J. Agric. Sci. Mansoura Univ., 28 (2): 1463- 1471, 2003

SUSCEPTIBILITY OF TWO IMPORTANT COTTON INSECTS, *Spodoptera littoralis (Boisd) AND Agrotis ipsilon (Huf.)* TO THE ENTOMOPA-THOGENIC FUNGUS *Metarhizium anisopliae*

Aly, Safaa H.
Agricultural Research Center, Plant Protection Research Institute, Pesticides Tests of cotton Pests Department, Egypt

ABSTRACT

Laboratory bioassay of the fungus *Metarhizium anisopliae* included different stages of two important cotton insects, *Spodoptera littoralis(Boisd)* and *Agrotis ipsilon(Huf.)*, under different concentrations of fungus. The fungus proved virulent against eggs and larvae of both insects. Treatment eggs of both insects pests reduced rate of hatchability. It was evident that eggs and larvae of both insects were susceptible to fungul infection, and most of hatched larvae from treated eggs failed to devleop to adults and dead in both *S.littoralis* and *A.ipsilon*. LC_{50} for *S.littoralis* eggs was 1.7×10^3 spores/ml and 2.5×10^3 spores/ml for larvae. LC_{50} for *A.ipsilon* eggs was 2.5×10^3 spores/ml, and 5.5×10^3 spores/ml for larvae. *S.littoralis* appeared to be more susceptible than *A.ipsilon* through all the stages, and recorded more reduction in larvae, pupae, and adults during larval development of the newly emerging larvae from the treated eggs.

INTRODUCTION

Spodoptera littoralis and *Agrotis ipsilon* are an important insect pests causing a great damage to many agricultural economic crops especially cotton, their chemical control in the field faces serious difficulties because they developed resistance to most pesticides and caused harmful effect on environment. Lacey, et. al (2001), found that the entomopathogens are safety for humans and other non-target organisms and reduction of pesticides residues in food preservation of other natural enemies and increased biodiversity in managed ecosystems.

The Deutomycetes entomopathogen, *Metarhizium anisopliae* has a wide host range which includes representative of *Lepidoptera* (Veen, 1968), Wallengren and Johansson (1929), Kodaira (1961) and Roberts (1966) *M. anisopliae* attaks many species of insects including *Cleoptera, Lepidoptera* and *Orthoptera*, Goettel, (1992). Ignoffo & Garcia, (1979), Mueller koegler, (1965), Stephan *et al.* (1996), and recently against *homoptera* insect. Gindin, *et al.*, (2000), used *M. anisopliae* against looper larvae, *Aphodius sp., and Bemesia tabaci*,.

Synergistic effects of *M. anisopliae* on a *Lepidopteroan* species indicated another approach for integrated pest control, Brousseau, *et. al.*, (1998).

M. anisopliae Var. anisopliae was chosen as one of the most virulent strains to *S. Littoralis* for farther investigations. It is used also against anather *Lepidopteran* insects such as budworm, Brousseau *et al.* (1998).

Investigations about the effect of *M. anisopliae* on *S. Littoralis iridicated that* high mortality levels and were obtained thus affered good prospects for reducing the pesticide input, Anke Skrobk, (2001). Ignoffo & Garcia (1981) used *M. anisopliae* against black cutworm, and they proved the susceptibility of larvae of the black cutworm to species of entomopathogenic bacteria, fungi, protozoa and viruses. Also Hassani, *et. al.*, (1998). studied the effect of different strains of the fungi against two cotton pests *S.littoralis* and *Helicoverpa armigera (Lepidoptera: Noctuidae).*

The aim of this study is to investigate possibility of using *M. anisopliae* as a biological control agent against the both important insects, *S. littoralis* and *A. ipsilon,* such a fungus has been known attaking *Lepidopterous* insect as *Bomby mori* (Ferron 1981 and Kodaira 1961) and moribund hosts (Roberts 1966), Criquet pelerin (Veen 1968), *Pyrausta nubilalis* (Wallengren and Johnansson 1929), black cutworm (Ignoffo and Garcia, 1979), budworm (Brousseau *et. al.*, 1998), and *S. littoralis* (Hassani, *et al.* 1998, and Anke Skrobk, 2001).

