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INTEGRAL ACTION OF *Metarhizium anisopliae*, *Nosema locustae* AND CHLORFLUAZURONE AGAINST THE BERSEEM GRASSHOPPER, *Euprepocnemis plorans plorans* (CHARP.) IN THE FIELD

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ABSTRACT

The integrated action of the fungus *Metarhizium anisopliae* var. *acridum* isolate IMI 330189 (Green Muscle), *Nosema locustae* (Nolo-Bait) and the antimoulting compound chlorfluazurone (Atbrone 5%) was investigated in a field at Atmida village- Met-Ghamr, Dakahlia Governorate, against the nymphal instars (3rd, 4th and 5th) of the berseem grasshopper, *Euprepocnemis plorans plorans*. The obtained results revealed that the integration between *M. anisopliae* and 25% of the recommended dose of chlorfluazurone exhibited the highest integral action, recording 60% population reduction after 10 days of application and 100% after 19 days posttreatment. On the other hand, the integral action between *N. locustae* and 25% of the recommended dose of chlorfluazurone induced 40% reduction after 10 days posttreatment and 90 % after 19 days. Chlorfluazurone spray was more potent than chlorfluazurone bait. The fungus *M. anisopliae* treatment was the most effective treatment than chlorfluazurone and *N. locustae* when tested separately. The fungus alone and nosema induced moderate percentages of reduction in the population of this pest after 11 days posttreatment. Therefore, chlorfluazurone was chosen to test the integrated action with *M. anisopliae* and *N. locustae*.

Keywords: Integral action, *Metarhizium anisopliae, Nosema locustae,* chlorfluazurone, *Euprepocnemis plorans plorans,* field.

INTRODUCTION

The berseem grasshopper, *Euprepocnemis plorans plorans* (Charp.) is considered one of the most economic species that caused a serious damage to many agricultural crops (Abdel-Fattah, 2002). Chemical pesticides had become less attractive for numerous reasons including increased cost, the development of pesticide-resistant insects and weeds, concerns raised about human health hazards, and deleterious effects upon non-target organisms (Evans, 2008). Effective alternatives that offer improved safety could have repaid and favorable environmental and economic impact. The biological control is regarded as a desirable for controlling insects, due to its minimal environmental harmful impact and preventing the development of resistance in vectors, (Eilenberg *et al.*, 2001). Field trails showed promise for *M. anisopliae* as a biocontrol agent against grasshoppers and locust (Arthurs and Thomas, 2000; Metaweh *et al.*, 2001). Abdel-Fattah (2005) reported that a integrated action of the fungus *M. anisopliae* var. *acridum* isolate IMI 330189 (Green Muscle) with low doses of some insecticides on the desert locust, *Schistocerca. gregaria*. All these insecticides were not toxic to the fungus and not inhibited its effect on locust. The activity of *M. anisopliae* when mixed with the growth inhibitor (antimoulting hormone) Consult 10% (fungus 10^{10} spores/ml + 100 mg/l of Consult) or (fungus 10^{9} spores/ml + 0.1mg/l of Consult) against the desert locust *S. gregaria* was more effective than the used fungus alone (El-Dydamony, 2011). El-Gammal *et al.* (2004) studied the integrated action of the fungus *M. anisopliae*

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var. *acridum* with the antimoulting agent Consult and the antifeedant (Azadirachtin) in fields of Shark El-Uwainat area against the last instar nymphs of *Locusta migratoria migratorioides*. They mentioned that the infection of *L. migratoria migratorioides* by *N. locustae* increased mortality percentage of instar nymphs early with the increased application of *N. locustae* and decreased during the next generation and the following. The application of *N. locustae* had no effect on the distribution of infection during the developmental stages of locusts (Zhang-Long *et al.*, 2001). *N. locustae* is a pathogen of orthopterans with an unusually wide host range, so it is the only microsporidian that has been developed as a microbial control agent (Sokolova and Lange 2002). The present work aimed to investigate the integral action of *M. anisopliae* var. *acridum* isolate 330189 (Green Muscle), *N. locustae* and 25% of the recommended dose of the antimoulting agent (chlorfluazurone) against the grasshopper, *E. plorans plorans* in the field.

