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EFFECTS OF THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* (METSCH.) AND GRANULOSIS VIRUS (GV)COMBINATIONS ON THE POTATO TUBER MOTH *Phthorimaea operculella* (ZELLER) (LEPIDOPTERA : GELECHIIDAE)

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ABSTRACT

The efficacy of the entomopathogenic fungus *Metarhizium anisopliae* and the granulosis virus (GV) on the potato tuber moth *Phthorimaea operculella* was investigated under the experimental conditions . Laboratory bioassays were performed to assess the susceptibility of *P. operculella* larvae to three fungal isolates . *P. operculella* showed high susceptibility to these fungal isolates. The present results indicated that the impact of a combination of *M. anisopliae* and GV on *P. opercuella* was variable, ranging from synergistic to antagonistic effects depending on the concentrations. The rate of infestation and damage by *P. operculella* were significantly decreased when the fungus at high concentration and GV at low concentration were applied together . This combination also affected the development of *P. operculella* . The present study suggests that the two agents can be synergitic and that a combination of both could be an efficient means of controlling *P. operculella* infestation and reducing the concentration of GV usually required for such control .

Key words *: combined effect , granulosis virus (GV), Metarhizium anisopliae , potato tuber moth.*

1. INTRODUCTION

The potato tuber moth *Phthorimaea operculella* (Zeller) is a major pest of potatoes in both field and stores. In Egypt 70% of seasonal potato production is kept in rustic, none - refrigerated stores (Nawallas) during the summer period. The Nawalla conditions are ideal for the development of large *P. operculella* populations . This, damage is particularly severe in these nawallas. Insecticides have been extensively used to reduce storage losses from *P. operculella*. The development of a high level resistance to most chemical insecticides and the negative impact on environment need to develop alternative means of control. The biocontrol element is much needed to control this pest. *Bacillus thuringiens*is is now registered and recommended as a control agent against *P. perculella* in Egypt. However, the granulosis virus, (GV) is one of the most promising agents for controlling *P. operculella* in Egypt. (Abol- Ela *et al.*, 1996). It is evident that the entomopathogenic fungi constitute one of the most important mortality factors among pest insects. The fungus *Metarhizium anisoplia* (Metsch.) is a common fungal pathogen of insects (Veen, 1968). It infects a wide range of insects including some lepidopteran species(Fargues *et al.*, 1976 and Sewify, and Sharaf 1994). This fungus could be mass produced on artificial nutrient media and sterile grains(Zimmermann, 1992). New formulations containing fungal spores in oil have largely eliminated dependence of the infection on humidity, so that a better, more reliable result can now be obtained in a short time (Bateman *et al.*, 1993).

The present work aims to investigate the possibility of using the entomopathogenic fungus *M. anisopliae* combined with entomovirus, GV to increase the efficacy of GV and reduce its required doses for *P. operculella* control and period of lethal infection and describes the interaction between the fungus *M. anisopliae* and entomovirus GV in a host *P. operculella*.

2. MATERIALS AND METHODS

2.1. Insect maintenance

Eggs and larvae of *P. operculella* were obtained from the " *Phthorimaea* mass rearing unit " at the Faculty of Agriculture, Cairo University (Entomovirology Lab.) ,Giza and kept at 25 ± 1°C , and 16 h light.

2.2. Fungal preparation and bioassay procedure

Three isolates of *Metarhizium anisopliae* Ahm_1, Ahm_2 and Ahm_3 used in the experiments were originally isolated from soil in Egypt by Sewify (1997). These fungal isolates were passaged twice through *P. operculella* 4th larval instar and then grown on autoclaved Potato Dextrose Agar medium (PDA). The inoculated agar medium (PDA) with fungal spores was incubated for 2 weeks at 27°C. Spores were harvested by rinsing with sterilized distilled water. Collected spores were filtered through cheese cloth to reduce clumping. Spores suspended in sterilized water were counted using a haemocytometer. To determine the lethal concentration (LC_{50}) of the three fungus isolates, five concentrations of each were used ($5x10^5$, 10^6 , $5x10^6$, 10^7 and $5x10^7$ spores/ml.). For each concentration, pieces of potato tuber cortex (10g) were immersed for 30 sec. in the spore suspension, and then transferred to small plastic vials. Each vial was infested with 20 *P. operculella* newly hatched larvae and kept at 25°C. A control of untreated larvae was inoculated in the test. Both untreated and treated larvae were daily observed and mortality percentages were assessed 8 days after treatment.

