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The Potential of Native Palestinian *Nomuraea rileyi* Isolates in the Biocontrol of Corn Earworm *Helicoverpa (Heliothis) armigera*

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ABSTRACT

Four native isolates of the entomopathogenic fungus *Nomuraea rileyi* were isolated from soil samples collected from 210 different irrigated and non-irrigated tomato-growing fields in the West Bank- Palestine. The four isolates were recovered from soil samples collected from the Jenin area during August 2006 (Nr 3, Nr 5, Nr 17 and Nr 18), and were then deposited at the US Agriculture Research Service collection of Entomopathogenic Fungi (ARSEF 7794, ARSEF7793, ARSEF7792 and ARSEF 7791). The isolation was carried out by using dilute plate technique on modified selective medium. The medium used in the study was based on the standard Saboraud's maltose agar yeast extract, and modified by adding Tween® 80 and the fungicide dodine® (SMAYTD).

The effect of temperature on sporulation, spore germination and mycelial growth of the entomopathogenic fungus, *N. rileyi*, were studied *in vitro*. The fungus favored relatively moderate temperature (15–25°C) for germination, sporulation and mycelial growth. The growth of isolates was completely inhibited at 35°C. The virulence of *N. rileyi* native isolates against *Helicoverpa armigera* was investigated in terms of lethal time (LT_{50}) and lethal concentration (LC_{50}). The results showed that the LT_{50} for these isolates at the inoculum concentration of 10^8 spore ml^{-1} were in the range of 7.9 to 9.4 days. The LC_{50} was in the range of 10^5 and 10^6 spore ml^{-1}. The virulence of the most promising isolate (ARSEF 7794) compared to the insecticide Thyonex® was evaluated on *H. armigera* under tomato open field conditions; the results showed that the fungus and the insecticide reduced tomato infestation by 46%, and 88% respectively, compared to the control.

Keywords: *Helicoverpa armigera*, *Nomuraea rileyi*, Biological Control, Virulence, Entomopathogenic Fungi.

1. INTRODUCTION

The corn earworm, *Helicoverpa (Heliothis) armigera* (Hubner) is a polyphagous insect pest attacking many crops, and considered as economic pest in different geographical regions with worldwide distribution. The control of this pest depends mainly on the application of insecticides. However, *H. armigera* developed considerable resistance to many insecticides and become one of the most difficult insect pests to manage (Ranga and Shanover, 1999).

Nomuraea rileyi (Farlow) Samson is a deuteromycetous entomopathogenic fungus and recognized as the key mortality factor in several noctuid populations under certain environmental conditions. There are more than 30 species of lepidopterous insect pests listed as susceptible to *N. rileyi* (Ignoffo, 1981). Caterpillars belonging to the family Noctuidae are included among its most sensitive hosts. The fungus was found to be safe to human beings and other nontarget organisms including insect parasites and predators (Ignoffo, 1981). *N. rileyi*, caused 90.5–100% mortality in fourth-instar larvae of the corn earworm, *H. armigera*, when applied at 10^7 spore/ ml to corn silks, leaves of soybean, tomato and chrysanthemum; the LT_{50} was 5.9–6.7 days (Li-chang and Roger, 1998). The fifth-instar larvae of *H. armigera* showed a mortality of 94.6% on soil with 20% water content when the soil surface was sprayed with 10^8 spore ml^{-1} suspension of *N. rileyi* (Li-chang and Roger, 1998). It was further found that injection or spraying of (10^5-10^7) spore ml^{-1} suspension into corn ears was effective in controlling larvae of corn earworm resulting in 75.9%-85.8% marketable ears, and

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was not statistically different from carbofuran treatment (Li-chang and Roger, 1998).

In India, *S. litura* larvae exhibited >80% mortality, 19 days after spraying of castor plants with aqueous suspension of *N. rileyi* (Vimala, 1994). In Taiwan, spraying of corn plants with aqueous suspensions of the fungus significantly reduced *H. armigera* larvae, resulting in significant increase of marketable corn ears (Tang and Hou, 1998). In Ecuador's humid costal plain, application of aqueous sprays of *N. rileyi* against *Anticarsia gemmatalis* and *Pseudoplusia includens* in soybean revealed limited increase in larval mortality (Stansly *et al*., 1990). It has been reported however, that eggs, pupae and adults of *Spodoptera littoralis* were not infected by *N. rileyi* (Boucias *et al*., 1984), but larvae emerging from contaminated eggs became infected by the fungus (Fargues, 1984). Commercial products of *N. rileyi* (Ago-Biocontrol *Nomuraea* 50) were registered for control of Lepidoptera in ornamentals and vegetables in Columbia (Shah and Goettel, 1999).

