

DIFFERENTIAL EFFICACIES OF *NOMURAEA RILEYI* AND *ISARIA FUMOSOROSEA* ON SOME SERIOUS PESTS AND THE PESTS' EFFICIENT PREDATOR PREVAILING IN TOMATO FIELDS IN EGYPT

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Abstract: The efficiency of the two microbial control agents *Nomuraea rileyi* and *Isaria fumosorosea*, were evaluated against *Bemisia tabaci* and *Myzus persicae* pests in tomato cultivations. The safety levels of the agents, to the predator *Coccinella undecimpunctata*, were also studied under laboratory and field conditions. Results showed that under laboratory conditions, LC_{50} values for *N. rileyi* and *I. fumosorosea* were 103.7×10^4 and 139.4×10^4 spores/ml against *B. tabaci*, respectively, while the corresponding values for *M. persicae* were 89.1×10^4 and 149.8×10^4 spores/ml, respectively. Under the field conditions, the percentages of infested plants with *B. tabaci* and *M. persicae* were significantly decreased after treatments with both fungi as compared with the corresponding controls. At the El-Esraa farm (Nobaria region), the weights of the tomato yield were 2,417 and 2,911 kg/feddan when *I. fumosorosea* and *N. rileyi* were applied respectively, as compared with 2,010 kg/feddan in the corresponding controls. The corresponding yields in El-Kassaseen were 2,699 and 2,999 kg/feddan, respectively, as compared to 1,990 kg/feddan in the control. The present study showed that *C. undecimpunctata* exhibit relatively high and reasonable resistance to *N. rileyi* and *I. fumosorosea* at the highest lethal concentration (1×10^8 spores/ml) for both tested preys.

Key words: *Bemisia tabaci*, *Coccinella undecimpunctata*, *Isaria fumosorosea*, microbial control, *Myzus persicae*, *Nomuraea rileyi*

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most important Solanaceous vegetable crops in Egypt. The tomato plants are currently infested with many serious pests. The most destructive pests are the green peach aphid, *Myzus persicae*, and whitefly, *Bemisia tabaci*. They transmit several viruses to the plant, causing great damage and disease to leaves and fruits (Namba and Sylvester 1981; Berry 1998; Filotos *et al.* 2004).

Integrated Pest Management programmes which include chemical insecticides, pollute the environment, reduce beneficial insects, develop insecticidal resistance in the major associated pests and consequently, cause inevitable outbreak (Lowery and Sears 1981).

Recently, many research studies have advocated the use of entomopathogenic fungi as a biotic alternate. Contrary to the other specific microbial insecticides, entomopathogenic fungi have successfully controlled a wide range of insect pests (Sabbour and Shadia Abd El-Aziz 2002; Sabbour and Sahab 2005, 2007; Thungrabeab and Tongma 2007; Sahab and Sabbour 2011). The two microbial control agents, *Nomuraea rileyi* and *Isaria fumosorosea*, proved highly pathogenic to aphids and whiteflies (Espinel *et al.* 2008). The fungus *N. rileyi*, exhibits host preferential infections in lepidopterous larvae (Ignoffo *et al.* 1976). Concerned with the effect of entomopathogenic fungi on natural enemies,

Thungrabeab and Tongma (2007) reviewed the research of several authors dealing with the differential susceptibilities of many natural enemies to various fungal species. They concluded that some genera or species of fungi could be specific and might inflict only certain types of hosts. They also mentioned that *Beauveria bassiana* was not pathogenic to *Coccinella undecimpunctata* and *Chrysoperla carnea*, while *M. anisopliae* was found pathogenic to them. Shanthakumar *et al.* (2010) reported that *N. rileyi*, despite the fact that it can cause an epizootic in various pest insect populations, was confirmed safe to *Trichoderma japonica* and *T. chilonis* and did not cause reduction in their parasitization capacity nor did it cause an imbalance in the male and female ratio. In this respect, James and Lighthart (1994) considered that *M. anisopliae* has the ability to infect certain species of *Coccinella* but *N. rileyi* did not (Thungrabeab and Tongma 2007). Haseeb and Murad (1997) and Farag (2008), however, found that *B. bassiana* was relatively injurious to *C. undecimpunctata* when applied at a relatively high concentration.