2. 3. Viral preparation

A granulosis virus isolated from *P. operculella* (Tunisian isolate PTMGV), provided by El- Bedewi (International Potato Center, Egypt) was multiplied in larvae reared under laboratory conditions and used in experiments. GV infected larvae of *P. operculella* were homogenized using the polytron on Ultra-Turrax in distilled water (Abol Ela *et al.*, 1994). After grinding, the undesired material was removed by filtering the homogenite through several layers of muslin (Tompkiens, 1991).

2. 4. Determination of the compatibility of GV with fungus *M. anisopliae*

To determine the interaction between GV and the fungus *M. anisopliae*, three experiments were carried out as follows :

Experiment 1 : Plastic boxes (17x 17 x 9 cm) covered with muslin were provided with a bottom 1cm layer of sand. Medium sized potato tubers free from *P. operculella* infestation were sprayed with a suspension of fungus alone (10^9 spores/ kg), a mixture of four viral concentrations 2,4,6, and 8 diseased larvae equivalents /kg. and fungal spores (10^9 spores / kg) for each and for GV alone (8 virus diseased larvae/ kg). Treated potatoes were transferred to plastic boxes (½ kg/ box), and then infested with 150 eggs of *P. operculella* (150 eggs/½kg./box).Four replicates were made for each treatment and all treatments and untreated control were held at 25 °C± 1 °C .

Experiment 2 : Potato tubers were treated with a mixture of four concentrations of the isolate Ahm2 at 5x 10^7 ,10^8 , 5x10^8 and 10^9 spores / kg and GV (4 virus diseased larvae / kg.) for each fungal concentration. The application was carried out as above .

Experiment 3 : The plastic cages (80 x 30 x 30cm) covered with muslin were prepared with a layer of sand. Potato tubers were sprayed with a mixture of fungal isolate Ahm_2, (5x10^9 spores/ kg) and GV (4 virus diseased larvae / kg).The tubers were introduced to two cages (4kg./cage), then infested with 500 eggs of *P. operculella* (500eggs/4kg /cage).Four replicates were carried out for every treatment; all treatments and control were held at 25°C ± 1 °C .

In all experiments the number of tubers infested with *P. operculella,* the number of tunnels / tuber,the number of holes / tuber and the pupated insects were scored 24 days after application.

3. RESULTS

3.1. The susceptibility of *P. operculella* (1st larval instar) to *M. anisopliae* isolates

Newly hatched larvae of *P. operculella* were highly susceptible to the three *M. anisopliae* isolates Ahm_1 , Ahm_2 and Ahm_3, with LC_{50} of 3.45 x 10^6, 8.37 x 10^5 and 4.13 x 10^6 spores / ml respectively . The bioassay results showed differences among the three fungal isolates : Ahm_3 was less pathogenic to the first larval instar compared with the

two other isolates . The data showed that isolates Ahm_2 caused high mortality in a shorter time than any other tested isolate (Table1).

Table (1) : LC_{50} (spores / ml) and LT_{50} for the first instar larvae of potato tuber moth *P . operculella* **with three** *M. anisopliae* **isolates .**

Fungal isolates	LC_{50}	95 % Fiducial limits	Slope	LT_{50} (days)
***M. anisopliae* Ahm 1**	3.45×10^6	1.97×10^6-4.45×10^6	-1.5	3.4
***M. anisopliae* Ahm 2**	8.37×10^5	5.09×10^5 1.39 - $\times 10^6$	-2.8	1.6
***M. anisopliae* Ahm 3**	4.13×10^6	3.08×10^6 - 5.58×10^6	-5.6	3.7

LT_{50} at concentration of 10^6 spores / Kg .

3.2.Effect of combination of fungus *M. anisopliae* **and GV on infestation and damage caused by** *P. operculella*

In the first experiment, three isolates of entomopathogenic fungus *M. anisopliae* were tested separately and in combination with different concentrations of GV. Each agent alone failed to cause a significant protection against *P. operculella* infestation . Among the three tested fungal isolates, Ahm_2 was more effective against *P. operculella* followed by the two other isolates. The obtained results showed that the combinations of the fungus at a concentration of 10^9 spores / kg. and GV at a concentration of 2 virus diseased larvae / kg. revealed significant reduction of *P. operculella* infestation than the other combinations. The combination of fungal isolate Ahm^2 (10^9/spores/kg.)and GV(2 virus diseased larvae/kg.) caused a maximum reduction of infestation with *P.operculella* that reached 31.5% (Table 2). The mean number of holes and tunnels caused as a result of *P. operculella* infestation followed the same trend.

