

A NEW INTEGRATED STRATEGY FOR CONTROLLING THE DESERT LOCUST SCHISTOCERCA GREGARIA (FORSKAL)

ABDEL-FATAH, T. A.¹, M. KH. EL-DYDAMONY¹,
A. A. FRRAG² and T. M. ABDEL-GHANY²

1. Department of Locusts and grasshoppers, Plant Protection Research Institute, Agricultural Research Center
2. Botany and Microbiology Department, Faculty of Science, Al-Azhar University

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Abstract

Nowadays, there is an upsurge in the locust and grasshopper populations in many parts of the world. Any way despite of the increase use of agrochemicals, that reduced the attack by insects. These chemicals are still represents a high risk to field workers and consumers. Accordingly, the use of biological control in these days as a particle solution is too desirable as the only alternative solution to the problem of chemical pesticides usage for long time. Our study revealed that the mortality percentage of the first nymphal instar of the desert locust was 100% after 7, 10 And 19 days when treated with mixture of conc. 5×10^{10} added to 100ppm μ l of 10% consult (inhibitor compound), and *Metarhizium anisopliae* fungal spores at concentrations 5×10^{10} , 5×10^9 Spores/ml., respectively. On the other hand, mortality percentage reached 98 and 80% after 6 days by treatment with 100ppm and 0.1ppm of the inhibitor compound respectively. Also, the mortality percentage of the second nymphal instar attained 100% after 20 days by treatment with Conc. 5×10^9 Spores/ml. of fungal spores. Amazingly, the mortality percentage was 100% after 17 days for the third nymphal instar, this by mixing the fungal spores at higher Conc. 5×10^{10} Spores/ml. With the insecticide at Conc. of 100ppm interestingly, the third and fourth nymphal instar, were less sensitive toward lower concentration of insecticide than that of fungal spores usage. Any way, the biochemical pattern revealed that the carbohydrate concentration in haemolymph of infected adult locust was higher than that in the adult non infected control.

On the other hand lipid contents and total cholesterol, of the growth inhibitor treated adult insect was sharply increased after 3 days of treatment. Moreover, results showed that, the treatment with fungal spores reduced both the lipid and total cholesterol contents in the insect nymph, while total protein in the nymph of adult locust insect was increased with treatment by both fungal spores and growth inhibitor as a mixture. Also results revealed that the mortality percentage was 100% in both cases of adult and immature insect after 35 days and, 72% after 24 days. Moreover the treatment with mixture of both fungal spores and growth inhibitor reduced the hatchability percentage up to 47.2% in case of treated immature insects compared with 95.5 to the control. However, results also showed that the percentage was 16.9% in case of adult when compared with control. Also sterility percentage was 93.12% in case of immature insect to control, and 97.82% for mature ones compared with the control.

INTRODUCTION

The locust and grasshopper cause significant economic damage to crops forages and range in the Canadian prairies and result in extensive pesticide application Susan *et al* (2008). Also Locust and grasshopper generally have very high reproductive rates and are able to respond to unfavorable climatic conditions with rapid population increase (Bateman *et al.*, 1993). Population of locusts and grasshoppers are monitored and treated as soon as out_breaks threaten (Showler and Potter, 1991).

National authorities have adequate capacity to conduct preventive measures to control the out breaks at an early stage through the use of chemical pesticides in countries such as Argentina, Australia, China, Niger, and South Africa. Concern over the impact of chemicals on human health and the environment has been the driving force for investigation to the use of entomopathogenic micro-organisms for control such pests. However, over the last 25 years ago, chemical pesticides have 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).

The biological control is regarded as a desirable technique for controlling insects, due to its minimal environmental impact and preventing the development of resistance in vectors, (Eilenberg *et al.*, 2001). The locust is susceptible to the Deuteromycete pathogenic fungus, *Metarhizium anisopliae* (Lomer. 1997). Field trials show promise for *M. anisopliae* as a biocontrol agent of grasshoppers and locust (Arthurs and Thomas. 2000). Although previously conducted field trials of indigenous agent against grasshoppers have yielded divergent results, mostly attributed to timing and environmental condition. Any way, the Food and Agriculture Organization (FAO) of the United Nations (UN) has ranked Green Muscle® as the top insecticide in the two categories of human and environmental safety also, in the absence of desert locust swarms, the product is being used on a large scale against other locust and grasshopper in Africa. Finally, the study goal is to find out a more efficient and environmentally safe way to strongly control the outbreak of such harmful insect.