MATERIALS AND METHODS

Host Insects:

S littoralis and *ipsilon* were reared on the host plant cotton leaves at laboratory. Adults were put in Jars to lay eggs on the plant leaves at 25°C . the insect newly deposited eggs and 2nd instar larvae were treated with M. anisopliae serial concentrations.

Fungus and Bioassay Procedures:

1- The sample of *M. anisopliae.*

The sample which was used in this work is obtained from Prof. Dr. Taborsky, Fungies laboratory at Agriculture University of Prague.

2- Cultivation of the fungus. Sabourauds agar:

Preparation of sabourauds agar by weighting pepton (8.0 g), glucose (18.0 g), maltose (18.0 g) and agar (15.0 g). all were mixed in a jar and completed to 1.000 ml. Of distilled water, sterilized at 120°C for 30 minutes in autoclave and then poured into petri dishes, which is then sterilized at 120°C for 30 minutes, cooled and again sterilized at the same conditions.

i- Incoluation of petri dishes in flowbox after sterilization for 30 minutes. The desk was cleaned by 70% ethyl alcohol and inoculation started by suspension of the conidia. For better inoculation we need liquid culture of fungus which was cultivated in the same medium without agar.

ii- Incubation in the petri dishes after inoculation. Incubation was in the termostat at 25°C for 9-14 days.

iii- Harvest of condia: the wet method was adopted by using solution of 0.02 tween- 80 (sterilized distilled water), then we put 30 ml. Or 50 ml . in the small or the big dish, and moved it by brush on the surface and then collected the suspensions of conidia to flask. After that filtered to remove the wastes from the suspension by cotton in funnel, then filtered again by

muslim, and examined a drop on slide to assure that it is pure, and then collected the pure conidia by centrifuge.

iv- Preparation of the suspension of conidia.

The number of conidia was calculated by Hemocitometer to determine the lethal concentration (LC_{50}), four concentrations were used (2 x 10^3), (3 x 10^3),(4x10^3)and (6 x 10^3) spores/ml. for *S.littoralis* and (11 x 10^3), (7 x 10^3), (5 x 10^3), and (2 x 10^3) spores/ml for *A. ipsilon* four replicates were made for each treatment.the eggs and larvae were treated using spraying method During all tests pieces of moist cotton were placed in the petri dishes to keep the relative humidity at 100% after applications. The treated stages were examined at 1,2, 3 and 4,days after treatments. Newly emerging larvae from treated eggs were maintained in order to examine the mortality during larval development. All treatments were incubated at 25°C. Percentage mortality was assessed at 72 hours after treatments.

RESULTS AND DISCUSSION

Present work indicates that the eggs and larvae of *S. littoralis* and *A. ipsilon* are susceptible to the fungus, *M. anisopliae*, more over eggs appear to be more susceptible than larvae in both insects. The successful infection by *M. anisopliae* in the two cotton insect was also reported for both Lepidopteran insects by Ank Skrobek, (2001), who studied the pathogenicity of *M. anisopliae* to *S. littoralis* and gave high mortality level. Also the succesful infection by *M. anisopliae* against the black cutworm species was reported by Ignoffo & Garcia, (1981). Extensive network of hyphac on the egg and larvae was appeared clearly through 2 days after putting in 100% moisture at 25°C after treatments as recommended by Butt & Goettel (2000). The fungus was observed clearly under the microscobe with dark green clour on the infected larvae and eggs, this explains the phenominon which was demonstrated in host insects by Rodrigue-Reudan and Fargues (1980), who suggested two ways to contamination of newly hatched larvae; first, fungal germination on the chorion surface and penetration of the eggs integument before hatching; second, conidia on the eggs cuticle could be an infective inoculum for neonate larvae upon chorion, and this mostly happened in Lepidopterous eggs.