Virulence and persistence of entomopathogenic fungi is always affected by environmental factors such as temperature, humidity and light (Ignoffo *et al*., 1985, Hajeck *et al*., 1990 and Vimala *et al*., 2003). High temperature, low RH and solar radiation are known to be major factors limiting development and field persistence of entomopathogenic fungi in nature (Uziel and Shtienberg, 1993). Inspite of these facts, *N. rileyi* was isolated from North Jordan Valley during summer months, under relatively hot conditions (Kenneth and Olmert, 1975). It has been found that the optimal temperature for infection of *N. rileyi* was 20ºC which resulted in 95% host larval mortality and LT_{50} of 5.8 days. Temperature below 26ºC decreased the development rate of the infection process; lower or higher temperatures than 26 ºC caused lesser mortality and recorded higher LT_{50} values (Boucias *et al*., 1984). Spore germination rates were best at 20 and 25 ºC (Tang and Hou, 2001). Spore survival may be reduced under field conditions after exposure to solar radiation (Carruthers *et al*., 1988). High humidity is essential for spores germination and penetration into hemocoel after attachment to cuticle (Hajek *et al*., 1990). In addition, spore survival and viability are greatly influenced by humidity (Daoust and Roberts, 1983; Ignoffo *et al*., 1985).

Entomopathogenic fungi can be isolated indirectly from the soil by live baiting with insects such as larvae of *Galleria* spp (Zimmermann, 1986), and/ or directly by extraction using a dilute plate technique, in conjunction with selective medium (Beilharz *et al*., 1982). The standard growth medium used to day for multiplication of *N. rileyi* is Saboraud's Maltose Agar Yeast extract medium (SMAY) (Bell *et al*., 1982). SMAY has been further modified by fungicides amendment for isolation of *N. rileyi* from soil (Barakat and Iqteit, unpublished data).

The objective of this study was to assess the potential of four native isolates of *N. rileyi* against *H. armigera* under laboratory and field conditions.

2. MATERIALS AND METHODS

2.1. Helicoverpa (Heliothis) armigera Culture

H. armigera cultures were established and the neonate larvae were fed individually on an artificial diet as described by (Vimala Devi, 1994). Two hundred fifty grams of beans (*Phaseolus vulgaris*) were soaked overnight in 1 liter of deionized water. The soaked beans were placed in biosafety bags, and autoclaved. The following ingredients were then added to the autoclaved mixture: dried alfalfa meal, 102 g; nipagin (Clariant Division functional chemicals, Germany), 8.1g; sorbic acid (ICN Biomedicals Inc., USA), 205 g; yeast extract (Biolife Italiana S.R.I., Italy), 81 g; ascorbic acid (Pacific Ring chemical Co., UK), 8 g; chloramphenicol (Sigma, USA), 1 g and 10 ml of formaldehyde in 40 ml water. Autoclaved fourty grams agar in 1 liter water were added manually to the previous mixture to reach the volume of 2.5 liters. The mixture was then poured into plastic container, and covered with aluminum foil. The diet was kept in refrigerator and cut into pieces for larvae feeding when needed.

H. armigera adults after emergence were fed on 10% sucrose solution in saturated cottons. Within three to five days, adults started laying eggs on cotton placed inside the plastic container. Cotton with insect eggs were transferred to another plastic container containing the artificial diet prepared earlier for feeding larvae. Eggs hatched within nearly five days at 25 ºC. The neonate larvae were fed on artificial diet provided and until they were separated to feed individually later on. When larvae reached 5^{th} stage and after fifth molt, they were transferred to wood bran for pupation. After pupation, the pupae were placed in Petri dishes inside plastic containers covered with glass board for adult emergence. All insects' life stages were incubated at 25 ±1ºC. Healthy larvae of

the desired instars were used for various experiments.

2.2. Nomuraea rileyi Isolation from Soil

Composite soil samples were collected from 218 different irrigated and non irrigated tomato growing fields in the West Bank (WB) during August 2006. Samples were collected at the depth of 2 -15 cm from the rhizosphere of tomato plants. Samples were collected from tomato fields which showed damage due to *H. armigera* from various areas in the WB: 120 from Hebron area, 28 Jericho, 40 Jenin, 10 Tulkarm, and 20 samples from Bethlehem.