MATERIALS AND METHODS

1- Tested micro-organisms, entomopathogenic fungus:

The entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum* isolate (IMI330189) was obtained from the International Agricultural Center, Nairobi, Kenya, and the commercial name is Green Muscle®. The conidia of fungus were suspended in

sterilized (boiling and then left until cooling) water and to be emulsified adding Little of (Tween 80). We making two concentration 10^{10} and 10^9 , the control insects applied with sterilized water only.

2- Tested insect:

Nymphal instars and adult of the desert locust *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae) 2 days after ecdysis were used in the experiments. The insects were taken from the stock culture maintained for several generations at the Locust Research Section, Plant Protection Research Institute (PPRI), ARC, Dokki, Giza. The insects were reared in the laboratory in framed cages measuring (90×90×100cm) according to (Robert *et al* 2002). These cages made from wood and sides were made from glass and top from wire and a small door in the front side for cleaning, feeding and handling, this cages were kept at 30 ± 2 °C and 30-50 % of relative humidity. In the bottom of each cage put a sand layer with 20 cm deep for egg laying, and kept until eggs hatching, this layer are sprayed with water from time to other to keep humidity in side the sand layer, in order to allow the female of locust to lay the eggs. Each cage was illuminated and heated by two bulbs each of them are 100 watts. When eggs hatching, the nymphs were transferred to another cages measured (60×60×70cm) without sand layer for rearing the progeny and carry out the experiments using them. Under legs of the cages, suitable containers which filled with water were placed to protect nymphs from ants attack. The daily routine work includes removing the previous uneaten food, faeces and dead nymphs. The insects (nymphs and adult) are fed on trefoil, *Trifolium alexanderinum* and in some cases leaves of lettuce or cabbage and in summer on sesban plant and mixture from dry wheat and yeast powder which put on Petri dishes.

3- The anti moulting agent:

The anti-moulting agent was used is Consult 10% E.C. [3,5-dichloro-4-(1, 1, 2, 2-tetrafluoroethoxy) phenyl]-3-(2, 6-difluorobenzoyl) urea. Two concentrations were used, the first is lethal dose 100 ppm and the other is 0.1 ppm. Only 5µl of sterilized water was used for the control insects.

4- Bioassay of the Entomopathogenic fungus to nymphal instars and adult of desert locust, *S. gregaria*:

Instar nymphs (1st, 2nd, 3th, 4th, and 5th) and adult of 2 days after ecdysis were inoculated with 5µl of conidial suspension under ambient condition in the laboratory using micropipettes for topical application beneath the dorsal pronotal shield for each nymph. Ten nymphs were kept in an opened plastic cylinder (diameter 8 cm and length 25 cm) at both ends which covered with a sheet of cloth for ventilation.

Two concentrations were prepared and replicated 5 times. Each replicate contained 10 individuals. Treated insect were kept at 30 ± 2 °C and 30-50 % of relative humidity.

5-Biochemical changes the haemolymph of the infected locust:

5-1- Preparation of insect

Adult immature insect after 2 days of moulting are inoculated with 5µl from two types of suspension. (1) Conidial suspension with concentration 10^{10} . (2) Mixture from conidial suspension with concentration 10^{10} and consult suspension with sublethal dose and control insects are treated with (water + Tween 80). Treated and control insects were kept in an incubator at 30 ± 2 °C and 30-50 % of relative humidity with a 12-12h lighting (lamp as a source of light).

5-2- Sample collection

Samples of the haemolymph from the previous treatments and control were taken at 3, 6, 9 and 12 days after inoculation. The haemolymph was collected through a fine puncture from beneath the dorsal pronotal shield membrane and transferred into dry centrifuge tubes. Few crystals of phenylthiourea were added to prevent melanization before analysis (Metaweh *et al.*, 2001).