MATERIALS AND METHODS

According to surveys carried out in summer seasons of 2007, 2008 and 2009, the berseem grasshopper, *E. plorans plorans* (Charp.) was the most prevailing insect pest in Sharkia, Dakahlia, Domiatta and Kafr El-Shiekh Governorates. So, this insect was chosen to evaluate the efficiency of the proposed agents, during the summer season of 2009. A suitable area cultivated with clover *Trifolium alexandrinum* and infested with the berseem grasshopper was selected at Atmida village, Met-Ghamr, Dakahlia Governorate to conduct this experiment.

Pathogens and Test Chemical

1. Entomopathogenic tested fungus, *Metarhizium anisopliae* var. *acridum* isolate IMI 330189 (Green Muscle) was obtained from the company of biological control products SAPTY South Africa. Its application rate was 50g/ha (1gram powder contains 5 x 10^{10} spores). It is a virulent strain against locust and grasshopper.
2. The spores of *Nosema locustae* (Nolo-Bait Canning preparation) were obtained from Evans Biocontrol, Inc. 895, Interloken Pky., Unit Abroom Field, Colorado 80020 (203) 4601780. The concentration of *N. locustae* spores in this formulation was one billion spores per 454 grams (0.05%).
3. The antimoulting agent used was chlorfluazurone (Atabrone E.C. 5%) supplied by Dow Agro sciences, Egypt. It was used at the rate of liter/feddan.

Experimental Design

The experimental field (heavily infested with 3rd, 4th and 5th nymphal instar of *E. plorans plorans* more than 50 nymphs/m^2) was divided into plots of about, 25 m x 25 m = 625 m^2 with a wide belt of 10 x 25 = 250 m^2) to prevent immigration of treated nymphs to the other plots and avoid the drift of spray. Plots laying up wind of treatments were used for the control and sprayed with water containing 0.05% Tween 80. Each treatment was represented with five replicates of cages (0.5 x 0.5 x 0.5 m) each. The insects were collected randomly from each treatment directly after insecticidal application by using sweep net and placed in the cages; the cages were maintained on the area of treatment under field conditions. The nymphs (50 per cage) were fed daily on treated berseem plants. Mortality counts were recorded daily until the 20th day posttreatment. Grasshoppers that died during the bioassay were washed by water, sterilized by using ethanol 70% and placed in Petri dishes on damp tissue paper. Died grasshoppers due to the pathogen was only recorded if the entomopathogen developed on the cadavers.

To study the integrated action between *M. anisopliae* var. *acridum* isolate IMI 330189 (Green Muscle), *N. locustae* and chlorfluazurone were applied according to the following design. Powder formulation of *M. anisopliae* var. *acridum* isolate IMI 330189 Green Muscle) spores were sprayed in a separate plot after dilution with water + 0.05% Tween 80 as an emulsifible agent using ULV technique on berseem plants. *N. locustae* (Nolo-Bait) was applied in a separate plot at the rate of 20 kg bait wheat bran/fed. chlorfluazurone was sprayed alone at the recommended dose using ULV technique or combined with the entomopathogenic fungus or *N. locustae* at the rate of 25% of the recommended dose in water and 0.05% Tween 80 was added. ULV hand-held battery sprayer operated by a spinning disc provided with batteries was used (El-Gammal and Hindy, 1992).

Application Equipment

- Sprayer: the ULVA+

* Nozzle: Red nozzle was used in all treatments, spraying height: 0.5 m above the plants.

- Walking speed: 40 m/min = km/hr.

- Swath width: 3 m according to wind velocity

- Weather conditions at applications.

* Wind: 3-5 m/sec.

* Humidity: 30-40%

*Temperature: 30-32°C maximum and 18-20°C minimum

*The sun rise clearly: the spraying was done between 8 and 11 am early in the morning.

Assessments

In the cages, routine work was carried out daily includes removing the uneaten food, faeces, dead nymphs and counting the living insects before introducing the fresh food. Mortality data were summarized as estimates of the median lethal time (MLT).