The Saboraud's maltose agar yeast extract medium (SMAYTD), amended with Tween® 80 (Sigma, Germany) and the fungicide dodine® (Efal chemical industries Ltd.) modified for the isolation of the fungus from soil contained (g l^{-1}): Maltose (Sigma, USA), 40; Peptone (Difco, USA), 10; Yeast extract (Biolife, Italy), 10; Agar (Difco, USA), 20; chloramphenicol (Sigma, USA), 0.25; Tween 80, 1 ml and Dodine, 90 mg. Five grams of each collected soil sample were suspended in 45ml 0.1% Tween 80 solution and shaked for 30 minutes. Two soil dilutions (10^{-2} and 10^{-3}) were prepared using 0.1% Tween 80. From each dilution, 300 µl was seeded on SMAYTD medium Petri plates with four replicates for each dilution. Plates were then incubated at 25ºC under dark conditions for three days and then transferred to full light. Plates were inspected daily for *N. rileyi* growth. The isolates were sub-cultured on SMAY medium, and later identified according to Kisk *et al*. (1974) and Tzean *et al*. (1993).

2.3. Effect of Temperature and Light on Growth Rate, Spore Germination, and Sporulation of N. rileyi

To evaluate the effect of temperature and light on the mycelial growth rate, seven plates of SMAY medium were inoculated with single spores of each isolate taken from 21- day old cultures. Four plates were incubated under light conditions and three incubated under dark conditions and incubated at 15, 20, 25, 30 and 35 ºC. Colony diameters were measured at 7 and 9 days from inoculation. The mycelial growth rate was calculated by the following formula:

Rate = $(D_2 - D_1)/ (T_2 - T_1)$ were D_2= the second reading of the growth diameter, D_1= the first reading of the growth diameter, T_2= the time of the second reading, and T_1= the time of the first reading.

The experimental design was completely randomized with four replicates (plates) under light conditions and three replicates under dark conditions for each temperature and isolate, and the experiment was repeated.

To evaluate the effect of temperature and light on spore's germination, spores were harvested in distilled water from 30-days old cultures of the isolates. Spores suspension concentrations were calibrated to 660 spores ml^{-1}. For each isolate, 500 µl spore suspension were seeded on seven SMAY medium plates; the plates were then incubated at 15, 20, 25, 30 and 35 ºC. The experimental design was completely randomized with four replicates under light conditions and three replicates under dark conditions for each temperature and isolate, and the experiment was repeated. Numbers of growing colonies were counted at six and ten days after inoculation.

For measuring spore's production, the same preparation procedure used in the spore's germination experiment was used. Mycelial disks (0.196 cm^2) were then taken from each plate (replicate) after 17 days and homogenized in 10 ml of 95% ethanol. Haemocytometer was used to determine spore numbers per unit area of each plate. The experimental design was completely randomized with four replicates per treatment under light conditions and three replicates were under dark conditions.

2.4. Virulence of N. rileyi Isolates

Virulence of *N. rileyi* isolates was assessed by inoculating 4th stage larval instars of *H. armigera*; larvae were dipped in 10^7 spore ml^{-1} distilled water harvested from 21-days old cultures of the four isolates. The larvae were then fed on artificial diet inoculated with the same spore suspension; control treatment involved using only distilled water. The treated larvae were then incubated at 25ºC under natural light, and 100 % R.H. Larval mortalities were recorded at 10, 12, 13, and 14 days after inoculation. The experimental design was completely randomized with nine replicates.

In another experiment to test virulence, seventeen-day-old cultures of *N. rileyi* isolates were harvested in sterile distilled water; the spore concentration was determined using a haemocytometer. The spore concentration was calibrated to reach the dilutions of 10^5, 10^6, 10^7 and 10^8 spore ml^{-1}. Fourth larval instars of *H. armigera* were fed on tomato leaves dipped in the prepared spore suspensions for 48 h before they were fed on regular artificial diet. Larvae of the control treatment

were fed on tomato leaves dipped in sterile distilled water. The experimental design was completely randomized with nine replicates for each treatment. The treated larvae were then incubated at 25ºC under natural light, and 100 % R.H. Larval mortalities were recorded daily for thirteen days.

2.5. Field Experiment

The field experiment was conducted at Al-Aroub Agriculture Experimental Station (10 Km north of the city of Hebron - Palestine) during summer 2007. Completely randomized block design was used, with four replicates; each block had three plots (1.5 X 3 m) and each with two rows and four tomato plants per row. The plants were drip irrigated, feritigated and after ten weeks, experimental treatments were applied. Plants were treated with spore suspension of *N. rileyi* isolate (ARSEF 7794), with the insecticide Thyonex® (Makhteshim chemical works Ltd.) (Endosulfan; hexachloro-hexahydro-methano-benzodioxathiepin-oxide) and the last treatment with water agar suspension (CK). For the first treatment, spores of *N. rileyi* native isolate (ARSEF 7794) were harvested from 25- day- old cultures, and suspended in 0.1 % agar solution at the concentration of 10^8 spore ml^{-1}. Ten weeks- old- tomato plants were then sprayed with the spore suspension prepared earlier (60 ml for each tomato plant). For the second treatment, plants were sprayed with 60 ml per plant of the insecticide Thyonex® at the concentration (700 mg ml^{-1} a.i). Control plants were sprayed with 60 ml of 0.1% water agar suspension per plant. Each plant incorporated in the experiment was artificially infested earlier by three first and second stage larvae of *H. armigera*. Treated plants were covered with agriculture muslin under tunnel cultivation with 50 cm height. The percentage of infested tomato fruits were recorded after eight days.

