb (bait)	Fipronil (bait)
0	9.00	9.50	9.25	8.75	8.75	8.50	9.50	9.50
1	9.25	8.75	8.75	8.25	1.00*	1.25*	4.50*	4.50*
2	9.25	8.25	7.75*	8.00	0.00*	1.00*	2.25*	2.00*
3	9.75	8.00	8.50	7.50*		0.00	1.00*	1.00*
4	9.50	7.00*	7.75*	7.50*			0.50*	0.75*
5	9.50	6.00*	7.50*	7.75*			0.00	0.75*
6	9.75	5.25*	7.25*	8.00				0.00
7	9.00	5.00*	7.00*	7.00*				
8	9.25	4.00*	6.25*	7.50*				
9	9.50	4.00*	5.75*	7.75*				
10	9.75	3.25*	5.75*	7.00*				
11	10.00	2.75*	5.25*	5.25*				
12	10.00	2.25*	5.00*	4.25*				
13	10.00	1.25*	4.25*	3.50*				
14	9.75	1.00*	3.75*	2.00*				

Means with * are significantly lower than control

Table (3) Accumulative corrected mortality percentages of caged insects from second trial

Treatments Days	<i>M. acridum</i> + Indoxacarb (10 % of recommended dose)	Indoxacarb (10% of recommended dose)	<i>M. acridum</i> + Fipronil (10 % of recommended dose)	Fipronil (10% of recommended dose)
0	0.00	0.00	0.00	0.00
1	37.00	16.00	43.00	17.00
2	64.00	28.00	66.00	31.00
3	88.00	42.00	89.00	43.00
4	95.96	48.48	95.96	50.51
5	95.92	51.02	96.94	53.06
6	95.92	54.08	97.96	57.14
7	98.98	59.18	100.00	60.20
8	100.00	59.79		63.92
9		67.71		66.67
10		73.96		69.79
11		75.00		

Table (4) Mean number of grasshoppers per square meter from second trial

Treatments Days	Control	<i>M. acridum</i> + Indoxacarb (10 % of recommended dose)	Indoxacarb (10% of recommended d dose)	<i>M. acridum</i> + Fipronil (10 % of recommended dose)	Fipronil (10% of recommended dose)
0	9.00	9.50	9.25	8.75	8.75
1	9.25	4.00*	6.50*	4.00*	5.25*
2	9.25	3.00*	5.75*	3.00*	4.50*
3	9.75	2.50*	5.25*	2.50*	3.75*
4	9.50	1.75*	4.50*	1.50*	3.50*
5	9.50	1.50*	3.50*	0.50*	3.00*
6	9.75	0.50*	3.00*	0.00	2.75*
7	9.00	0.00	1.75*		1.75*
8	9.25		0.50*		0.50*
9	9.50		0.00		0.00
10	9.75				

Means with * are significantly lower than control

Table (5) Accumulative corrected mortality percentages of caged insects from third trial

Treatments \ Days	<i>N. locustae</i> + Indoxacarb (10 % of recommended dose)	Indoxacarb (10% of recommended dose)	<i>N. locustae</i> + Fipronil (10 % of recommended dose)	Fipronil (10% of recommended dose)
0	0.00	0.00	0.00	0.00
1	26.00	14.00	20.00	12.00
2	40.00	25.00	36.00	24.00
3	52.53	36.36	49.49	36.36
4	67.35	43.88	56.12	43.88
5	77.55	47.96	62.24	47.96
6	82.65	52.04	72.45	52.04
7	83.51	57.73	79.38	56.70
8		59.38	83.33	63.54
9		68.75		69.79
10		75.00		72.92

Table (6) Mean number of grasshoppers per square meter from third trial

Treatments \ Days	Control	<i>N. locustae</i> + Indoxacarb (10 % of recommended dose)	Indoxacarb (10% of recommended dose)	<i>N. locustae</i> + Fipronil (10 % of recommended dose)	Fipronil (10% of recommended dose)
0	9.00	8.25	8.25	8.75	8.50
1	9.25	7.00*	7.25*	7.50*	6.50*
2	9.25	6.50*	6.00*	6.25*	5.75*
3	9.75	5.75*	5.25*	5.25*	5.25*
4	9.50	4.25*	4.50*	2.75*	4.00*
5	9.50	2.25*	3.75*	2.25*	3.25*
6	9.75	1.50*	2.75*	1.00*	3.00*
7	9.00	0.75*	2.75*	0.00	2.75*
8	9.25	0.00	2.25*		2.00*
9	9.50		2.00*		1.75*
10	9.75		1.50*		1.67*
11	10.00		1.00*		1.00*
12	10.00		0.00		0.00