The first aim of the present study was to evaluate the efficacy of the two fascinating entomopathogenic fungi *N. rileyi* and *I. fumosorosea* against two serious pests of tomato plants (*M. persicae* and *B. tabaci*) under laboratory and field conditions. The second aim was to evaluate the main efficient predator *C. undecimpunctata*, (prevailing in tomato cultivations), also in laboratory and field conditions.

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MATERIALS AND METHODS

Insect cultures

Pests

Whitefly *B. tabaci* and *M. persicae* were reared on small potted tomato plants inside cylinder glass cages (15 cm diameter × 40 cm high), under controlled conditions (26±2°C and 65±5% RH – relative humidity). The cages were covered with muslin.

Predator

The stock culture of the ladybeetle *C. undecimpunctata*, was started with adults that were collected from aphid-infested tomato cultivars in the village of Manawat, Giza governorate, Egypt. Glass jars (2 l) held 10 adults each. The jars were supplied with fresh *duranta* leaves infested with aphids for feeding. They were covered with muslin cloth which was held in position with rubber bands. Food was renewed every other day. The jars were checked daily for egg deposition. The eggs were collected and transferred to Petri dishes (19 cm diameter) till hatching. Neonate larvae were transferred individually to plastic cups till the larvae reached the proposed experimental nymphal stage (2nd nymphal instar). The cups contained an ample amount of *Ephestia kuehniella* eggs. Unused nymphs were left in 2 l glass jars (5/each) with small *duranta* branches carrying different stages of aphids, till maturation.

Source and production of fungi

The fungi *N. rileyi* and *I. fumosorosea* were kindly obtained from Dr. Alain Vey (Prof.), Mycology Unit, Pasture Institute, France, and reproduced in the Microbiology Department, National Research Centre, Cairo, Egypt. They were primarily purified using the mono-spore technique. Then, the fungi were propagated in Petri dishes (9 cm) on Potato Dextrose Agar medium (PDA) enriched with 1% peptone, 4% glucose, and 0.2% yeast extract and incubated at 26°C. Seven-day-old cultures with well developed spores, were harvested by washing with 10 ml sterilized water. Then 3 drops of Tween-80 were added to 100 ml with water. It was used as stock suspension and kept refrigerated at 4°C. From this stock, dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 l cell-culture glass bottles according to Rombach *et al.* (1988) (modified by El-Husseini *et al.* 2004).

Laboratory tests

Treatment of pests

Concentrations of *N. rileyi* and *I. fumosorosea* ranged from 1×10² to 1×10⁸ spores/ml. These concentrations were prepared by a 1–10 fold dilution from the main stock culture and tested under controlled conditions (26±2°C and 65±5% RH) against *B. tabaci* and *M. persicae* third instar nymphs. Fresh tomato leaves were sprayed with the desired fungus concentration (3 shots as spurts/leaf) (Matter

et al. 1993), left to dry and placed in 1 l plastic containers (one/each). Then, twenty nymphs of either species were placed on each leaf. Five containers (replicates) were used/ concentration/microbial pathogen/species. Each container was covered with muslin and incubated at 25°C. After this, the untreated leaves were put in the plastic containers to allow for the gentle transfer of survivors into them, and the previous treated leaves were discarded. Untreated leaves were placed in plastic containers sprayed with water only, and used as the control treatment. The experiment was replicated 4 times. The percentages of mortality were calculated after seven days and corrected according to Abbott, (1925), while LC₅₀ was calculated through probit analysis according to Finney (1964).

Treatments of the predator

One-day-old adults and 2nd instar nymphs of *C. undecimpunctata* were used for the evaluation of the pathogenicity and efficacy of *N. rileyi* and *I. fumosorosea* spores. The following techniques were used:

A – spray technique to evaluate contact effect,

B – feeding technique to evaluate oral toxicity, either required which meant there was no choice (there was exposure to those prey treated only), or free-choice which meant there was exposure to both infected and uninfected prey. This was done to see whether the predator had the ability to distinguish infected from uninfected prey.