2.6. Statistical Analysis

Data of the fungal growth parameters, fungal virulence and the field experiment data were statistically analyzed using one way analysis of variance (ANOVA), and the Fisher LSD test ($P \leq 0.05$) was used for mean separations (Sigma Stat® 2.0 program, SPSS Inc., USA).

3. RESULTS

3.1. *N. rileyi* Isolation from Soil

Four native isolates of *N. rileyi* were recovered from soil samples collected from Jenin area during August 2006 (Nr 3, Nr 5, Nr 17 and Nr 18) and classified and deposited at the ARSEF (US Agriculture Research Service collection of Entomopathogenic Fungi) under the accession numbers (ARSEF 7791, ARSEF 7792, ARSEF 7793 and ARSEF 7794), respectively.

3.2. Effect of Temperature and Light on Growth Parameters of *N. rileyi*

All *N. rileyi* isolates were able to grow at temperatures between 15- 30ºC (Table 1). The optimum temperature for mycelial growth of most isolates were in the range of 20- 25ºC with no significant differences between light and dark treatments. However, mycelial growth of all isolates was completely inhibited at 35 ºC. Germination percentages of *N. rileyi* isolates followed the same trend of mycelial growth behavior. Spores of all isolates were able to germinate at the temperature range of 15-30 ºC (Table 2). The optimum temperature for conidial germination of most isolates was in the range of 20-25ºC with slight variations and no significant differences between the light and dark treatments. No germination, however, took place at 35 ºC. The optimum range for sporulation, however, was from 15 to 25 ºC with low sporulation rates at 30 ºC and spores production totally stopped at 35 ºC with no obvious significant differences between the light and dark treatments. The highest sporulation rate ($6.8x10^7$ spores cm^{-2}) was recorded by *N. rileyi* isolate ARSEF 7791 (Table 3).

3.2. Effectiveness of *N. rileyi* Isolates in controlling *H. armigera*

Experiment I

In this experiment, all *N. rileyi* isolates were able to induce significant mortalities among *H. armigera* 4th stage larvae when dipped in fungal spore suspension and fed on artificial media inoculated with the same spore suspensions. Mortality percentages recorded by the isolates ARSEF 7794, ARSEF 7793, ARSEF 7792 and ARSEF 7791 were 83.3%, 25%, 42%, and 62.5%, respectively, compared with the control (Fig. 1).

Experiment II

The 4th stage larvae of *H. armigera* were fed on tomato leaves dipped in different concentrations of *N. rileyi* spore suspensions and showed various mortality levels. Mortality of 4th stage larvae started at day 6 when *N. rileyi* inoculum concentrations were 10^7 and 10^8 spores

ml^{-1}; mortality percentages ranged from 10% to 100% depending on inoculum concentration and the fungal isolate (Fig. 2and 3). Lethal time required to kill 50% of larval population (LT_{50}) decreased as the concentration of fungal inoculum increased (Table 4), with minimum LT_{50} of 7.8 days at the high concentration (10^8 spore ml^{-1}), and more than 13 days at the low inoculum concentration (10^5 spore ml^{-1}) (Table 4).

Maximum mortality (100%) was observed after 10 days from inoculation of tomato leaves with 10^8 spore ml^{-1} of ARSEF 7794 and ARSEF 7791, while 100% mortality required 11 days for the isolates ARSEF 7793 and 7792 at the same inoculum concentration (Fig. 2 and 3).

The lethal inoculum concentration required to kill 50% of tested *H. armigera* larvae (LC_{50}) was measured from regression equations of the curves in (Fig. 3). LC_{50} was between 10^5 and 10^6 spore ml^{-1} for the isolates ARSEF 7794, ARSEF 7793 and ARSEF 7791 while was more than 10^6 for the isolate ARSEF 7792. LC_{100} was achieved at the inoculum concentration of 10^7 spore ml^{-1} of the isolate ARSEF 7794, while achieved at the inoculum concentration of 10^8 spore ml^{-1} of the other three isolates.