Susceptibility of *S. littoralis* eggs to *M. anisopliae*:

Data in table (1) show that the eggs of *S.littoralis* susceptible to the fungus, and high susceptibility appeared at the higher concentration than those at the lower concentration, most of the larvae hatched from the treated eggs were dead. LC_{50} value for the eggs of *S. littoralis* was 1.7 x 10^3 spores/ml. and LC_{90} was 6.3 x 10^3 spores/ml, 2 fig (1). These data are in agreement with Aly and Rashad, (1997), who recorded that the LC_{50} of *Earias insulana (Lep. Noctuidae)* was 1.5 x 10^3 spores/ml for eggs treated with *M. anisopliae*. Also these results are in agreement with Rashad & Aly, (1994), who proved the susceptibility of *Pectinophora gossypiella (Lepidoptera)* eggs to *M. anisopliae*, and LC_{50} of eggs was 1.8 x10^3 spores/ml.

Table (1): **Pathogenicity of *M. anisoplia* at different concentrations against the eggs of *S. Littoralis*.**

Treatment Concentration	No. of treat. Eggs	Hatching (%)	Larval Mort. (%)	Correct Mort. (%)
6 x 10^3 spores/ml	50	4	96	91.2
4 x 10^3 spores /ml	80	25	75	71.2
3 x 10^3 spores/ml	100	37	63	59.9
2 x 10^3 spores/ml	50	45	55	52.3
Control	100	95	5	0.0

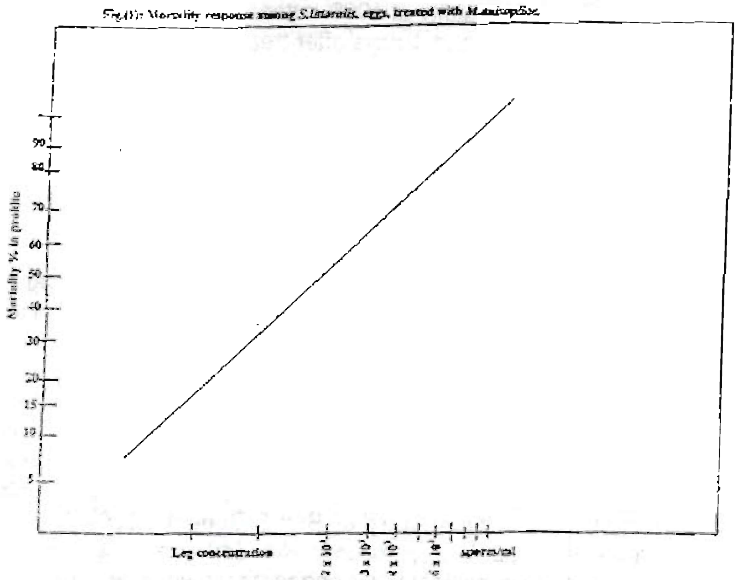


Table (2): **Pathogenicity of *M. anisoplia* at different concentrations against the eggs of *A.Ipsilon*.**

Treatment Concentration	No. of treat. Eggs	Hatching (%)	Larval Mort. (%)	Correct Mort. (%)
11 x 10^3 spores/ml	100	14	86	81.7
7 x 10^3 spores /ml	50	18	82	77.9
5 x 10^3 spores/ml	80	35	65	61.7
2 x 10^3 spores/ml	50	51	49	46.6
Control	100	95	5	0.0