The percentages of pupated larvae previously exposed to fungal isolates Ahm_1, Ahm_2 and Ahm_3 during larval stage were 38.7%, 23.7% and 60.7% pupae, respectively. However, the larvae exposed to GV alone or combined with the fungus failed to develop to pupal stage .

In the second experiment, combinations of the fungal isolate Ahm_2 at different concentrations and GV at 4 virus diseased larvae / kg. were tested. The obtained results (Table 3) indicated that the

Table (2) : Effect of various combinations of fungus *M. anisopliae* isolates [(a)] and GV on *P. operculella* infestation under laboratory conditions.

Treatment	Mean percentage of infestation	Mean No. of holes / tuber	Mean No. of tunnels/ tuber	% of pupated larvae
Untreated control	89.3a	7.2 a	2.8a	100%
GV alone (8 virus diseased larvae/kg.)	63.5 bcd	2.8bc	1.6 cde	8.6
Isolate Ahm_1 alone	62.3 bcd	3.8bc	1.8 bcde	38.7
Isolate Ahm_1 + 2 virus diseased larvae/kg	50.0 def	2.3bc	1.3 cde	0.0
Isolate Ahm_1 + 4 virus diseased larvae/kg	52.0 de	2.3bc	1.5 cde	0.0
Isolate Ahm_1 + 6 virus diseased larvae/kg	86.3a	3.4bc	1.4 cde	0.00
Isolate Ahm_1 + 8 virus diseased larvae/kg	86.3a	4.0 b	1.1 de	0.00
Isolate Ahm_2 alone	57.3bcd	3.3bc	1.9bc	23.7
Isolate Ahm_2 + 2 virus diseased larvae/kg	31.5 f	2.1 c	1.0 e	0.0
Isolate Ahm_2 + 4 virus diseased larvae/kg	35.8 ef	2.7 bc	1.7 bcde	0.0
Isolate Ahm_2 + 6 virus diseased larvae/kg	56.8 bcd	2.7 bc	1.7 cde	0.0
Isolate Ahm_2 + 8 virus diseased larvae/kg	54.8 cde	3.5 bc	1.8 bcd	0.0
Isolate Ahm_3 alone	57.8 bcd	3.4bc	2.5 ab	60.7
Isolate Ahm_3 + 2 virus diseased larvae/kg	63.5 bcd	3.8 bc	1.6 cde	8.6
Isolate Ahm_3 + 4 virus diseased larvae/kg	67.0 bcd	3.2 bc	1.8 bcde	2.7
Isolate Ahm_3 + 6 virus diseased larvae/kg	72.0 abc	3.0 bc	1.7 cde	4.1
Isolate Ahm_3 + 8 virus diseased larvae/kg	75.3 ab	3.4 bc	1.8 cde	2.5

(a)Fungus at the concentration of 10^9 spores / kg.

Means whithin columns followed by the same letter (s) are not significantly different ($p < 0.05$) by Duncan.

percentage of infestation with *P. operculella*, mean number of holes and tunnels varied significantly due to the increase of fungal concentration in the combinations. The combination of the fungus at a concentration of 10^9 spores/kg and GV at 4 virus diseased larvae led to a maximum reduction of infestation with *P. operculella* which reached 35.8 %.

3.3. Small scale trials

In the third experiment, the combination of the fungus isolate Ahm_2 (5x 10^9 spores/kg) and GV (4 virus diseased larvae / kg) successfully reduced *P. operculella* infestation and consequently reduced damaged tubers when compared with the control (Table 4). The percentage of infestation , holes / tuber and tunnel / tuber were reduced significantly compared with the untreated control. The results indicated that the combination of the fungus and GV affected the development of *P. operculella* .

4. DISCUSSION

A granulosis virus (GV) gave promising results in reducing storage infestation of the potato tuber moth (Ben Salah and Aalbu , 1992 , Das , *et al.*, 1992 , Doss *et al.*, 1994 and Abo Ela *et al* ., 1996) . This pathogen causes prolonged larval development (at low concentrations) and the infected larvae died 12 – 21 days after viral infection . However, the recent bioassay results revealed that the *P. operculella* first larval instar was highly susceptible to the fungus *M. anisopliae* and the LT_{50} ranged from 1.6 to 3.7 days .