5-3- Determination of total Lipids

Total lipids were estimated by the method of (Knight *et al.*, 1972) by preparation of phosphovanillin reagent and preparation of standard solution. After 45 min. the developed color was measured at 525 nm. (250 µl) was treated in the manner as the sample solution the amount of

$$\text{mg lipids} = (\text{absorbance of test sample} / \text{absorbance of standard}) \times \text{absorbance of standard.}$$

5-4- Determination of total Carbohydrate

Total carbohydrate was determined in acid extracts by the phenol sulfuric acid reaction (Dubios *et al.*, 1956). The blank were prepared by substituting distilled water for the sugar solution. The yellow-orange color solution is measured at 490 nm against blank. Carbohydrate concentration are expressed as mg glucose / 100 ml haemolymph.

$$\text{mg carbohydrate} = (\text{absorbance of test} / \text{absorbance of standard}) \times \text{absorbance of standard.}$$

5-5- Determination of total Protein

Total proteins were determined by the method of (Bradford 1976). Sample solution (haemolymph) 50 µl were pipetted into test tube and the same volume of standard solution pipetted into test tube the absorbance at 595 nm was measured after 2 min. and before 1 hour.

mg protein = (absorbance of test / absorbance of standard) × absorbance of standard.

5-6- Determination of total Cholesterol

Total cholesterol was determined by the enzymatic colourmetric method of (Richmond, 1973). All reagents used in this determination were supplied by Ames Division Miles Lab.Inc, England.

RESULTS

As show in table (1) the mortality % of the 1st nymphal instar as the effect of entomopathogenic fungal spores at concentration of 5×10^{10} gave 100% mortality of desert locust after 10 days while spores concentration of 5×10^9 gave the same percentage mortality after 19 days of infection. On the other hand, the effect of growth inhibitor at concentration 100 ppm was gave 98% mortality after 6 days while at concentration of 0.1 ppm at this time was gave only 80% mortality . Results also showed that the effect of mixture of entomopathogenic fungal spores and growth inhibitor at concentration (5×10^{10} spores + 100 ppm growth inhibitor) give 100% of mortality after 7 days while concentration (10^9 spores + 0.1 ppm growth inhibitor) gave 88% mortality after 22 days of infection. However, the mottality percentage of 2nd nymphal instar as affected by entomopathogenic fungal spores at concentration of 5×10^{10} spores/ml give 94% of mortality after 20 days of infection while concentration of 5×10^9 spores/ml gave 100% mortality after 20 days of infection. On the other hand the effect of growth inhibitor at concentration of 100 ppm give 96% mortality after 19 days of infection while concentration of 0.1 ppm give 84% mortality after 21 days of infection . However, results also showed that the effect of mixture from entomopathogenic fungi and growth inhibitor at concentration of (10^{10} spores + 100 ppm growth inhibitor) give 98% mortality after 7 of infection days while concentration of (10^9 spores + 0.1 ppm growth inhibitor) give 90% mortality after 21 days of infection (Table 1). Also, the mortality percent of the 3rd nymphal instar, reached to 100% after 17 days when treated with mixture of 5×10^{10} spores and 100ppm of inhibitor compound (Table 2). At the same time 3rd and 4th nymphal instar were less sensitive to lower concentration of growth inhibitor compound than fungal spores, where the mortality percent don't exceed than 54%, up to 22 days of treatment with inhibitor compound, while ascending with increasing days of treatment with fungal spores and their mixture with inhibitor compound.