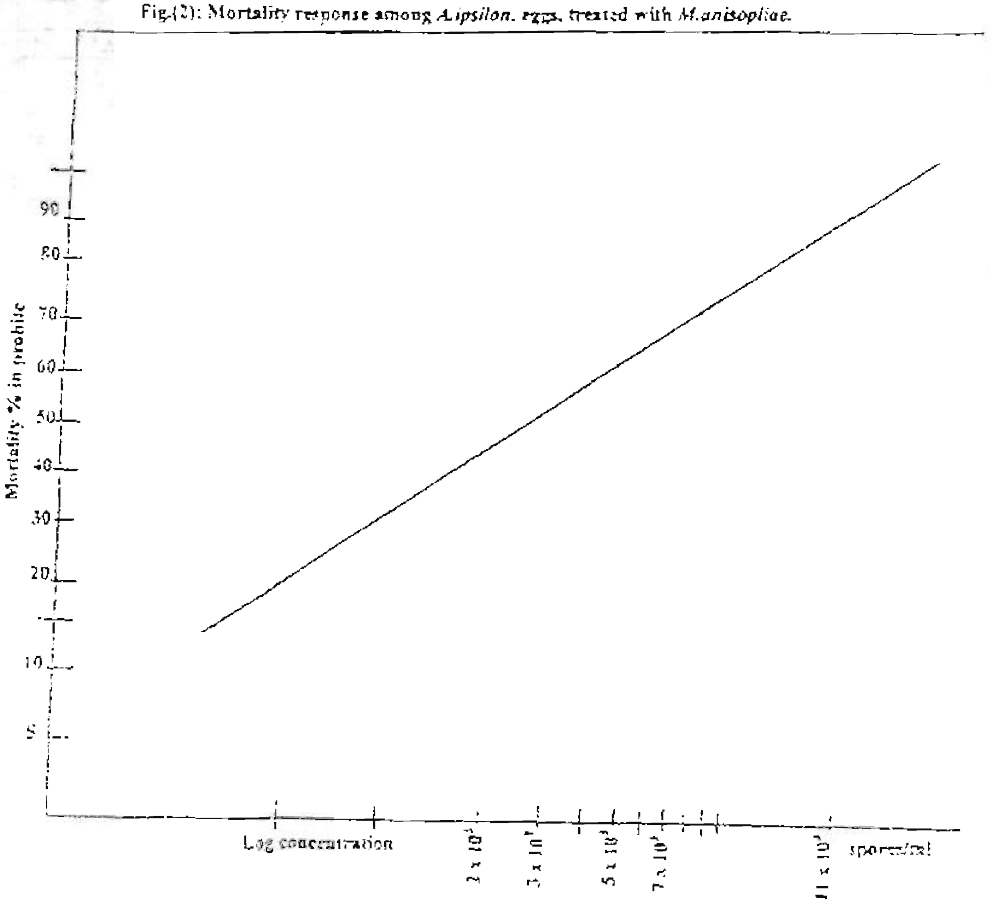
Susceptibility of *A. ipsilon* eggs to *M. anisopliae*:

Data in table (2) demonstrate the susceptibility of *A. ipsilon* eggs to the fungus, and high susceptibility appeared at the higher concentration than those at the lower concentration, also most of the larvae hatched from the treated eggs dead LC_{50} value for the eggs was 2.5 x 10^3 spores/ml, and LC_{90} was 10.8 x 10^3 spores/ml, (fig 2).

These data are in agreement with Ignoffo & Garcia, (1981), who proved the susceptibility of black cutworm to species of fungi.

The data of both insects indicated that the eggs of *S.littoralis* were more susceptible for the fungus than *A.ipislon* eggs.

Fig.(2): Mortality response among *A.ipsilon*. eggs, treated with *M.anisopliae*.



Susceptibility of *S.littoralis* larvae to *M.anisopliae:*

Data in table (3) and (Fig 3) show that the second-instar larvae of *S.littoralis* susceptible to the fungus, and the high susceptibility appeared at the higher concentration than those of the lower concentration. LC_{50} value for the second-instar lavae of *S.littoralis* was 2.5×10^3 spores/ml after 72 hours of treatment with fungus, and LC_{90} was 6.8×10^3 spores/ml. These data are in agreement with Aly and Rashad, (1997), who recorded that the LC_{50} of *Earias insulana* (Lep) was 3×10^3 spores/ml for larvae treted with *M. anisopliae*. These data are in agreement with those of Rashad & Aly (1994), who recorded LC_{50} of 2.5×10^3 spores/ml for larvae of *Pectinophora gossypiella* (Lep.) with *M. anisopliae*.