A – spray technique

There were 20 predators of one-day-old adults or one-day-old 2nd nymphal stage predators per group. Each group was placed in a petri dish (19 cm diameter) and sprayed with the fungus at a 1×10⁸ concentration level using small atomizer. Three shots as spurts were given (Matter *et al.* 1993). The shots were directed at the insects at a 15 cm distance. Then, using tweezers, the insects were gently transferred individually to plastic cups (5 cm diameter and 12 cm high). The cups had a small water-moistened filter paper and an aphid infested tomato plant leaf. The cups were covered with muslin as mentioned, and incubated at 25°C. The filter paper, and branches carrying aphids, were renewed every other day. Five groups (20 individuals/group) from each stage were used /each pathogen. The cups of each group were checked daily for insects showing signs of fungus infection. The death toll was recorded for two weeks post-treatment, and mortality percentages were calculated in each case.

B – required and free-choice feeding techniques

Groups of twenty individuals of either adults or 2nd instar nymphs per group were exposed to either a required contaminated diet (pathogen treated aphids), or selectively to pathogen treated and untreated aphids, for 24 h. In the case of the free-choice feeding, five groups were used/pathogen/predator stage. The predator nymphs were starved for 4 and 6 hours for nymphs and the predator adults were starved for 6 h. Then, each group was introduced in the middle of 5 l glass jar in which had been placed two branches of *tafla* carrying ample amounts of the pests. One branch was previously sprayed with the fungus, while the other branch was sprayed with water

only. The two branches were placed on both sides of the glass jar, facing each other, to allow the predator individuals free choice to feed on either the treated or untreated aphids. Five glass jars (replicates) were used/ each pathogen. Regarding the required feeding, the same number of predators in each of the 5 glass jars were used as mentioned above, but these predators were offered only treated aphids. In both trials, the exposure period was 24 h. Then, the predators from all the treatments as well as the control, were transferred individually to plastic cups, offered untreated aphids, and checked daily for 14 days

Field experiments

Pests

The experiments were carried out to study the effectiveness of the tested fungi against the target insect pests in two different areas. Each area has different climatic and soil factors. These two areas were: El-Esraa (El-Nobaryia region) with dry weather and sandy soil, and El-Kassaseen (Ismailia) with wet weather and clay soil. Tomato plants (var. Bio-Bride) were planted on the first of April in an area of about 1,200 m², and divided into 12 plots of 100 m² each. Four plots were assigned for each pathogen, while 4 plots were treated with water and used as the controls. *N. rileyi* and *I. fumosorosea* were applied at 1x10⁸ spores/ml concentration and 5 l/plot. Treatments were performed in a randomized plot design at sunset. A five-litre sprayer was used to spray on the treatments. Three applications were made at one week intervals, at the commencement of the experiment. Twenty plant samples were randomly collected at certain time intervals from each plot and transferred to the laboratory for examination. The average number of each of the tested pests/ sample/plot/treatment was calculated 20, 50, 90 and 120 days after the 1st application. The infestation of whiteflies and aphids were then estimated in each case.

After harvest, the yield of each treatment was weighed as kgs/feddan. Yield loss was calculated according to the following equation:

$$\text{Yield loss} = \frac{\text{potential yield} - \text{actual yield} \times 100}{\text{potential yield}}$$

Potential yield was that of *N. rileyi*, which gave the best results among the tested pathogens, and was taken as a base for comparison with the other treatments.

Predator

Seedlings of tomato plants were sown in rows (Ca 50 cm from each other) in ca half a feddan located in the village of Manawat, Giza governorate. One-month-old plants were found highly infested with the *M. persicae* aphid, and white fly *B. tabaci*. The cultivated land was divided longitudinally into 3 areas (Ca Kirat/each), separated from each other by uncultivated land (4 m width). One area was used for each entomopathogen and the check as well. Each pathogen was sprayed at the rate of 250 l/feddan, using a high pressure hand held gun. The concentration of the fungus was about (1x10⁸) conidia/ml. This concentration had previously achieved more than