3.3. Field Experiment

This experiment was conducted to test the virulence of *N. rileyi* isolate ARSEF 7794 in controlling *H. armigera* under field conditions in comparison with the insecticide Thyonex®. Data showed that the application of *N. rileyi* isolate (ARSEF 7794) resulted in 46 % reduction in tomato fruit infestation compared to the control, while Thyonex resulted in 88% reduction in tomato fruit infestation compared to the control. Both treatments were significantly different from the control treatment (Table 5).

4. DISCUSSION

Infection, sporulation, spore germination and growth rate of entomopathogenic fungi are influenced by environmental factors, especially temperature and humidity (Tanada and Kaya, 1993). In addition, spore survival and persistence are dramatically influenced by temperature (Hajek *et al*., 1990). In this study, it was found that the isolates of *N. rileyi* collected from the Northern Jenin area favored by relatively moderate temperatures (15- 25ºC) for germination, sporulation and mycelial growth. Growth, sporulation and germination of these fungi were completely inhibited at 35 ºC. Similarly, Tang and Hou (2001) found that the spore germination of *N. rileyi* was optimum at 20 and 25 ºC when tested on SMAY. Furthermore, Mohamed *et al*. (1977) found that the infection of *N. rileyi* was best at 20- 25ºC, when tested against *Heliothis zea*. In the same context, Tang and Hou, (2001) showed that *N. rileyi* favored moderate temperature particularly at 20 ºC for spore germination and infection. The high temperature of 35ºC was unsuitable for spore germination of *N. rileyi* (Boucias and Pendland, 1984). Accordingly, it seems that the optimum temperature for *Nomuraea* spp is an isolate dependent. The same conclusion was documented by (Boucias *et al*., 1984).

Virulence against the target insect pest is the most important criterion for commercial exploitation of fungal pathogens (Vimala *et al*., 2003). Several parameters for virulence of entomopathogenic fungi have been documented (Butt, and Goettel, 2000); of these are mortality percentage, LT_{50}, and LC_{50}. In this study, the isolates ARSEF 7794 and ARSEF 7791 were capable of causing significant larval mortalities (78 % and 89 %) of *H. armigera* after 9 days, whereas the isolates ARSEF 7793 and ARSEF 7792 caused mortality percentages of 75 % and 78 % after 10 days, respectively. The LT_{50} for all isolates were more than 13 days at the spore concentration of 10^5 spore ml^{-1} and was reduced to be in the range of 7.8 - 9.4 days at the spore concentration of 10^8 spore ml^{-1}. These variations in LT_{50} values within isolates could be due to genetic variations between different isolates.

In addition; it was reported that the difference in larval mortality percentages and LT_{50} due to *N. rileyi* infection, depends on environmental factors especially temperature and humidity (Tanada and Kaya, 1993). Geographical isolates of entomopathogenic fungi vary in their virulence to target insects (Ignoffo *et al*., 1985). Tang and Hou (2001) found that the infection of *H. armigera* by *N. rileyi* resulted in approximately 95 % mortality of 4^{th} stage larvae, and LT_{50} of only 5.8 days, at the inoculum concentration of 5 x 10^6. They further found that *N. rileyi* infection resulted in 94.3% mortality of 3^{rd} instars and LT_{50} of 4.59 days, at the inoculum concentration of 5 x 10^8 spore ml^{-1}. However, 100% 4^{th} larval instars mortality with LT_{50} of 7.2 days occurred at the incubation temperature of 20ºC, and LT_{50} of 6.8 days at the incubation temperature of 25ºC.

In this study, and in the same direction, the lethal concentrations (LC_{50}), for *N. rileyi* isolates were between 10^5 and 10^6 spore ml^{-1} for the isolates ARSEF 7794, ARSEF 7793 and ARSEF 7791, while was slightly more than 10^6 spore ml^{-1} for the isolate ARSEF 7792. However, the LC_{100}, were between, 10^7 and 10^8 spore ml^{-1}. In this respect, it has been indicated that the LC_{50} of *N. rileyi*, tested against 2nd instars of *S. litura* populations ranged between $2.23x10^8$ and $2.2x10^7$ spore ml^{-1} (Vimala, 1994).

In conclusion, the recommended dose could range between 10^7 and 10^8 spore ml^{-1} of *N. rileyi* against *H. armigera*. This variation in lethal concentrations could be due to isolates genetic variation in virulence, and spore germination, in addition to other factors such as efficacy in laboratory bioassays (El- Sayed *et al*., 1989).

Concerning the use of *N. rileyi* isolate in the field, this study showed promising results in which tomato fruits infestation was reduced by 46% compared to the control, but in no mean close to the reduction in fruit infestation due to the application of the insecticide Thyonex®. This however may be important in an integrated control approach for management of the corn earn worm *H. armigera* where the introduction of entomopathogenic fungi can always be assisted by a reduced dose of the selective insecticide. This is obviously an important tool towards the required reduction in the levels of insecticides used under modern intensive agriculture for the benefit of the environment and the consumer's safety.

