Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Pathogenicity of *Metarhizium rileyi* (Farlow) Kepler, S.A. Rehner and Humber isolates against *Spodoptera litura* (Fabricius) and their extracellular enzymatic activities

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Abstract

Background: *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a serious agricultural pest that infests many commercially important crops of Southeast Asian countries. Indiscriminate use of chemical pesticides has led to various health hazards as well as insecticide resistance. Entomopathogenic fungi (EPF) provide an important alternative as biological control agents. *Metarhizium rileyi* is an EPF with a specific host range for lepidopteran pests. The present study aimed to identify virulent *M. rileyi* isolate against *S. litura* larvae and analyse their extracellular cuticle-degrading enzyme activities.

Results: Three *M. rileyi* isolates viz *M. rileyi* NIPHM, *M. rileyi* MTCC 4254 and *M. rileyi* MTCC 10395 formulations were evaluated at different concentrations against 2nd instar larvae of *S. litura*. A maximum percent mortality of 63.33% was recorded in *M. rileyi* NIPHM (12 g/l), followed by *M. rileyi* MTCC 4254 (58.33%) at the same concentration, 10 days post-treatment. Maximum means of chitinase, protease and lipase activities (0.44, 1.58 and 2.95 U/ml, respectively) were recorded in the case of *M. rileyi* NIPHM. Correlation analysis was positive between enzyme activity and larval mortality.

Conclusions: *Metarhizium rileyi* NIPHM recorded the highest enzymatic activity and exhibited the maximum mortality rate against 2nd instar larvae of *S. litura*, suggesting the possible role of these enzymes in the pathogenicity of the fungus. Further knowledge in this regard may help in the development of enzyme-based screening methods for selecting virulent fungal isolates for the eco-friendly management of crop pests.

Keywords: *Spodoptera litura*, Entomopathogenic fungi, Chitinase, *Metarhizium rileyi*, Protease, Lipase, Mortality

Background

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae), commonly known as tobacco caterpillar, is a polyphagous pest, which feeds on different species of plants and is widely distributed in various parts of the world. Crops like soybean, oilseeds, pulses, cotton and vegetables are seriously affected by this pest, which causes great yield losses (Srivastava et al. 2018). Indiscriminate use of conventional insecticides has led to the development of resistance leading to pest resurgence and crop failures (Ahmad et al. 2007). With the increasing emphasis being given to organically produced food, environmental protection, conservation of biodiversity and sustainable agriculture, the use of biopesticides has become an important tool that may provide an alternative to conventional pesticides (Dhakal and Singh 2019). Biopesticides are safe and eco-friendly and can be used for controlling pest populations organically under protected conditions (Singh and Joshi 2020). They have a specific activity only towards target pests and result in lower

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exposure and rapid decomposition without leaving any residues behind (Namasivayam and Arvind 2015).

The fungus *Metarhizium rileyi* (Farlow) Kepler, S.A. Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler et al. 2014), is an EPF that has been studied as an alternative method for the management of lepidopteran pests belonging to different genera (Boucias et al. 1982). The main barrier to the invasion of fungi is the host cuticle, which is a thick layer made up of chitin, lipids, proteins and other biomolecules. The germinating conidia degrade this layer by releasing cuticle-degrading enzymes such as chitinase, protease and lipase during penetration (Liu et al. 2019). Virulence of various EPF may be associated with cuticle-degrading enzymes that are considered important in the infection process. Establishing a correlation between the production of these enzymes and virulence could be useful for the production of more effective mycoinsecticides (Petrisor and Stoian 2017). So, the present study was undertaken to evaluate the extracellular enzymatic activities and pathogenicity of different *M. rileyi* isolates against *S. litura* so as to identify virulent *M. rileyi* isolate for *S. litura* management.

Methods

Fungal isolates

Two fungal isolates viz *M. rileyi* MTCC 4254 and MTCC 10395, procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India, and one *M. rileyi* NIPHM isolate procured from the National Institute of Plant Health Management (NIPHM), Hyderabad, India, were used in the present study. The three isolates were grown and maintained on Sabouraud maltose agar media with yeast extract (SMAY). These were then stored at refrigeration temperature for further use.

Production of mycoformulations

M. rileyi isolates were individually inoculated and incubated on sterilized broken sorghum grains for 14 days at 25 ± 2 °C and formulated to talc formulation according to the methodology of Kaur and Joshi (2014) with some modification. The formulations had a viable colony count of 1×10^8 cfu/g.