Table (3): Pathogenicity of *M. anisoplia* at different concentrations against the second- larvae instar larvae of *S.littoralis*

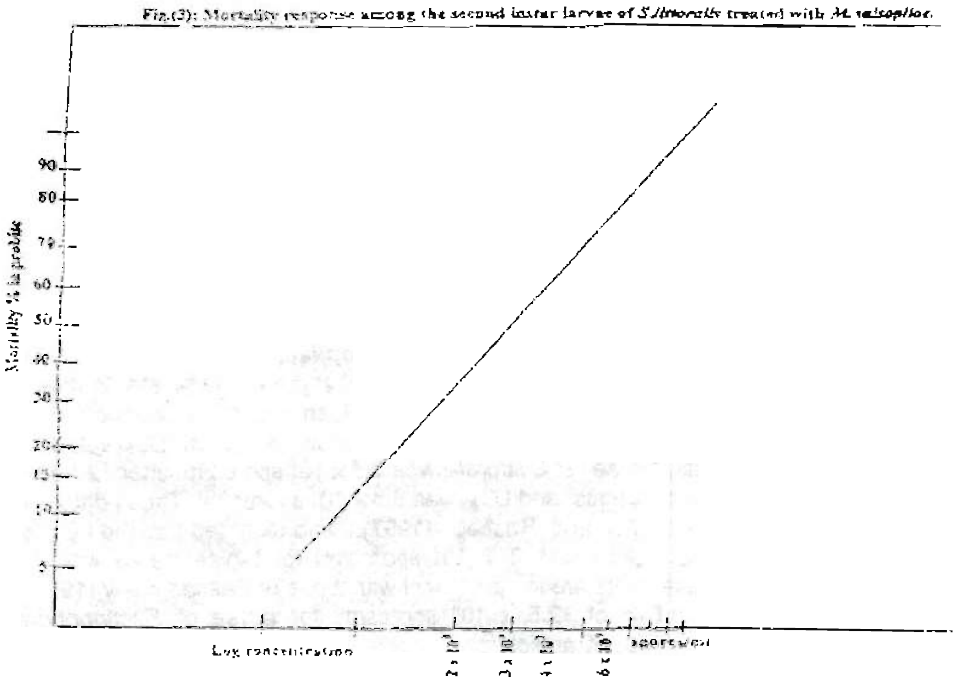
Treatment Concentration	No. of treat. larvae	Mort. (%)	Correct Mort. (%)
6 x 10^3 spores/ml	50	84	80.6
4 x 10^3 spores /ml	50	72	69.1
3 x 10^3 spores/ml	50	52	49.92
2 x 10^3 spores/ml	50	40	38.8
Control	50	4	0.0

Susceptibility of *A. ipsilon* larvae to *M. anisopliae:*

Data in table (4) and (Fig. 4) indicated that the second-instar larvae of *A.ipsilon were* susceptible to the fungus, and the high susceptibility appeared at the higher concentration than those at the lower concentration. LC_{50} value for the second-instar larvae of *A. ipsilon* was 5.54 x 10^3 spores/ml, and LC_{90} was 11.8 x10^3 spores/ml. after 72 hours of treatment with fungus.

Table (4): Pathogenicity of *M. anisoplia* at different concentrations against the second- larvae instar larvae of *A.Ipsilon.*

Treatment Concentration	No. of treat. larvae	Mort. (%)	Correct Mort. (%)
11 x 10^3 spores/ml	50	72	69.1
7 x 10^3 spores /ml	50	56	53.8
5 x 10^3 spores/ml	50	40	38.4
2 x 10^3 spores/ml	50	31	29.8
Control	50	4	0.0



The results are in agreement with those of Ignoffo and Garcia, (1981), who proved the susceptibility of black cutworm larvae to species of fungi.

The obtaind data of both insects indicate that the second- larvae instar larvae of *S.littoralis* are more susceptible for the fungus, *M.anisopliae* than the larvae of *A.ipsilon, and* are in agreement with Hassani, *et al.*, (1998), who studied the effect of different strains of the fungi against two cotton pests, *S.littoralis and Helicoverpa ormigera (Lepidoptera)*. Also these data are in agreement with Anka Skrobk, (2001), who studied the susceptibility of *S.littoralis* larvae against *M.anisopliae* by using concentration of 5×10^7 spores/ml for third-instar larvae.

The effects of *M.anisopliae* on the devlopment of the newly emerging larvae of *S.littoralis* from treated eggs:

Data in table (5) demonestrate the percentage of mortality during the life duration of larvae emerged from the treated eggs. Data explain that 71.63% total mean of larvae, 80% total mean of pupae and 98.13% total mean of adults are failed to maintained and dead.