Table 1. Mortality percentage of the 1st and 2nd nymphal instar of the desert locust, *Schistocerca gregaria* treated with different concentrations of entomopathogenic fungal spores, growth inhibitor and their mixture

Days after infection	Mortality percentage after infection with Entomopathogenic fungal spore				Mortality percentage after infection with Growth inhibitor				Mortality percentage after infection with Mixture				Control	
	5×10 ¹⁰ spores/ml		5×10 ⁹ spores/ml		100 ppm		0.1 ppm		5×10 ¹⁰ + 100 ppm		5×10 ⁹ + 0.1 ppm			Water+ Tween 80
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd		
5	76	44	58	70	94	80	70	50	96	84	46	54	0	
6	84	72	72	70	98	82	80	58	96	94	50	68	0	
7	92	76	80	70	98	82	80	58	100	98	52	70	0	
8	94	80	88	74	98	84	80	64	---	98	64	72	0	
9	98	82	96	86	98	84	80	72	---	98	67	72	0	
10	100	82	96	90	98	84	80	72	---	98	78	76	0	
11	---	84	96	90	98	88	80	72	---	98	78	76	0	
12	---	84	96	90	98	88	80	72	---	98	78	84	0	
13	---	86	96	90	98	88	80	72	---	98	78	84	0	
14	---	86	96	90	98	88	80	72	---	98	78	88	0	
15	---	86	96	90	98	88	80	76	---	98	80	88	0	
16	---	86	96	90	98	88	80	78	---	98	84	88	0	
17	---	86	96	90	98	90	80	80	---	98	84	88	0	
18	---	92	96	90	98	92	80	80	---	98	84	88	0	
19	---	92	100	98	98	96	80	82	---	98	86	88	0	
20	---	94	---	100	98	96	80	82	---	98	86	88	0	
21	---	94	---	---	98	96	80	84	---	98	86	90	0	
22	---	94	---	---	98	96	80	84	---	98	88	90	0	

Table 2. Mortality percentage in the 3rd and 4th nymphal instar of the desert locust, *Schistocerca gregaria* treated with different concentrations of entomopathogenic fungus spores, growth inhibitor and their mixture

Days after infection	Mortality percentage after infection with Entomopathogenic fungal spore				Mortality percentage after infection with Growth inhibitor				Mortality percentage after infection with Mixture				control Water+ Tween 80
	5×10 ¹⁰ spores/ml		5×10 ⁹ spores/ml		100 ppm		0.1 ppm		5×10 ¹⁰ + 100 ppm		5×10 ⁹ + 0.1 ppm		
	3 rd	4 th	3 rd	4 th	3 rd	4 th	3 rd	4 th	3 rd	4 th	3 rd	4 th	
5	44	34	22	28	84	80	34	36	66	56	46	34	0
6	46	50	30	32	92	84	38	36	76	56	46	52	0
7	50	62	46	38	92	86	40	36	86	56	58	64	0
8	54	62	50	44	96	88	44	36	86	56	62	66	0
9	58	62	52	52	96	88	44	36	86	58	62	66	0
10	62	62	54	56	96	90	44	42	90	58	68	66	0
11	62	64	56	58	96	90	44	42	92	60	68	66	0
12	62	72	58	62	96	92	50	44	92	66	76	66	0
13	62	84	58	66	96	92	50	52	92	72	80	78	0
14	62	84	58	68	96	94	50	52	98	76	80	90	0
15	62	84	62	70	96	94	50	52	98	80	80	94	0
16	70	84	62	72	96	94	52	54	98	80	80	94	0
17	70	84	64	76	96	94	52	54	100	80	80	94	0
18	70	84	66	78	96	94	52	54	----	80	80	94	0
19	70	86	72	82	96	96	52	54	----	80	80	94	0
20	78	86	76	82	96	96	52	54	----	80	80	94	0
21	78	86	76	84	96	96	52	54	----	80	80	94	0
22	78	86	76	84	96	96	52	54	----	84	80	94	0

Results obtained from Table (3) showed that the effect of treatments on the 5th instar as affected by entomopathogenic fungal spores at concentration of 5×10¹⁰ spores/ml was 100% mortality after 7 days while concentration of 5×10⁹ spores/ml was 96% mortality after 13 days. On the other hand, the effect of growth inhibitor at concentration of 100 ppm gave 90% mortality after 8 days while concentration of 0.1 ppm ml/l was 46% mortality after 15 days. Results also showed that, the effect of mixture of entomopathogenic fungal spores and growth inhibitor at concentration of (10¹⁰ spores + 100 ppm growth inhibitor) gave 46% mortality after 20 days while concentration of (10⁹ spores + 0.1 ppm growth inhibitor) was 54% mortality after 21 days.