80% mortality of both pests, in laboratory experiments. Three applications were made at one week intervals. Then, the *C. undecimpunctata* (nymphs and adults) were carefully scrutinised and counted, on site, in each of the treated and untreated tomato plant plots. The methods used were vision, hand picking, and also, a sweeping net (25 cm diameter) was used. The counts were made just before the 1st application, and 1, 2, and 3 weeks after the last application. After each count, the predators were once again placed on their previous location at the corresponding plant site. Fifty tomato shrubs (10 each from 5 rows) per each treated area as well as the control, were arbitrarily chosen for each time interval. The average number of predators/50 plants/time interval was calculated in each case. The increase or decrease in the population density of the predator/50 plants as compared with the check, was calculated according to Henderson and Tilton's (1955) equation, as follows:

$$\% \text{ increase or decrease in population density} = \frac{(Ca)}{(Cb)} \times \frac{(Tb)}{(Ta)} - 1 \times 100$$

where:

Ca – population density in the treated area before treatment

Cb – population density in the treated area after treatment

Ta – population density in the treated before treatment

Tb – population density in the treated area after treatment.

RESULTS AND DISCUSSION

Pests

Under laboratory conditions

Under controlled laboratory conditions, the LC₅₀ values for the fungus *N. rileyi* were 103.7x10⁴ and 89.1x10⁴ against *B. tabaci* and *M. Persicae*, when treated with different concentrations of each fungus, respectively (Table 1). The corresponding LC₅₀ values for *I. fumosorosea* were 139.4x10⁴ and 149.8x10⁴ spores/ml, respectively.

Under field experiments

In both the EL-Esraa and the El-Kassaaeen farms, the percentages of infested plants by *B. tabaci* or *M. psicae* in plots treated with *N. rileyi* or *I. fumosorosea*, were significantly lower than the check, for all the post-treatment days. However, in almost all cases, it was found that the infestation of either of the two pests was much less in plots treated with *N. rileyi* than in plots that were treated with *I. fumosorosea* fungus (Table 2).

Field application of the bioinsecticides, showed that in the control plots, the estimated yield weight was 2010±36.82 kg/feddan. While in the *N. rileyi* and *I. fumosorosea* treated plots, the estimated weights of the tomato yields were 2911±34.31 and 2417±42.57 kg/feddan, respectively in the El-Nobaria region. In El-Kassasin (Ismailia), the untreated plots recorded 1990±80.54 kg/feddan but the weight showed a significant increase after the *N. rileyi*

Table 1. Effect of the fungi *N. rileyi* and *I. fumosorosea* against *B. tabaci* and *M. persicae*, under laboratory conditions (26±2°C and 65±5% relative humidity)

Insects	Fungi	LC ₅₀	95% confidence limits
<i>B. tabaci</i>	<i>N. rileyi</i>	103.7×10 ⁴	69.7–128.6×10 ⁴
	<i>I. fumosorosea</i>	139.4×10 ⁴	92.0–155.0×10 ⁴
<i>M. persicae</i>	<i>N. rileyi</i>	89.1×10 ⁴	67.1–137.6×10 ⁴
	<i>I. fumosorosea</i>	149.8×10 ⁴	92.1–179.0×10 ⁴

Table 2. Percentage of infested plants with *B. tabaci* and *M. persicae* after treatment with the fungi *N. rileyi* and *I. fumosorosea* under field conditions in farms from two regions

Treatments	Days after 1st application	El-Esraa (Nobarya)		El-Kassaseen (Ismailia)	
		<i>B. tabaci</i>	<i>M. persicae</i>	<i>B. tabaci</i>	<i>M. persicae</i>
The control	20	45±3.6	52±3.6	56±7.8	63±5.9
	50	70±7.7	70±6.7	79±8.8	188±5.7
	90	89±5.7	92±7.9	95±5.9	197±5.6
<i>I. fumosorosea</i>	20	18±1.8	13±4.7	12±5.3	9±3.9
	50	27±2.7	18±2.8	35±6.6	27±4.3
	90	38±4.9	33±4.2	43±4.2	36±4.1
<i>N. rileyi</i>	20	0	0	4±0.3	5±0.2
	50	12±3.6	8±0.6	18±4.5	11±1.7
	90	24±2.8	17±1.3	21±4.9	20±3.4
F test		28.3		23.7	
LSD 5%		12.2		19.5	