Means with * are significantly lower than control

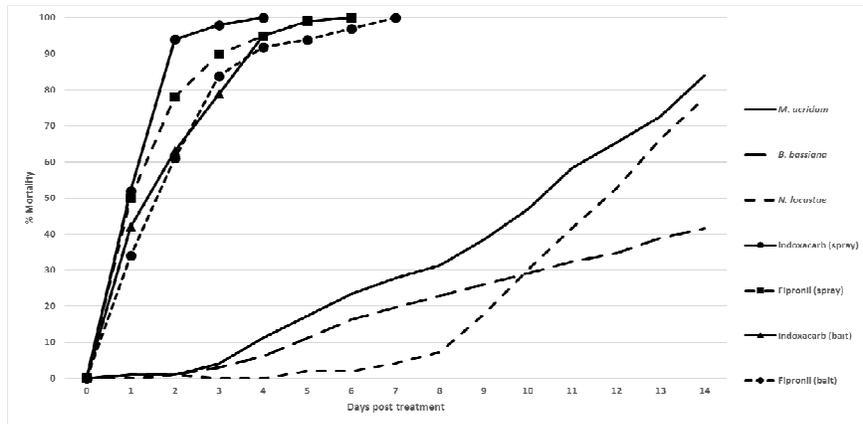


Figure (1) Accumulative corrected mortality percentages of caged insects from first trial

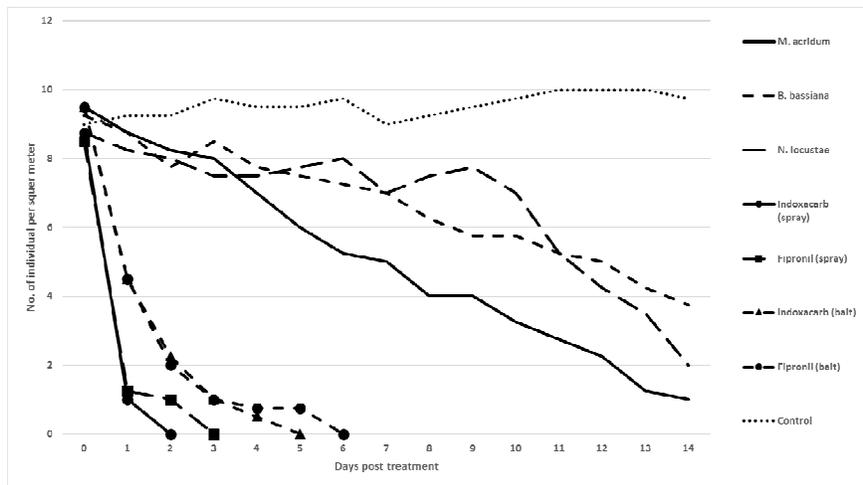


Figure (2) Accumulative corrected mortality percentages of caged insects from first trial

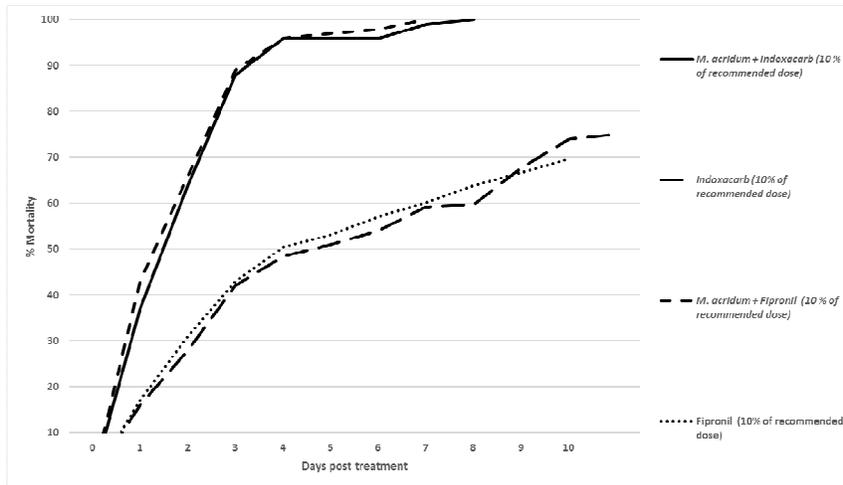


Figure (3) Accumulative corrected mortality percentages of caged insects from second trial