The present study indicated that the impact of combination of *M. anisopliae* and GV on the *P. operculella* was variable ranging from synergism to antagonism, depending on the concentration of GV. The reduction of infestation and damages by *P. operculella* were significantly decreased when the two pathogens were applied together at low concentrations of GV. It was evident that the combinations of the fungus at high concentrations and GV at low concentrations induced a synergistic effect. However , an antagonistic effect occurred when the fungus and GV at high concentration of each were combined. Malakar *et al.*, (1999) reported that synergistic effects are not uncommon among

Table (3) :Effects of various combination of the fungus *M. anisopliae* isolate Ahm_2 and GV[a] on *P.* operculella infestation under experimental conditions .

Treatment	Mean percentage of infestation	Mean No. of holes/tuber	Mean No. of tunnels/tuber	% of pupated larvae
Untreated control	96.5a	6.4 a	4.6 a	100
M. anisopliae ($5x10^7$ spores/kg.) + GV	83.b	4.4 b	2.9 ab	13.6
M. anisopliae ($5x10^8$ spores/kg.) + GV	80.8 b	4.9 ab	3.9 a	4.8
M. anisopliae ($5x10^9$ spores/kg.) + GV	77.2 b	5.1 ab	3.0 ab	3.8
M. anisopliae ($5x10^9$ spores/kg.) + GV	35.8 c	2.6 c	1.9 b	0.0

a : GV at concentration of 4 virus diseased larvae/kg .
Means within columns followed by the same letter(s) are not significantly different ($p<0.05$) by Duncans .

Table (4) :Combined effect of the fungus *M. anisopliae* [(a)] isolate Ahm2 and GV [(b)] on *P.operculella* infestation under a small scale trial .

Treatment	Mean percentage of infestation	Mean No. of holes/tuber	Mean No. of tunnels/tuber	% of pupated larvae
Untreated control	69.7	3.0	2.0	100
Fungus +GV	16.9**	1.9**	0.5**	0

(a) Fungus at a concentration of $5x10^9$ spores/kg.
(b) GV at a concentration of 4 virus diseased larvae/kg.
** indicate highly significant differences at 1% level of probability.

insect pathogens and the interactions between two pathogens depend not only upon the time of inoculation but also on the dose of the pathogen(s) applied. For example, when *Melolontha melolontha* grubs were treated with *Beauveria bassiana* in peat soil , one month after exposure to *Entomoxvirus melolonthae*, Ferran and Hurpin (1974) observed a higher mortality among the grubs than when they were treated with these pathogens separately . The obtained results are important because they could allow a reduction in the amount of GV required for *P. operculella* control .

It seems, therefore, that GV under low concentrations may act as a stressing agent, increasing the susceptibility of *P. operculella* to the fungus, while GV at high concentrations inhibits the mycosis development.Other factors , such as depletion of essential resourses by one pathogen or changes in the chemical environment in the host during infection , may affect the outcome of the pathogen interaction and should be investigated . In the present study, it appears that in all combinations between the fungus and GV , the exposed *P. operculella* larvae failed to develop. This effect is due to viral infection . Ben Salah and Aalbu, (1992) mentioned that field application of the GV reduced the development of *P. operculella* in stored potato .

From the above results, it could be concluded that the combination of the fungus and GV (at low concentrations)could protect the potato tubers when applied in stores (Nawalla).

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التأثير المشترك لفطر المسكاردين الأخضر وفيروس الجرانيولوسز الممرضين للحشرات على فراشة درنات البطاطس

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ملخص

تناولت الدراسة تأثير استخدام خليط من فطر *Metarhizium anisopliae* والفيروس الحبيبى (GV) على يرقات فراشة درنات البطاطس تحت الظروف المعملية. وقد أظهرت الاختبارات الحيوية حساسية يرقات فراشة درنات البطاطس لثلاث عزلات من هذا الفطر . وأوضحت تجارب استخدام خليط من هذين العنصرين عند تركيزات مختلفة منهما أن هناك تأثيراً متبايناً يتراوح بين تأثير منشط توافقي عند استخدام الخليط في تركيز عالي من الفطر ، و تركيز منخفض من الفيروس وتأثير مثبط غير توافقي عند استخدام الخليط في تركيزات عالية من الفطر مع تركيزات عالية من الفيروس . وتوضح تجارب المعاملة بهذا الخليط التوافقي لدرنات بطاطس مصابة ببيض فراشة درنات البطاطس حدوث خفض معنوي في نسبة الإصابة باليرقات ، وفى كمية الضرر الحادث وعدم قدرة اليرقات على استكمال تطورها . وتبرز الدراسة الحالية إمكانية استخدام هذا الخليط في مكافحة تلك الآفة داخل النسوالات مع تقليل تركيزات الفيروس المستخدمة حالياً.

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