Table 1. Effect of temperature on mycelial growth of *N. rileyi* incubated under light and dark conditions

Temp.(°C)	Growth rate (mm day^{-1})							
	ARSEF 7794		ARSEF 7793		ARSEF 7792		ARSEF 7791	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
15	0.63* bc	1.25 ab	1.13 bc	2.33 ab	1.38 a	1.38 a	0.38 c	0.5 ce
20	2 a	1.38 ab	1.3 bc	2.88 ab	2.33 a	2.17 a	3.33 a	2.5 ad
25	1.88 a	1.38 ab	2.75 ab	3.63 a	2.33 a	1.5 a	2 bd	1.25 be
30	1.38 ab	2.25 a	1.75 bc	0.25 c	0.25 b	0.13 b	0.5 ce	0.25 c
35	0 c	0 c	0 c	0 c	0 b	0 b	0 c	0 c

* Means followed by the same letter within the same column are not significantly different according to Fisher LSD method ($p \leq 0.05$).

Table 2. Effect of temperature on spore's germination of *N. rileyi* grown on SMAY media incubated under light and dark conditions

Temperature (°C)	% Germination							
	ARSEF 7794		ARSEF 7793		ARSEF 7792		ARSEF 7791	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
15	40 b	64 a	65 b	90 a	58 b	84 a	63 b	85 a
20	60 a	63 a	69 b	66 b	76 ab	84 a	84 ab	82 ab
25	63 a	67 a	70 ab	67 b	98 a	95 a	86 a	88 a
30	15 c	69 a	37 d	65 b	60 b	88 a	53 b	89 a
35	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c

* Means followed by the same letter within the same column are not significantly different according to Fisher LSD method with $p \leq 0.05$.

Table 3. Effect of temperature on sporulation of *N. rileyi* grown on SMAY media and incubated under Light and dark conditions

Temperature (°C)	Sporulation (10^6 spore cm^{-2})							
	ARSEF 7794		ARSEF 7793		ARSEF 7792		ARSEF 7791	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
15	58 a	50 a	4 bc	37 a	16 b	42 a	48 ab	45 ab
20	50 a	56 a	6 bc	6 bc	13 b	22 ab	68 a	51 ab
25	50 a	41 a	12 b	5 bc	4 b	20 ab	60 a	56 a
30	5 b	12 b	1 c	3 bc	2 b	4 b	20 bc	8 c
35	0 b	0 b	0 c	0 c	0 b	0 b	0 c	0 c

* Means followed by the same letter within the same column are not significantly different according to Fisher LSD method with $p \leq 0.05$.

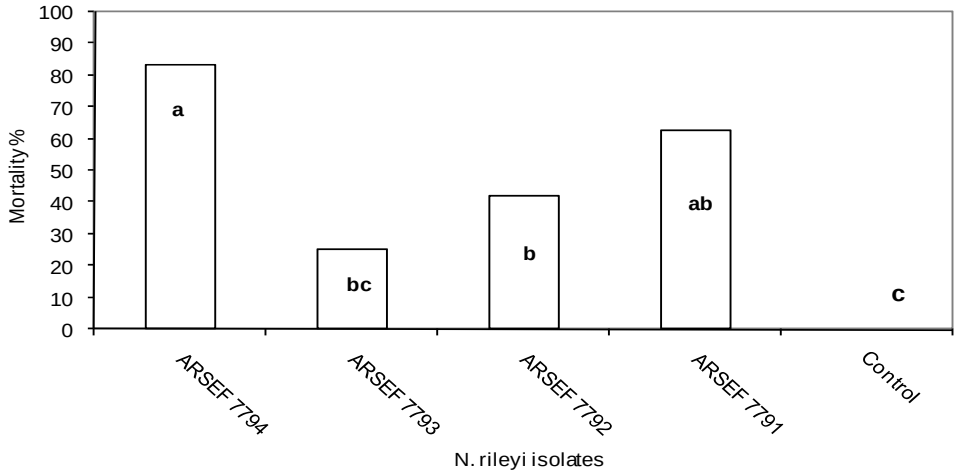
Table 4. The LT_{50} of 4th instar larvae of *H. armigera* at different inoculum (spore) concentrations of *N. rileyi*

Inoculum concentration (spore ml^{-1})	LT_{50} (days)			
	ARSEF 7794	ARSEF 7793	ARSEF 7792	ARSEF 7791
0 (Control)	-	-	-	-
10^5	-	-	-	-
10^6	10.2	11	-	10.9
10^7	7.8	9.9	10.7	9.7
10^8	8.3	9.4	8.9	7.9

