Research article

Efficacy of the Entomopathogenic Fungus *Nomuraea rileyi* in the Biological Control of Vegetable Pest *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract Eight strains of the entomopathogenic of the fungus *Nomuraea rileyi* were screened for control of the common cutworm, *Spodoptera litura*. The results showed that *N. rileyi* was pathogenic to *S. litura*, and that pathogenity significantly varied among the strains. The mortalities ranged from 2.5 to 92.5% at 7 d after inoculation. The strains tested were classified into three groups according to mortality percentage. High pathogenicity level was defined as a mortality greater than 75.79%, moderate pathogenicity was a mortality between 75.79 to 49.50%, and low pathogenicity level less than 49.50% mortality. The high pathogenicity level contained two strains, BCC 14653 and BCC 14671, which were identified as highly virulent and used in a secondary bioassay against various larval stages. *Nomuraea rileyi* could become an important biopesticide agent in an integrated pest management program for insect pest control.

Keywords *Nomuraea rileyi*, *Spodoptera litura*, Entomopathogenic Fungi, Biological control

INTRODUCTION

The common cutworm, *Spodoptera litura* F (Lepidoptera: Noctuidae), is a highly polyphagous migratory lepidopteran pest species. This insect is also known as the tobacco budworm, oriental leafworm moth, tropical armyworm, and taro caterpillar. It causes extensive losses in over 120 different plant species including many vegetables, fruits and ornamental crops. Some examples are: asparagus, beets, broccoli, cabbage, carrots, chrysanthemum, corn, cruciferous crops, eggplants, grapes, lettuce, orchid, potatoes, radish and sunflowers.

Insecticides for control *S. litura* have become ineffective because of the development of resistance, and cause toxic residues in the crops.(Rajan and Muthukrishnan, 2009). Insecticide resistance and the demand for reduced chemical inputs in agriculture have provided an impetus for the development of alternative methods of pest control. Biological control offers an attractive alternative or supplement to the use of chemical pesticides. Microbial control agents are naturally occurring organisms and perceived as being less damaging to the environment. Furthermore, their generally complex mode of action makes it unlikely that resistance could develop to a bio-pesticide. Biological pest control agents include viruses, bacteria, fungi, and nematodes. The use of microorganisms as selective pesticides has had some notable successes (Hong Wan, 2003).

Nomuraea rileyi is an important naturally-occurring, mortality-causing agent of many lepidopterous pests in a variety of crop ecosystems throughout the world (Vimaladevi et al, 1996). Natural occurrence of *N. rileyi* has been widely reported. *N. rileyi* has occurred in epizootics on *Spodoptera exiqua* in black gram, *S. litura* in tobacco, *Tricoplusia ni* and *Heliothis zea*

(Srisukchayakul, 2005). Furthermore, its occurrence has been reported on *Helivoverpa armigera* in tomato and on *S. litura* in soybean, potato and cabbage (Patill and Abhilash, 2014).

OBJECTIVES

The objective of this study was to determine the pathogenicity of *Nomuraea rileyi* strains on larvae of *Spodoptera litura* under laboratory conditions.

METHODOLOGY

Insects

Spodoptera litura populations including egg masses, larvae and adults were collected from cruciferous vegetable crop plantations in Chiang Mai, Thailand. All stages were maintained under laboratory conditions. The pupae were kept and used as a stock to build up new colonies. The adults were reared in cages and fed with a 5% honey solution.

Fungal Preparations and Pathogenicity

Nomuraea rileyi strains from adult insects in the order Lepidoptera collected in natural environments of Thailand were obtained from the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani, Thailand. (Table 1). The fungal strains were cultured on SMAY (Sabouraud's maltose agar medium supplemented with 1% yeast extract) in 90 mm Petri dishes and incubated for 12-15 d at 25±1°C (Fig. 1). Conidial suspensions were prepared by scraping conidia from petri plates into an aqueous solution of 0.002% Tween 80. The conidial suspension was filtered through several layers of cheesecloth to remove mycelial mats. Viability of conidia was assessed by germination tests before preparation of suspensions. The concentration of conidia in the final suspensions was determined using a hemocytometer. The conidial suspension used for the bioassays was adjusted by diluting conidia with the Tween 80 solution to final concentrations of 6x107, 6x108 and 6x109 conidia/ml. To determine pathogenicity, each of the conidial suspensions were sprayed on larvae of different instars (I-III). Three replications were used with 10 larvae per replication including a non-treated control. The larval mortality was recorded at 7 d after inoculation.