and *I. fumosorosea* treatments. The percentages of yield loss in the untreated plots were 30 and 33% in the El-Nobaria region and the El-Kassasin region, respectively (Table 5). These findings are in accordance with those of Sabbour and Shadia Abd El-Aziz (2002 and 2010). The results of Abdel-Rahman and Abdel-Mallek (2001), Abdel-Rahman (2001) and Abdel-Rahman and Abdel-Mallek (2001), Abdel-Rahman *et al.* (2004, 2006) also showed control of cereal aphids with entomopathogenic fungi under laboratory and field conditions. Moreover, Sabbour and Sahab (2005, 2007), Sahab and Sabbour (2011) found that entomopathogenic fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

Effects of *N. rileyi* and *I. fumosorosea* on *C. undecimpunctata*

All laboratory investigations showed that the predator *C. undecimpunctata* proved less susceptible to *N. rileyi* than to *I. fumosorosea* (Table 3).

In general, adults predators showed greater resistance to both fungi, than predator nymphs did. Laboratory experiments showed that mortality percentages among adult predators exposed to the *N. rileyi* spray (contact effect) was about 0.62 times more than the mortality percentages obtained from *I. fumosorosea* fungus spray. The corresponding ratios, in the case of nymphs, was 0.67.

In indirect treatments by feeding, either on treated prey only (required) or by free-choice feeding on either

treated or untreated prey (selectivity), revealed that the required ingestion of *N. rileyi* – infected prey caused mortality percentages of 2.56 and 2.31 times that obtained from those given free-choice ingestion (selection treatment) for adult and nymph predators, respectively. The corresponding ratios for *I. fumosorosea* fungus were about 1.35 and 1.86, respectively. This indicated that the predator, particularly the adult predator, has a greater ability to distinguish between *N. rileyi* – treated prey and non-treated ones than *I. fumosorosea* fungus, which indicates that the adult predator can avoid *N. rileyi* infected prey much more often than the adult predator avoids *I. fumosorosea* – infected prey. It is worth mentioning that no death from fungus infection was encountered in the check within the experimental period.

In field experiments, the population density of the predator in the *N. rileyi* – treated area showed 36.22 and 19.81% reductions, one and two weeks after the last application, respectively. While the corresponding densities in the control area showed a 21.33 and 2.7% increase. However, the population, 3 weeks after the last application, surpassed that of the check, showing a 6.36% increase (Table 4). In the *I. fumosorosea* – treated area, severe reductions in the population densities (63.33 and 43.99%) were estimated in the 1st and 2nd weeks after the last application, respectively. There was less reduction (21.42%) estimated 3 weeks after the last application. However, the relatively higher reductions in predator densities in the treated areas, in the 1st weeks after the three applica-

Table 3. The effect of the entomopathogenic fungi *N. rileyi* and *I. fumosorosea* on some developmental stages of *C. undecimpunctata*

Pathogen	Mortality percentages of infected insects					
	adults			nymphs		
	Type of treatment					
	direct spray	ingestion of treated diet		direct spray	ingestion of treated diet	
required		selection	required		selection	
<i>N. rileyi</i>	19.20±7.61	18.4±2.71	7.2±1.5	28.6±2.19	29.6±3.49	12.8±1.96
<i>I. fumosorosea</i>	31.2±4.45	33.6±4.49	24.8±2.82	41.6±3.71	39.2±4.45	22.4±2.40
F test	29.2					
LSD 5%	11.2					

Table 4. The average number of *C. undecimpunctata* (all stages)/50 tomatos shrubs after a successive post fungal application period

After the application (weeks)	Treatments				
	Average number of the predator ±SE				
Just before the 1st application	the control	<i>N. rileyi</i>	<i>I. fumosorosea</i>	% increase(+) or decrease (-)*	
	18.75±3.36	21.00±2.55	20.00±1.58	<i>N. rileyi</i>	<i>I. fumosorosea</i>
One week after the last last application	22.75±1.70	16.25±2.25	7.5±1.71	-36.22	-69.09
Two weeks after the last last application	19.25±1.54	17.29±1.66	11.5±0.96	-19.81	-43.99
Three weeks after the last last application	17.00±2.12	20.25±1.65	14.25±1.93	+6.36	-21.42