Table 5. The effect of *N. rileyi* (ARSEF 7794) and the insecticide (Thyonex) on percentage of tomato fruits infestation by *H. armigera* under field conditions

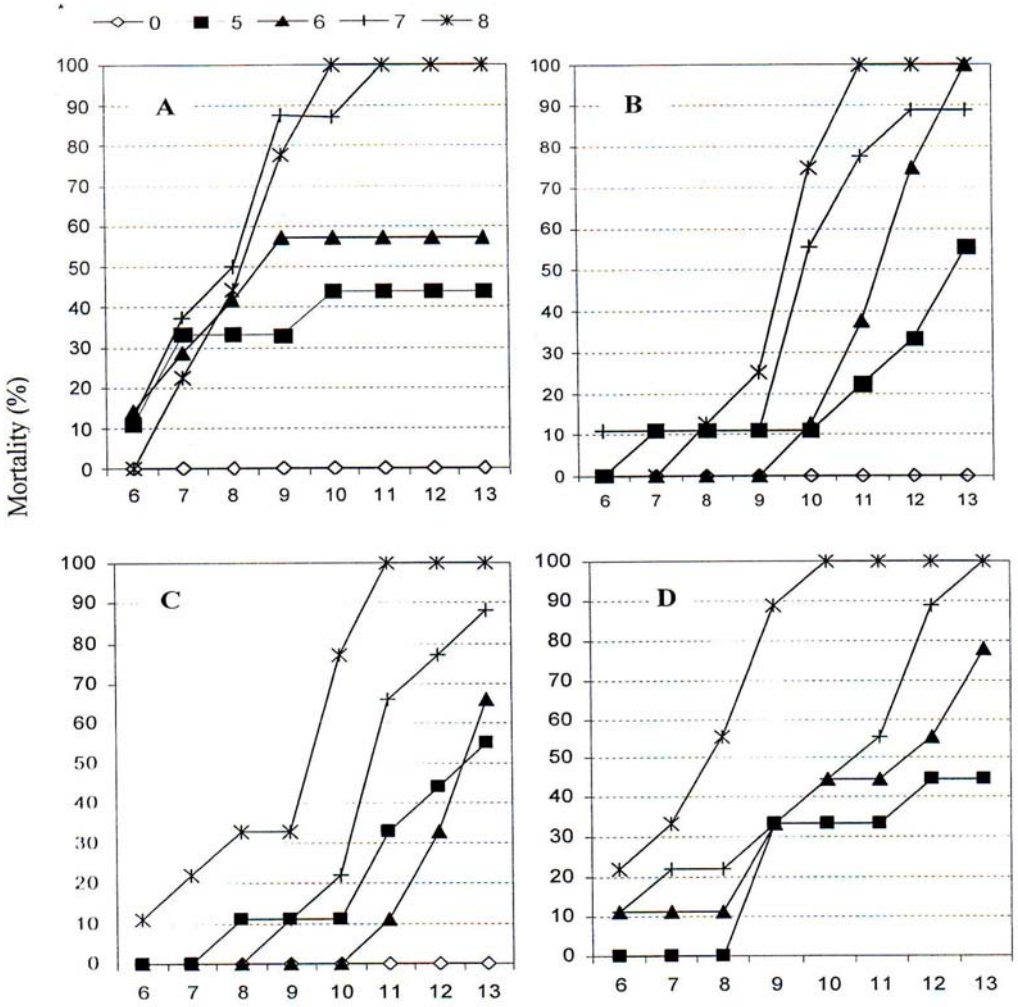
Treatments	Fruits infestation (%)
Control	100 a **
Thyonex	12 c
N. rileyi	54 b

**Means followed by the same letters are not significantly different by fisher LSD test with $p \leq 0.05$.



* Columns labeled by the same letter are not significantly different according to Fisher LSD method, with $p \leq 0.05$.

Fig. 1. Mortality % of 4th stage larvae of *H. armigera* inoculated with spore suspension of four native isolates of *N. rileyi.*



* legend presented the logarithm of spore concentrations; log 8 (--x--), log7 (--+--), log 6 (--▲--), log 5 (--■--), Control (--◊--)

Fig. 2. The mortality (%) of 4th stage larvae of *H. armigera* caused by native isolates of *N. rileyi* (A: ARSEF 7794, B: ARSEF 7793. C: ARSEF 7792, D: ARSEF 7791) at various inoculum concentration and incubation periods.