Table 1 Strains of the entomopathogenic fungus *Nomuraea rileyi* collected from Thailand and used in pathogenicity tests against larvae of *Spodoptera litura*

No	Strain number	TNCC number:	Fungus species	Host insect
1	BCC 14653	4522	*Nomuraea rileyi*	Lepidoptera - adult
2	BCC 14658	4685	*Nomuraea rileyi*	Lepidoptera - adult
3	BCC 14659	4686	*Nomuraea rileyi*	Lepidoptera - adult
4	BCC 14660	4687	*Nomuraea rileyi*	Lepidoptera - adult
5	BCC 14670	4327	*Nomuraea rileyi*	Lepidoptera - adult
6	BCC 14671	4328	*Nomuraea rileyi*	Lepidoptera - adult
7	BCC 14672	4329	*Nomuraea rileyi*	Lepidoptera - adult
8	BCC 14673	4318	*Nomuraea rileyi*	Lepidoptera - adult

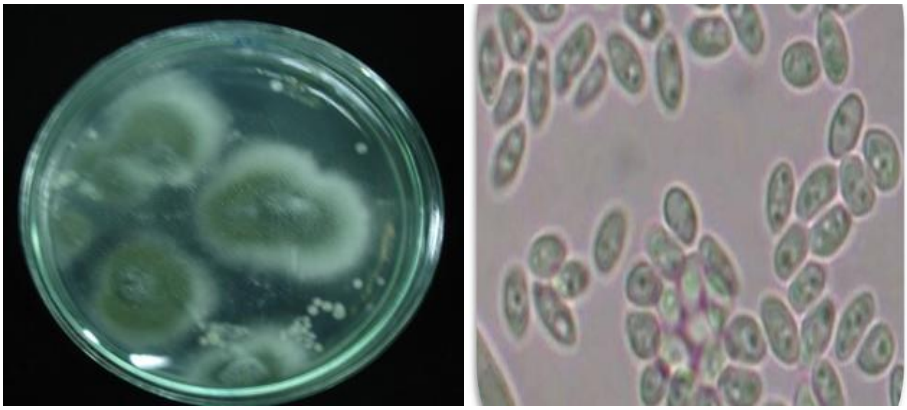

Fig. 1 Colony and conidial characteristics of *Nomuraea rileyi*

RESULTS AND DISCUSSION

The results showed that *N. rileyi* was pathogenic to *Spodoptera litura*; the cumulative mortalities ranged from 0 to 92.5% at 7 days after inoculation (Table 2). The corrected mortalities significantly differed ($P \leq 0.05$) between strains (Fig. 2).

Table 2 Cumulative mortality of the entomopathogenic fungus *Nomuraea rileyi* on each instar of *Spodoptera litura* at 7 d after inoculation

Nomuraea rileyi)conidia/ml(		% Mortality		
		I instar	II instar	III instar
Strain 1	$6x10^7$	82.5	72.5	72.5
	$6x10^8$	85.0	77.5	55.0
	$6x10^9$	92.5	92.5	70.0
Strain 2	$6x10^7$	35.0	20.0	20.0
	$6x10^8$	30.0	30.0	37.5
	$6x10^9$	37.5	32.5	32.5
Strain 3	$6x10^7$	2.5	7.5	7.5
	$6x10^8$	2.5	2.5	2.5
	$6x10^9$	0	0	0
Strain 4	$6x10^7$	7.5	10.0	10.0
	$6x10^8$	10.0	25.0	5.0
	$6x10^9$	5.0	22.5	2.5
Strain 5	$6x10^7$	47.5	30.0	2.5
	$6x10^8$	40.0	25.0	7.5
	$6x10^9$	40.0	25.0	25.0
Strain 6	$6x10^7$	87.5	77.5	75.0
	$6x10^8$	92.5	87.5	67.5
	$6x10^9$	92.5	80.0	82.5
Strain 7	$6x10^7$	47.5	32.5	12.5
	$6x10^8$	35.0	15.0	15.0
	$6x10^9$	50.0	10.0	10.0
Strain 8	$6x10^7$	7.5	17.5	7.5
	$6x10^8$	17.5	7.5	2.5
	$6x10^9$	17.5	10.0	12.5
Control		0	0	0

In support of the present investigation, Padanad and Krishnaraj, (2009) observed that all ten isolates of *N. rileyi* were active against third instars of *S. litura*, resulting in 85 to 97% mortality. Patil et al., (2014) stated that early instars were highly susceptible with a mortality of 70.17 percent, which decreased significantly as the age of the larvae advanced. However, there were statistically no significant differences among the strains with respect to the pathogenicity levels. The strains tested could be classified into two groups according to mortality percentage (Table 3). Fungal strains BCC

14653 and BCC 1467 were identified as highly pathogenic and were used in the secondary bioassay against various larval stages. The remaining strains were characterized as having low pathogenicity (Table 4).