*percentages of increase or decrease in *C. undecimpunctata* population density as compared with the check according to Hendrson and Tilton (1955)

Table 5. Weight of harvested tomatoes and percentage of yield loss, after treatment with the fungi *N. rileyi* and *I. fumosorosea* against *B. tabaci* and *M. persicae*, in farms of the two regions

Treatments	El-Esraa (Nobarya)		El-Kassaseen (Ismailia)	
	weight of tomatoes [kg/feddan]	% yield loss	weight of tomatoes [kg/feddan]	% yield loss
The control	2010±36.82	30.6	1990±80.54	33.7
<i>N. rileyi</i>	2911±34.31	-	2999±65.32	-
<i>I. fumosorosea</i>	2417±42.57	16	2699±69.33	3.9
F values	31.42		32.40	
LSD 5%	81		80	

tions, might be attributed to the relative disappearance of the most preferable prey which had been affected by the highly used pathogen concentration in one part, and to migration of survivors of predator adults to the uncontaminated area.

The present study showed that *C. undecimpunctata* exhibit relatively high and reasonable resistance to the tested entomopathogenic fungi *N. rileyi*, and *I. fumosorosea* infections, respectively, even when exposed to the lethal concentration for the prey insects.

Thungrabeab and Tongma (2007) concluded that some genera of fungi could be specific and might inflict only on certain types of hosts. Moreover, James and Lighthart (1994) declared that the fungus (*N. rileyi*) exhibits host preferential infection in lepidopterous larvae. They also found that *M. anisopliae*, *B. bassiana*, and *P. fumosoroseus* fungi have the potential to infect *Hyppodamia* con-

verges (Coccinellidae), whereas *N. rileyi* did not. Goettel *et al.* (1990) found that some commercial formulations of the entomopathogenic fungi can control aphids and thrips with low impact on non-target insects. Todorova *et al.* (1994), found that different strains of *B. bassiana* fungus showed different effects on the two Coleoptrous predatory insects due to the host response of the insects. Poprawiski *et al.* (1998) found that *Serangium parcestosum* (Coccinellidae) had lower survival potential when sprayed with *B. bassiana* fungus than when sprayed with *P. fumosoroseus* fungus. Shanthakumar *et al.* (2010) considered that despite the great virulence of *N. rileyi* against *S. littura*, the pathogen proved reasonably safe for *T. chilonis*. *N. rileyi* did not cause reduction in the parasite percentages of *T. chilonis*.

The present results also indicated that the predator, *C. undecimpunctata*, particularly the adult predators, can

distinguish between fungus-infected prey and non-infected prey. *C. undecimpunctata* will almost always avoid the treated prey, especially if given free-choice feeding. This, however, was more pronounced in the case of *N. rileyi* than *P. fumosoroseus*. This phenomenon observed in our investigations was also noted by many other authors. It was mentioned that predators, when given free choice to feed on fungus – treated or untreated aphids, predation on the infected prey was less than predation on the uninfected ones (Baverstock *et al.* 2007). Also, Roy *et al.* (2010) and Goettel *et al.* (1990) proved that *C. septempunctata* adults avoid contact with leaf and soil surfaces innoculated with *B. bassiana* fungus and mycosed cadavers. The predator was more often positioned away from mycosed cadaver than from uninfected ones.

Nevertheless, some researches indicated several adverse effects of some entomopathogenic fungi against some natural enemies. Haseeb and Murad (1997) and Delete *et al.* (1995) consider *C. septempunctata* to be somewhat susceptible to *B. bassiana*. While, Farag (2008) consider that some entomopathogenic formulations of *B. bassiana* have deleterious effects on *C. undecimpunctata* if applied at high concentration levels. The various views about the safety of entomopathogenic fungi stated by many different authors, might be due to the relative efficacy of the fungus or its isolates on pests which exhibit different susceptibilities, bionomics, and characters. The various views may also be due to the types of assessment and application rates.

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