Table 3. mortality percentage in the 5th nymphal instar of the desert locust, *Schistocerca gregaria* treated with different concentrations of entomopathogenic fungus spores , growth inhibitor and their mixture

Days after infection	Mortality percentage after infection with Entomopathogenic fungal spore		Mortality percentage after infection with Growth inhibitor		Mortality percentage after infection with Mixture		Mortality percentage of control
	10 ¹⁰ spores/ml	10 ⁹ spores/ml	100 ppm	0.1 ppm	10 ¹⁰ spores/ml + 100 ppm	10 ⁹ spores/ml + 0.1 ppm	
5	70	26	26	28	14	10	0
6	94	42	66	28	14	30	0
7	100	54	86	28	16	46	0
8	-----	82	90	30	24	48	0
9	-----	88	90	34	24	48	0
10	-----	92	90	38	28	48	0
11	-----	92	90	40	28	48	0
12	-----	92	90	40	28	48	0
13	-----	96	90	42	28	50	0
14	-----	96	90	42	28	50	0
15	-----	96	90	46	28	50	0
16	-----	96	90	46	28	52	0
17	-----	96	90	46	34	52	0
18	-----	96	90	46	42	52	0
19	-----	96	90	46	42	52	0
20	-----	96	90	46	46	52	0
21	-----	96	90	46	46	54	0
22	-----	96	90	46	46	54	0

Table 4. Estimation of total carbohydrate lipid, protein and cholesterol of adult desert locust, *S. gregaria* after infection with fungus and a mixture from fungus and growth inhibitor

Biochemical profiles	carbohydrate				lipid				protein				cholesterol			
	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Control	14.73 ± 1.38 ^f	22.05 ± 0.79 ^e	41.85 ± 1.36 ^a	17.19 ± 1.25 ^f	550 ± 11 ^{cd}	458 ± 8 ^e	512 ± 8 ^{de}	581 ± 12 ^{bc}	4040 ± 87 ^e	5183 ± 45 ^c	5386 ± 83 ^b	5111 ± 80 ^c	42.6 ± 1.6 ^d	41.16 ± 0.7 ^d	48.8 ± 2.7 ^c	53.7 ± 1.44 ^b
Fungal spores	21.5 ± 0.85 ^e	36.19 ± 1.14 ^c	39.69 ± 1.41 ^{ab}	38.01 ± 1.8 ^{bc}	289 ± 14 ^g	898 ± 40 ^a	395 ± 10 ^f	629 ± 21 ^b	4610 ± 26 ^d	5774 ± 83 ^a	4460 ± 55 ^d	3480 ± 48 ^f	32.3 ± 2 ^e	55 ± 2.15 ^b	23.9 ± 1.7 ^f	61.1 ± 2.57 ^a
Mixture(fungal spores + growth inhibitor)	20.59 ± 1 ^e	21.15 ± 0.7 ^e	31.26 ± 1.9 ^d	30.78 ± 2.21 ^d	569 ± 12 ^{bcd}	162 ± 7 ^h	203 ± 5 ^h	154 ± 16 ^h	3191 ± 18 ^g	3198 ± 33 ^g	3000 ± 105 ^h	2800 ± 52 ⁱ	34.2 ± 1.96 ^e	8.9 ± 0.85 ^h	12.57 ± 0.75 ^{gh}	16.87 ± 1.66 ^g