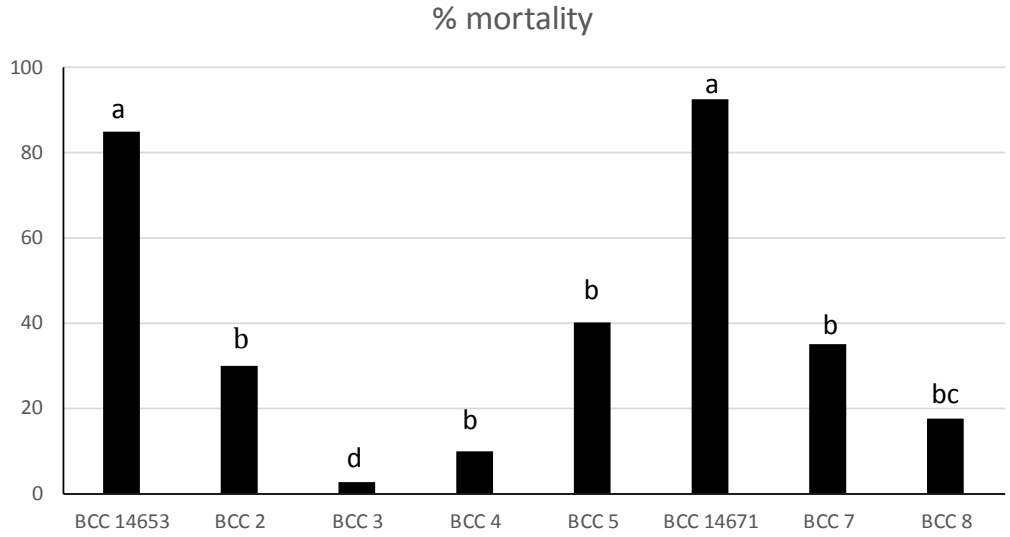

Fig. 2 Percentages of corrected mortality of *Spodoptera litura* treated with different strains of *Nomuraea riley* at a concentration of 6x108 conidia/ml at 25 ±1°C

Table 3 Pathogenicity levels of different strains of *Nomuraea rileyi* toward *Spodoptera litura* as expressed by percentage mortality

Pathogenicity level	Mortality (%)	Strain No.
High pathogenicity	>75.79	BCC 14653, BCC 14671
Moderate pathogenicity	75.79 – 49.50	-
Low pathogenicity	<49.50	BCC 14658, BCC 14659, BCC 14660, BCC 14670, BCC 14672, BCC 14673

Table 4 shows the efficacy of two strains of *Beauveria bassiana* against various larval stages of *Spodoptera litura*. All strains tested were effective against all larval stages of *Spodoptera litura*, but the effectiveness varied with larval stage. There were significant differences ($P \leq 0.05$) among different larval stages. No significant differences were found among concentrations.

Means in columns with different small letters indicate significant differences among different concentrations of *Nomuraea rileyi* (one way ANOVA, $P \leq 0.05$; Duncan's multiple rang test). Means in the same row followed by the different capital letters indicate significant differences between instars of Spodoptera litura at $P \leq 0.05$ (T- test).

Table 4 Efficacy of two strains of *Nomuraea rileyi* against various larval stages of *Spodoptera litura*

Strains no.	Conc.	Corrected mortality of 1st instar (%±SE)	Corrected mortality of 2nd instar (%±SE)	Corrected mortality of 3rd instar (%±SE)
BCC 14653	$6x10^7$	82.5 ±5.77 bA	72.5±3.33 bB	72.5±5.77 aB
	$6x10^8$	85.0±8.81 bA	77. ±5.77 5 bB	55.0±8.81 bC
	$6x10^9$	92.5±3.33 aA	92.5±5.77 aA	70.0±5.77 aB
BCC 14671	$6x10^7$	87.5±6.67 bA	77.5±5.77 bB	75.0±5.77 abB
	$6x10^8$	92.5±0.00 aA	87.5±8.81 aAB	67.5±13.33 bBC
	$6x10^9$	92.5±5.77 aA	80.0±5.77 abB	82.5±5.77 aB

CONCLUSION

Research is needed to further characterize the use of *Nomuraea rileyi* as an effective biocontrol of *Spodoptera litura* in vegetable crops. The results presented here suggest that some strains of this fungus are effective in causing high mortality levels and (at sufficient dosages) could become an important part of an integrated pest management program. *Nomuraea rileyi* treatment could be useful in preventing the development of resistance if used in rotation with other effective therapeutic agents.

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