Data were analyzed using general linear model procedures (SAS, 1995).

RESULTS AND DISCUSSION

The effects of *M. anisopliae* var. *acridum*, *N. locustae* and Chlorfluazurone 5% were tested alone and in binary mixtures against 3rd, 4th and 5th nymphal instars of the grasshopper *E. plorans plorans* during summer season of 2009. Mortality data were summarized as estimates of the median lethal time (MLT), which was calculated as a number of days to achieve an accumulated 50% mortality using a linear interpolation of corrected daily mortalities.

Table 1 indicate that mortalities in the nymphal instars of the grasshopper occurred after 5 days of treatment with the fungus, *M. anisopliae* var. *acridum* alone. These mortalities percentages were 5 after 5 days, 50 after 10 days, 85 after 15 days and 92.5 after 20 days. In treatments of *N. locustae* alone mortalities appeared after 3 days. These mortality percentages were 20 after 5 days, 35 after 10 days, 60 after 15 days and 80 after 20 days. As respects the treatment of chlorfluazurone spray alone, mortality percentages were 20, 45, 65 and 80 after 5,10, 15 and 20 days respectively. Mortality percentages of chlorfluazurone (as bait) were 12.5, 27.5, 42.5 and 50 after 5, 10, 15 and 20 days respectively. Arthurs and Thomas (2000) tested the fungus, *M. anisopliae* var. *acridum* against the brown locust, *Locustana pardalia* and found that mortality rates of infected individuals in the field were significantly slower than those in the laboratory. Prior *et al* (1995) assessed virulence of the fungus, *M. anisopliae* isolate (IMI 168777ii) against *S. gregaria* and concluded that its speed of action was concentration dependent. Mortalities of 0-100% were achieved after 4-6 days. Abdel-Fattah (2005) showed that the use of the fungus, *M. anisopliae* var. *acridum* alone for controlling the desert locust, *S. gregaria* in the field, caused a mortality percentage of 98.8% after 16 days. Even very low concentrations of inoculums gave high levels of mortality periods longer than 10 days. Abdel-Fattah *et al.* (2003) found that peanut grasshopper, *Catantopus axillaris* was the most susceptible one, the exposure of its nymphs for 48 hr to Nolo bait resulted in 100% kill after 21 days of treatment. The moderate susceptible grasshopper was *E. plorans plorans* followed by the frog grasshoppers, *Chrotogonus homalodemis* which recorded 5% kill after 10 days of treatment.

Data presented in Table 2 illustrate, the integral action of the tested agents (fungus and *Nosema* and 25% of the recommended dose of chlorfluazurone). The mortality percentages of grasshopper nymphs caused by the fungus with 25% of chlorfluazurone were 20, 60, 90 and 100% after 5, 10, 15 and 19 days of application the recommended dose, respectively. The integral action of the 25% of the recommended dose of chlorfluazurone and the recommended dose of the fungus was sufficient to induce a complete kill of the insect population. Rabie and Risha (1994) reported that virulence of the fungal spores of *M. anisopliae* against the 3rd

Table 1. Efficiency of the fungus, *Metarhizium anisopliae var. acridum* isolate IMI 330189, *Nosema locustae* and chlorfluazurone against nymphs of the grasshopper, *Euprepocnemis plorans plorans* in the field when tested separately

	Cumulative mortality percentages after the indicated posttreatment periods (in days)																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total kill %
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fungus	-	-	-	-	5	10	30	35	40	50	60	70	75	80	85	87.5	87.5	90	90	92.5	92.5	92.5	95.1	95
N. locustae	-	-	15	17.5	20	22.5	22	25	30	35	40	45	50	55	60	62.5	65	70	75	80	80	82.5	82.5	82.5
Chlorfluazurone spray	2.5	10	15	17.5	20	30	30	40	42.5	45	50	55	60	62.5	65	70	75	75	80	80	82.5	85	85	85
Chlorfluazurone bait	-	5	10	10	12.5	15	15	20	25	27	30	35	35	40	42.5	45	45	47	50	50	52.5	55	55	55