Data obtained from Table (4) revealed that, carbohydrate content in haemolymph which collected from infected adult locust after 3 days was higher than that of control content, also the content of total carbohydrate in infected locust was 20.59 ± 1 for mixture and 21.5 ± 0.85 for fungus, After 6 days of infection with fungal spores the carbohydrate content was higher than that of control content, which reached 36.19 ± 1.14 but in case of mixture, the content of carbohydrate as compared with control content which is 21.15 ± 0.7 for mixture and 22.05 ± 0.79 for the control. But the carbohydrate content in case treatment with fungal spores and the mixture was less than control content, after 9 days from infection, as the content was 31.26 ± 1.9 for the mixture, 39.69 ± 1.41 for fungal spores as compared with 41.85 ± 1.36 for the control. After 12 days of infection the value of carbohydrate for fungal spores and mixture infected locust differed than later which was higher than control content, the value was 30.78 ± 2.21 for mixture and 38.01 ± 1.8 for fungal spores compared with 17.19 ± 1.25 for the control. On the other hand after 3 days of infection the lipid content in the insects treated with mixture was higher than that of the control insects, while the lipid content for insects treated with fungal spores was lower than that of the control insects. Any way, the content was 289 ± 14 for fungal spores, 569 ± 12 for the mixture compared with 550 ± 11 for the control. Moreover, results also showed that after 6 days, lipid content in insects treated with mixture was lower than that of the control content. The insect treated with fungal spores gave different content "high and low" after 6 days it was higher than control with content of 898 ± 40 . After 9 days the value is lower than the control which is 395 ± 10 and after 12 days the content rise to 629 ± 21 which is higher than that of control. In case cholesterol, results also showed that, in all periods tested, the cholesterol content in case of the insects which infected with mixture were lower than that of the content of the control, while in case of treatment with the fungal spores, the cholesterol content was lower than the control value after 3 and 9 days and higher than that of the control content after 6 and 12 days of the infection. Furthermore, the protein content after 3 days from inoculation of the adult insect was higher in case of fungal spores than control, but in case of mixture it was lower than the control. Any way, the content was 3191 ± 18 for mixture, 4610 ± 26 for fungal spores compared with 4040 ± 87 for the control. However, after 6 days from infection the protein value in the insect infected with fungal spores was higher than the protein content in control insects where the protein in the mixture was also lower than the control content. Results also clarified that after 9 days and 12 days from infection the protein content in the infected insect with fungal spores and mixture was lower than the protein content of the control insects.

Table 5. Fecundity of adult females of *Shistocerca gregaria*

	No. of egg pod/ female	No. of eggs/egg pod	No. of eggs/ female
Control immature	3.9	78.5	308
Treated immature	0.84	50.9	42.76
Control mature	3.8	76	292
Treated mature	1.24	28.7	35.64

Number of eggs per an egg pod:

As recorded in table (5) data revealed that the number of eggs per egg pod was influenced significantly by the mixture treatment on both immature and mature insects. Anyway, results showed a reduction in the number of eggs / egg pod as compared to the control, in this case the number of egg per an egg pod was 50.9 for treated immature and 28.7 for treated mature adult locust compared to 78.5 for immature control and 76 for mature control.

Total number of eggs per a female:

It is evident from the results shown in table (5) that treatment with mixture on immature and mature adult locust led to reduce the number of eggs / female compared with that of control. However, average number of eggs/female were 42.76 for immature and 35.64 for mature insect compared with 308 in immature control and 292 in mature control.

Table 6. Fertility of eggs laid by adult females of *Shistocerca gregaria*

	Hatchability%	Sterility%
Control	95	0
Treated immature	47.2	93.102
Treated mature	16.9	97.82

Data in Table (6) shown that treatment with mixture of fungal spores and growth inhibitor reduced the hatchability percentage to 47.2% in treated immature and 16.9% in treated mature compared with 95% in case of control. At the same time the treatment resulted in a very high rate of adult sterility. Sterility percentage were 93.1% for treated immature and 97.82% for treated mature compared with 0% in control

DISCUSSION

The younger nymphal instars were (in general) more susceptible than the developed later nymphal instas (Metaweh *et al.* 2001).