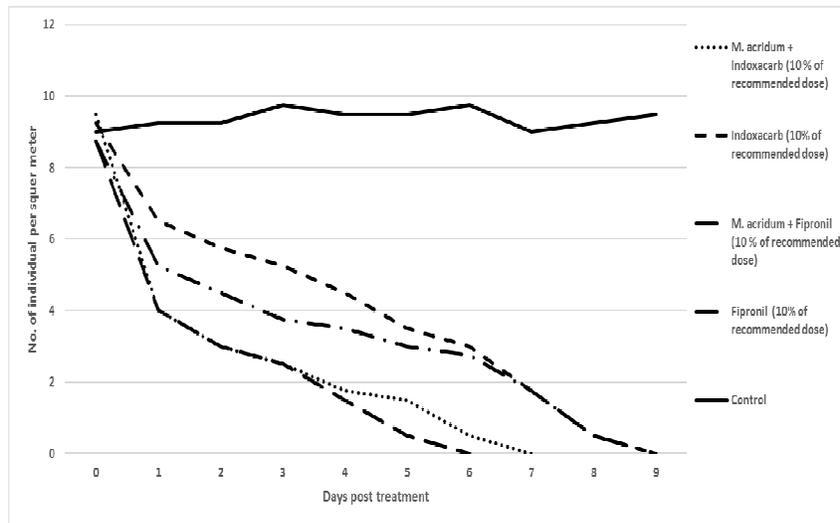


Figure (4) Mean number of grasshoppers per square meter from second trial

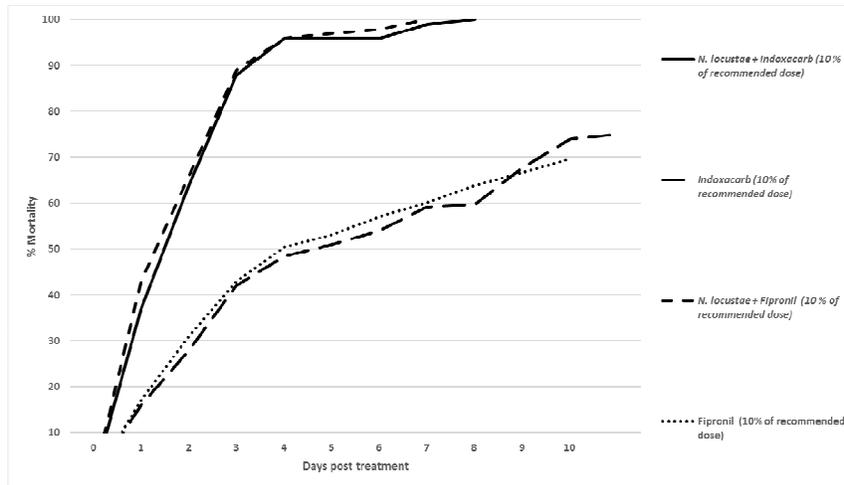


Figure (5) Accumulative corrected mortality percentages of caged insects from third trial

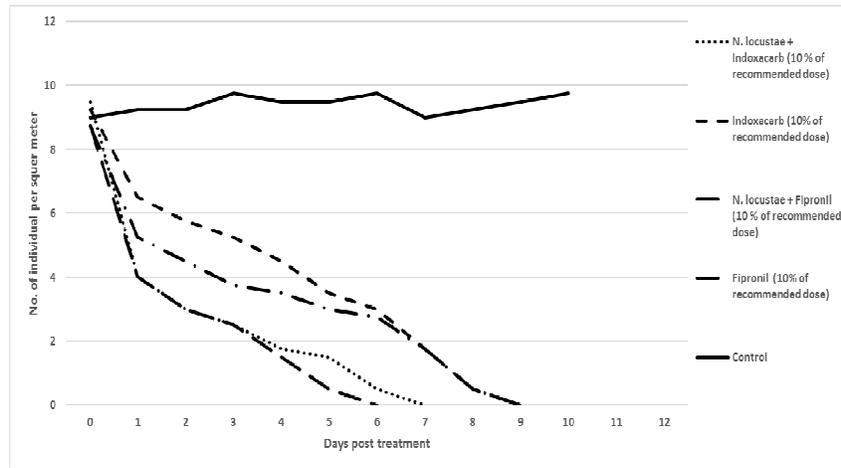


Figure (6) Mean number of grasshoppers per square meter from third trial

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تجارب حقلية لدراسة تأثير بعض مسببات المرضية الحشرية وبعض المبيدات الكيميائية
ضد آفة نطاط البرسيم (*Euprepocnemis plorans* (Charp.) (Orthoptera: Acrididae)

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أجريت ثلاث تجارب حقلية لتقييم تأثيرات ثلاثة مسببات مرضية حشرية (*Metarhizium acridum*, *Beauveria bassiana* and *Nosema locustae*) وكذلك مبيدات حشريان (*Indoxacarb* and *Fipronil*) ضد آفة نطاط البرسيم (*Euprepocnemis plorans*). أوضحت نتائج التجربه الأولى ان كل من *M. acridum* and *N. locustae* كانا من المسببات المرضية الناجحة لمكافحة النطاطات في الحقل ولكن فعلهم كان بطئ نسبيا, بينما كل من المبيدات الحشريان المستخدمان كانا ناجحان في مكافحة نطاط البرسيم في الحقل سواء تم استخدامهم كمحالييل رش او كطعوم سامة. من ناحية أخرى أظهرت نتائج التجربة الثانية ان خلط فطر *M. acridum* مع المبيدات المستخدمان (بعشر الجرعه الموصى بها) ادى الى تسريع موت نطاط البرسيم عن استخدام هذه المخاليط في صورة محالييل رش. بينما اظهرت نتائج التجربه الثالثة نجاح استخدام مخلوط البروتوزوا *N. locustae* مع كل من المبيدات المستخدمان (بعشر الجرعه الموصى بها) في مكافحة نطاط البرسيم في الحقل, مما يؤدي الى اجراء عمليات مكافحة ناجحة وأمنة بيئيا. ايضا اظهرت النتائج ان كل من *Indoxacarb* و *Fipronil* يمكن استخدامهما في مكافحة النطاطات كبديل للمبيدات الكيميائية التقليدية.