Table (5):The effects of *M.anisopliae* on the development of the newly emerging larvae of *S.littoralis* from the treated eggs.

Stage / Rep.	1	2	3	4	Total mean	Control
% Larvae mortality.	80	69	71	66.5	71.63	0.0
% Pupa mortality.	89	78	83	70	80	0.0
% Adult mort.ality.	100	95	100	97.5	98.13	0.0

The effects of *M.anisopliae* on the development of the newly emerging larvae of *A.ipsilon* from the treated eggs:

Data in table (6) demonestrate the percentage of mortality during the life duration of larvae from the treated eggs. Data explain that 52.02% total mean of larvae, 73.25% total mean of pupae and 97.13% total mean of adults are failed to maintained and dead.

The results of both insects indicate that S *littoralis* is more susceptible to the entomopathogen, *Metarhizium anisopliae* than *A ipsilon* to the same fungus and demonstrate that biological control using the entomopathogenic fungus *M.anisopliae* against *S.littoralis* and *A.ipsilon* is possible.

Table (6):The effects of *M.anisopliae* on the development of the newly emerging larvae of *A.ipsilon* from the treated eggs.

Stage / Rep.	1	2	3	4	Total mean	Control
% Larvae mortality.	55	40.5	62.7	50	52.02	0.0
% Pupa mortality.	70	82	75	66	73.25	0.0
% Adult mortality.	100	93.5	100	95	97.13	0.0

REFERENCES

Aly,S.H. and A.M. Rashad (1997). Effect of *Metarhizium anisopliae* on two stages of *Earias insulana*. Analele institutuluide de cercetari pentru Protectia Plantelor vol. XXVIII. Partea alla. Bucuresti.

Ank Skrobek, V.K. (2001), Investigations on the effect of entomopathogenic fungi on whiteflies. Genehmigte Inaugural-Dissertation zur Erlangung der Doktorwurde der Agrarwissenschaften (Dr. agr).

Brousseau, C.; G. Charpentier and S. Belloncik (1998). Effects of Bacillus thuringienis and destruxins (Metarhizium anisopliae mycotoxins combinations on spruce budworm (Lepidoptera: Tortricidae). Journal of Invertebrate pathology 72 (3), 262-268.

Butt, T.M. and M.S. Goettel (2000). Bioassays of entomogenous fungi. In: Navon, A and K.R.S. Ascher (eds.): Bioassays of entomopathogenic microbes and nematodes. CAB International, Wallingford, UK, 141-195.

Ferron, P. (1981). Pest control by the fungi Beauveria and Metarhizium in microbial control of pests and plasnt diseases 1970-1980. Ed.M.D.Burges, Academic Press, PP 465-483.

Goettel, M.S.(1992). Fungal agents for biocontrol. In: Biological control of locusts and grasshoppers. Proceedings of a workshop held at the International Institute of Tropical Agriculture, 29.April-1. May 1991, Contonou, Republic of Benin, 122-132.

Goettel, M.S.; G.D. Inglis and S.P. Wraight (2000). Fungi in field manual of techniques in invertebrate pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests. (L.A.lacey and H.K.Kaya, eds.), PP. 255-282. Kluwer Academic Publishers, Dordrecht.

Gindin,G, Geschtovt,N.u.,Raccah B., & Barah I.(2000). Pathogenicity of Verticillium Lacanii to different developmental stages of the silverleaf whitefly, Bemisia argentifolii. Phytoparasitica 28:3, 2000.

Hassani, M., Zimmermann, G.& Vidal, S.(1998). Effect of different strains of entomopathogenic fungi against two cotton pests: Spodoptera littoralis and Helicoverpa armigera (Lepidoptera: Noctuidae). In: British Mycological Society (ed.): The future of fungi in the control of pests, weeds and diseases, International Symposium of the British Mycological Society, 5-9. April 1998, Southampton University, UK, 117.

Ignoffo,C.M.(1981). The fungus Nomuraea rileyi as a microbial insecticide. In microbial control of pests and diseases 1970-1980, PP. 513-538, Ed.H.D.Burges, Academic Press, London.