Result of treatment showed that :- In the first nymphal instar the effect of growth inhibitor in the two concentrations used are higher than the use of entomopathogenic fungal spores, but by mixing with both at concentration of (10^{10} for fungus spores +100 ppm for growth inhibitor). The effect increased and that effect was appeared with no significant difference in the increase between both the mixture and the growth inhibitor alone. In the second nymphal instar the effect of entomopathogenic fungal spores at concentration of 5×10^9 spores/ml was more than the effect of growth inhibitor at the two concentrations, but in mixture of (10^{10} for fungi +100 ppm for the growth inhibitor) the dose effect increased and gave more mortality rate than in case fungal spores and growth inhibitor used separate, while in some case the use of mixture from (10^9 for fungal spores +0.1 ppm for growth inhibitor) the effect was appreciable as compared with growth inhibitor at concentration of 0.1 ppm. On the other hand in third nymphal instar the effect of fungal spores was less than the rest of other nymphal stages, and the results are in parallel to that of (Rabie and Risha 1994) who reported that the effect of *M.anisopliae* was non virulent against the third nymphal instar of the desert locust when used alone. However, in the fourth nymphal instar the use of mixture dose at concentration of (10^9 of fungal spores +0.1 ppm of growth inhibitor) gave similar mortality rate as growth inhibitor at concentration of 100 ppm while the entomopathogenic fungal spores at the two concentrations gave higher result than that of growth inhibitor at concentration of 0.1 ppm and mixture with concentration (10^{10} for fungi +100 ppm for growth inhibitor). Amazingly, that as the fifth nymphal instar used the effect of entomopathogenic fungal spores used at concentration of 5×10^{10} was better than the use effect of either growth inhibitor or the mixture from (entomopathogenic fungal spores and growth inhibitor) at the both concentrations used.

Finally we can generally conclude that, the use of mixture of (entomopathogenic fungal spores and growth inhibitor) was more effective that of the entomopathogenic fungal spores alone except in the case of the fifth nymphal instar.

Also, obtained results showed that insects treated with entomopathogenic fungal spores and a mixture of (entomopathogenic fungal spores and growth inhibitor), have higher carbohydrate content than that of control, except after 9 days of infection, the carbohydrate content decrease than that of control. However, results also clarified that, there is no difference between the use of mixture and control in its carbohydrate content after 6 days of treatment in case of mixture use the results are parallel with

(George *et al* 1996) who reported that , Hexaflumuron is highly toxic to II instar *Schistocerca gregaria* when applied as a single dose and exhibited important chronic effects such as extended intermoult periods, reduce mobility and feeding . the toxicity of its use under field conditions against locust will lead to faster reduction

On the other hand, treatment with mixture of (entomopathogenic fungal spores and growth inhibitor) resulted in decrease in the protein content than that of control, protein content of insect which treated with entomopathogenic fungal spores was higher than that of control content after 3 and 6 days of treatment and then decrease until the end of the experiment and the results are in parallel to studies of (Metaweh *et al* 2001) who found that treatment with *M.anisopliae* led to decrease in protein content in desert locust than control after 7 days.

Also results showed that in case of lipid and cholesterol the insect which treated with mixture of (entomopathogenic fungal spores and growth inhibitor) causing decrease in lipid content than that of control except after 3 days of treatment the content of lipid in mixture was higher than that of control content within small range in case of lipid only, but the content of lipid and cholesterol in insect which treated with entomopathogenic fungal spores was variable when compared with the control which decrease and then increase in a fluctuating manner which depend on the time of egg laying because cholesterol is very important to insect for egg formation .

Mortality rate was different in treated adults of locust from the control which not have mortality until end of the experiment. The results were in agreement with those obtained by (Douglas *et al*, 1995) who found an extensive mortality in oviposition grasshopper females when treated with *Bauveria bassiana* during oviposition.

Accordingly, the obtained results also showed a reduction in (number of egg pods / female, number of eggs per an egg pod and total number of eggs per a female), these results are in agreement with those of (El-Maghraby *et al* 2009).Which showed a reduction in number of egg pods / female and number of eggs per an egg pod when *Shistocerca gregariae* treated with *M. anisopliae* alone.

However, fertility of infected adult locust, as expressed, both hatchability and sterility increase in the sterility but decrease in the hatchability as compared with the fertility of control locust and the obtained results are in parallel with that obtained results (Abdelfatah 2002) who concluded that, the treated grasshoppers with entomopathogenic fungal spores *M. anisopliae* showed a decrease in hatchability and increase in sterility.