Table 2. Combined effect of the fungus, *M. anisopliae var. acridum*, *N. locustae* and chlorfluazurone at 25% of the recommended dose against mymphs of the grasshopper, *E. plorans plorans* in the field

	cumulative mortality percentages after the indicated posttreatment periods (in days)																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total kill %
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fungus+ Chlorfluazurone 25%	2.5	5	5	10	20	30	35	40	45	60	70	75	80	85	90	92.5	95	97.5	100	-	-	-	-	100
N. locustae + Chlorfluazurone 25%	2.5	2.5	5	7.5	10	15	20	25	35	40	50	60	70	80	85	90	90	90	90	92	95			95

instar nymphs of the desert locust, *S. gregaria* increased when sub-lethal doses of teflubenzuron were applied 10 hours before the fungal treatments. El-Gammal *et al.* (2004) investigated the integral action of *M. flavoviride*, antimoulting (Consult) and the antifeeding agent (Azadirachtin) in a field of Shark El-Uwainat area against the last instar nymphs of *Locusta migratoria migratorioides.* They found that, the integration between *M. flavoviride* and the recommended dose of Consult (37.8 g a.i./fed.) was the most effective inducing 67.2% population reduction after 5 days of application and 96.8% after 15 days. Abdel-Fattah (2005) studied the combined effects of the fungus, *M. anisopliae* var. *acridum* isolate IMI 330189 with sub-lethal dose of some insecticides on the desert locust, *S. gregaria* in Eastern desert of Egypt. He reported that all insecticides were not toxic to the fungus and not inhibited its effect on locust. The mortality percentages caused by *N. locustae* with 25% of the recommended dose of chlorfluazurone were 10, 40, 85 and 92% after 5, 10, 15 and 20 days of application, respectively. The integral action of 25% of the dose of chlorfluazurone with *N. locustae* was more effective than using of *Nosema* or using of chlorfluazurone alone to reduce the population of the nymphal instars of *E. plorans plorans*. Abdel-Fattah *et al.* (2003) tested *N. locustae* against the 3rd nymphal instar of the desert locust, *S. gregaria* and the grasshoppers, *E. plorans plorans*, *Catantopus axillaris* and *Chrotogonus homalodemis.* The obtained results revealed that *C. axillaris* was the most susceptible one while *E. plorans plorans* was the moderate susceptible. Also, the author mentioned that Nolo bait was effective against the reproductive potential of the desert locust and grasshopper in the laboratory.

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الفعل المتكامل بين فطر الميتاريزيم أنزوبلاى والنوزيما لوكاستا مع منظم النمو الحشرى كلورفلوزورون ضد نطاط البرسيم إيوبربيوكنمس بولورنس بولورنس فى الحقل

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تم دراسة الفعل التكاملى بين فطر ميتاريزيم أنيزوبلاى صنف أكريديم والنوزيما لوكاستا ومانع الانسلاخ الحشرى كلوروفلوزورون ٥٪ فى حقل برسيم فى قريه أتميدة – ميت غمر- دقهلية ضد حوريات العمر الثالث، الرابع والخامس لنطاط البرسيم العادى. أوضحت النتائج المتحصل عليها أن التكامل بين جراثيم الفطر ومانع الانسلاخ عند استخدامه بنسبة ٢٥٪ من الجرعة الموصى بها كان الأكثر فاعلية فى هذا التكامل حيث أدى لموت ٦٠٪ من الحوريات بعد ١٠ أيام من التطبيق و ١٠٠٪ بعد ١٩ يوم. ومن جهة أخرى وجد أن الفعل التكاملى للنوزيما لوكاستا ومانع الانسلاخ أدى لموت ٤٠٪ بعد ١٠ أيام و ٩٠٪ بعد ١٩ يوم من التطبيق. يتضح من ذلك أن الفعل التكاملى بين الفطر ومانع الانسلاخ كان أكثر فاعلية من الفعل المشترك بين النوزيما ومانع الانسلاخ.