Kodaira, Y., (1961). Biochemical studies on the muscardine fungi in the silkworms, Bombyx mori. J.Fac. Text. Sci.and Tech., Shinshu University, 29.J.Fac. Text. Sci.and Tech., Shinshu University, 29. 1-68.

Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P. (2001). Insect pathogens as biological control agents: Do they have a future? Biological control 21 (3), 230-248.

Mueller-Koegler, E.(ed.) (1965). Pilzkrankheiten bei Insekten. Verlag Paul Parey, Hamburg, Berlin, Germany.

Rashad, A.M., & Aly, S.H., (1994). Effect of Metarhizium anisopliae on two stages of Pectinophora gossypiella Sci Bull. U.P.B. Scrics B.Vol 56-57. Nr. 1-4 1994-1995.

Roberts, D.W., (1966). Toxins from the entomogenous fungus M.anisopliae. II. Symptoms and detection in moribund hosts. J.Invertebr . Pathol., 8:222-227.

Rodrigue-Reudan, D.and J.Fargues, (1980). Pathogenicity of entomopathogenic Hyphomycetes Paecilomyces, Fumosoroseus and Nomuraearieyi to eggs Noctuids, Mamestra brassicae and Spodoptera littoralis. J. Invert. Pathol. 361980, PP. 339-408.

Stephan, D.,Weiling, M. and G. Zimmermann (1996). Locust control with Metarhizium flavoviride: formulation and application of blastospores. In Smits, P.H. (ed): IOBC wprs Bulletin, Proceedings of the metting of the working group "Insect parasites and insect pathogenic nematods, 27. Aug-1. Sept 1995, Poznan, Poland, 232-235.

Veen, K.H.(1968). Recherches sur la maladie, due a Metarhizium anisopliae chez le criquet pelerin. Mededelingen landbouwhogeschool, Wageningen 68 (1), 1-77.

Wallengren, H., and R. Johansson (1229). On the infection of pyrausta nubilalis Hb. By Metarhizium anisopliea (Metsch.) Sor. Sci. Repts. Intern. Corn Borer Invest., 2:131-145.

حساسية كل من حشرة دودة ورق القطن الكبرى والدودة القارضة السوداء للفطر الممرض للحشرات *Metarhizium anisopliae*

صفاء حسنين علي

معهد بحوث وقاية النبات- مركز البحوث الزراعية- مصر

أجريت تجارب معملية باستخدام الفطر الممرض للحشرات *M. anisopliae* على أطوار مختلفة لكل من حشرة دودة ورق القطن الكبرى والدودة القارضة السوداء باستخدام تركيزات مختلفة من الفطر، وقد أثبتت التجارب أن الفطر نجح في إصابة بيض ويرقات الحشرات المعاملة، ومعاملة البيض أدى إلى لانخفاض نسبة اليرقات الفاقسة لكل من الحشرتين ومعظم اليرقات الفاقسة من البيض المعامل فشلت في أن تكمل دورة حياتها وماتت في كل من الحشرتين. وسجلت يرقات وبيض الحشرات المعاملة حساسية عالية ، وكانت LC_{50} لبيض دودة ورق القطن الكبرى ١,٧ × ١٠ ^{٣} كونيديا/ ملل ولليرقات ، ٢,٥ × ١٠ ^{٣} كونيديا/ملل . كما سجل LC_{50} لبيض الدودة القارضة السوداء ٢,٥ × ١٠ ^{٣} كونيديا/ملل ولليرقات ٥,٥ × ١٠ ^{٣} كونيديا/ ملل . وقد أظهرت دودة ورق القطن الكبرى حساسية أعلى للفطر الدودة القارضة السوداء لجميع الأعمار وسجلت انخفاض أكبر في اليرقات والعذارى والحشرات الكاملة خلال حياة اليرقات الفاقسة من البيض المعامل. الهدف من هذه الدراسة هو تأكيد إمكانية استخدام الفطر *M.anisopliae* كعامل للمكافحة البيولوجية ضد كل من دودة ورق القطن الكبرى والدودة القارضة السوداء .