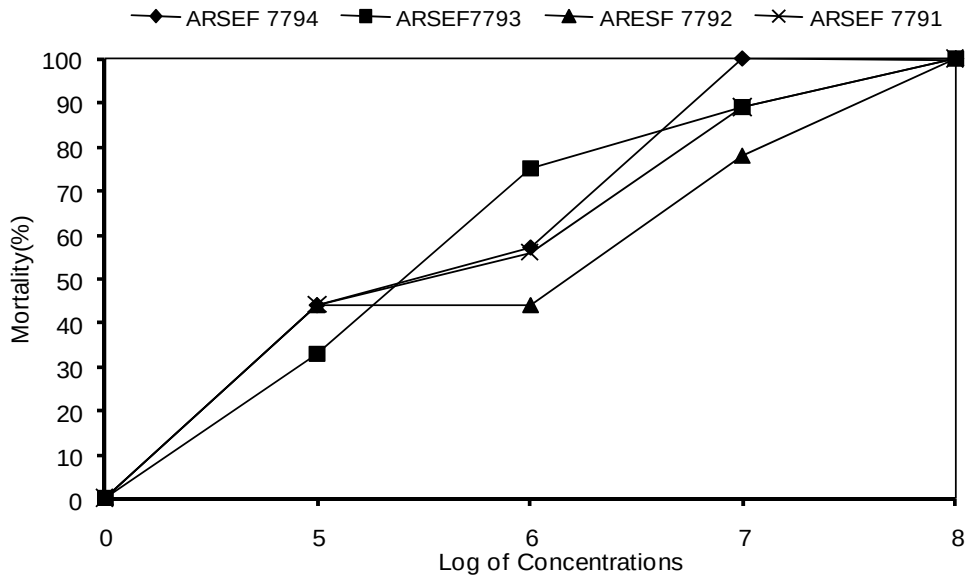


Fig. 3. Mortality percentages of 4th stage larvae of *H. armigera* induced by *N. rileyi* isolates using various inoculum concentrations.

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كفاءة عزلات محلية فلسطينية من الفطر *Nomuraea rileyi* في المكافحة الحيوية لعثة ثمار البندورة *Helicoverpa (Heliothis) armigera*

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ملخـص

هدفت الدراسة إلى استخدام عزلات محلية من الفطر الممرض للحشرات *Nomuraea rileyi* في المكافحة الحيوية لعثة ثمار البندورة *Helicoverpa* (*Heliothis*) *armigera*. وتم الحصول على أربع عزلات من الفطر المذكور بعد جمع 210 عينات من مناطق زراعة البندورة المروية والبعلية في الضفة الغربية- فلسطين خلال صيف 2006 باستخدام طريقة Dilute plate technique على بيئة غذائية خاصة SMAYTD، بعد تعديل البيئة المعروفة SMAY بإضافة المبيد الفطري دودين والمادة الناشرة توين 80. ألعزلات الأربع (Nr3, Nr5, Nr17, Nr18) تم جمعها من منطقة جنين شمال الضفة الغربية، وبعد تنقيتها وتصنيفها، تم إيداعها في المركز الأمريكي لجمع الفطريات الممرضة للحشرات المعروف باسم (ARSEF)، تحت أرقام الإيداع:

(ARSEF 7794, ARSEF7793, ARSEF7792 and ARSEF 7791)

أظهرت النتائج أثر درجات الحرارة المختلفة على معدل النمو، وإنبات وإنتاج الأبواغ؛ إلا إن الفطر ينمو بشكل جيد عند 15-25°م، ويتوقف عن النمو كلياً عند 35°م. هذا وأظهرت النتائج أيضاً ان للعزلات مقدرة عالية في المكافحة الحيوية ليرقات عثة ثمار البندورة وزيادة نسبة الموت معنوياً مقارنة مع الشاهد، وان الزمن اللازم لقتل 50% من اليرقات (LT_{50}) كان 7.9-9.4 يوم عند استخدام محلول الابواغ بتركيز 10^8 بوغ/ مللتر، وان تركيز الابواغ اللازم لقتل 50% من اليرقات (LC_{50}) تراوح ما بين 10^5- 10^6 بوغ/ مللتر، وهناك تفاوت معنوي بين العزلات. وكانت عزلة ARSEF 7794 الأكثر فاعلية، وعند مقارنة هذه العزلة مع المبيد الحشري Thyonex® في مكافحة الحشرة تحت ظروف الحقل، بينت النتائج أن المبيد قلل من الثمار المصابة بنسبة 88% بينما قللها الفطر بنسبة 46% مقارنة مع الشاهد.

الكلمـات الدالـة: عثة ثمار البندورة، فطر النومريا، المكافحة الحيوية، كفاءة، الفطريات الممرضة للحشرات.

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