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استراتيجيه متكامله جديده لمكافحة الجراد الصحراوي

ثروت عبدالمنعم عبدالفتاح¹، محمد خيرى الديدامونى¹،
ايمن فراج احمد دردير²، طارق محمد عبدالغنى²

١. قسم الجراد والنطاط - معهد بحوث وقايه النباتات - مركز البحوث الزراعيه
٢. قسم النبات والميكروبيولوجى - كلية العلوم - جامعه الازهر - القاهره

تتزايد فى هذه الأيام المخاوف من إنتشار حشره الجراد فى أنحاء عديده من العالم فعلى الرغم من أن ال استخدام المتزايد للمبيدات الكيماويه استطاع أن يقلل من هجوم تلك الحشرات. فما زالت تلك الكيماويات تشكل خطرا جثيما على مستخدميها فى الحقول وعلى المستهلكين ايضا. ولحسن الحظ فان لكل أفه حشريه ممرضات ميكروبيه مصاحبه لها وتبعاً لذلك فان استخدام المكافحه الحيويه فى هذه الأيام كحل علمى يعد مستحسناً للغاية كبديل لمشاكل استخدام المبيدات الكيماويه على المدى الطويل فقد أظهرت النتائج أن نسبة الموت للعمر الأول للجراد كانت 100% بعد فترات 9,10 و 7 أيام كنتيجة للمعامله بكل من استخدام جراثيم فطر ميتاريزيم عند تركيز 5×10^{10} , 5×10^9 ثم مخلوط من كل من جراثيم الفطر المختبره عند تركيز 5×10^{10} ومنظم النمو كونصلت بتركيز 100% على الترتيب. ومن ناحيه اخرى ف إن نسبة الموت لحشره الجراد وصلت الى 89% ، 80% بعد سته أيام فقط من استخدام منظم النمو عند تركيزات 100جزئ فى المليون و 0.1 جزئ فى المليون بالترتيب ومن ناحيه اخرى فان نسبة الموت بالنسبه للعمر الحورى الثانى كانت 100% بعد 20 يوم من استخدام الجراثيم الفطريه بتركيز 5×10^9 وان نسبة الموت كانت 100% بعد 17 يوما بالنسبه للعمر الحورى الثالث وذلك بخلط الجراثيم الفطريه عند التركيز 5×10^{10} مع منظم النمو بالنمو بتركيز 100جزئ فى المليون ومن الغريب أن العمرين الثالث والرابع كانوا أقل حساسيه باستخدام تركيزات متدنيه من منظم النمو عنه عند استخدام الجراثيم الفطريه ، وبينت الدراسه أن المحتوى الكربوهيدراتى كان أكثر فى حشره الجراد المعامله الغير بالغه عنه فى الحشره الغير معامله (الكنترول). ومن ناحيه اخرى ف إن المحتوى الدهنى الكلى لحشره الجراد الغير بالغه المعامله بالمخلوط قد زاد بشكل واضح بعد ثلاثه أيام فقط اما المحتوى الكوليسترولى فقد قل فى نفس المده بعد المعامله. ايضا فقد أظهرت النتائج أن المعامله بالجراثيم الفطريه قد خفض كلا من المحتوى الدهنى والكوليسترولى .وعند معاملة حشره الجراد الغير بالغه والمعامله بكل من المخلوط والجراثيم الفطريه وجد أن البروتين الكلى قد زاد ثم قل . إضافة الى ذلك فقد أظهرت النتائج ايضا أن نسبة الموت لحشره البالغه والغير بالغه كانت 100% بعد 35 يوم و 72% بعد 24 يوما على الترتيب وعلى اى حال فان المعامله بمخلوط من كل من الجراثيم الفطريه ومنظم النمو قد خفض نسبة التفريغ الى 47.2% بالنسبه لغير الناضجين مقارنة ب95% بالنسبه للكنترول ، كما اوضحت النتائج ايضا أن النسبه كانت 16.9% بالنسبه للناضجين فقط، مقارنة بنفس النسبه للكنترول. ايضا فان نسبة العمق بلغت 93.12% بالنسبه لغير الناضجين مقارنة بالنسبه المئويه للكنترول ، 97.82% بالنسبه للناضجين مقارنة بنسبه صفر للكنترول.