Rearing of *Spodoptera litura* in laboratory

Larvae and egg masses of *S. litura* were collected from cabbage and cauliflower field crops and reared on cabbage leaves in glass battery jars in the laboratory at suitable environmental conditions of 25 ± 2 °C. The open end of the glass jars was covered by a piece of muslin cloth and tied with a rubber band. The leaves were changed daily to provide adequate food. The 2nd instar larvae obtained from the 2nd generation of *S. litura* were used in the present experiment.

Pathogenicity against *Spodoptera litura* larvae

Talc-based formulations of *M. rileyi* at 3 different concentrations (8, 10 and 12 g/l) were tested for their efficacy against 2nd instar larvae of *S. litura* under laboratory conditions. The bioassay was carried out according to the methodology of Devi et al. (2003) with some modifications. The bio-formulation was sprayed on cabbage leaves to be tested. Ten treatments and 3 replications per treatment were used. Each replication had 20 larvae of *S. litura*, which were released onto the sprayed cabbage leaves kept in plastic insect rearing vials. The control treatment was maintained on leaves sprayed with distilled water. The larvae were kept at room temperature, and the mortality rate was recorded up to 10 days post-treatment.

Preparation of conidial suspension of *M. rileyi*

M. rileyi isolates were inoculated and incubated on SMAY media for 14 days at 25 ± 2 °C and 80% RH. The conidia were collected by scraping the conidia off the media plates. The volume of conidial suspension was made up to 100 ml with distilled water and to it Tween80 @ 0.1% was added. The suspension was vortexed for 5 min to obtain a uniform conidial suspension. The sample of this suspension was quantified in the Neubauer chamber. A suspension containing 10^8 conidia/ml was used for the analysis of enzymatic activity.

Production of cuticle-degrading enzymes

Production and activity of extracellular cuticle-degrading enzymes (CDEs) viz chitinase, protease and lipase productions were investigated from the isolates of *M. rileyi* according to the methodology of Dhawan and Joshi (2017) with some modifications. Each isolate of *M. rileyi* (having 10^8 spores/ml) was inoculated in 100 ml of chitin media. The composition of media (g/l) was KH_2PO_4, 3.0; K_2HPO_4, 1.0; $(NH_4)_2SO_4$, 1.4; NaCl, 0.5; $CaCl_2$, 0.5; $MgSO_4$, 0.7; bacto-peptone, 0.5; yeast extract, 0.5; chitin, 5.0; and olive oil, 5 ml/l. The flasks were incubated at 25 ± 2 °C at 150 rpm in an orbital shaking incubator. The enzyme assays were carried out on every alternate day up to 10 days. Flasks were removed from incubation on successive days, and the broth was centrifuged at 8000 rpm for 25 min to extract clear supernatant. This supernatant was used to determine the enzymatic activities.

Enzyme assays

Chitinase activity in the extracted supernatant was determined, using acid-swollen chitin as substrate (Nahar et al. 2004). Acid-swollen chitin was prepared by suspending 10 g chitin flakes (HiMedia) in 300 ml chilled o-

phosphoric acid (88% w/v) and kept at 4 °C for 1 h stirring occasionally. The mixture was poured into ice-cold distilled water and then filtered through Whatman filter paper. This was followed by washing with 1% (w/v) sodium bicarbonate solution and adjusting the pH to 7. The solution was then homogenized in a blender for 1 min, and the concentration of acid-swollen chitin was adjusted to 7 mg/ml by adding 50 mM acetate buffer (pH 5.0). The reaction mixture for the assay containing 1 ml of 0.7% acid-swollen chitin, 1 ml 50 mM acetate buffer (pH 5.0) and 1 ml crude enzyme extract was incubated at 50 °C for 1 h. The product formed and colour so developed was measured calorimetrically at 520 nm according to the methodology of Somogyi (1952) to estimate the N-acetylglucosamine residues produced. One unit of enzyme activity was expressed as 1 μg of N-acetylglucosamine produced per min per ml.

Protease activity was determined using casein as substrate (Nahar et al. 2004). Casein substrate contained 10 g casein in 100 ml of 0.2 mM sodium carbonate buffer (pH 9.7). The reaction mixture contained 1 ml casein substrate, 1 ml sodium carbonate buffer (pH 9.7) and 1 ml crude enzyme solution. This reaction mixture was incubated at 35 °C for 20 min and the reaction was then terminated by adding 3 ml of trichloroacetic acid (2.6 ml 5% TCA + 0.4 ml 3.3 N HCl). The absorbance of TCA soluble fraction was read at 280 nm. One unit of enzyme activity was expressed as 1 μg of tyrosine produced per min per ml.

Lipase enzyme activity was measured by using olive oil and gum acacia as substrate according to the methodology of Pignede et al. (2000). The substrate was prepared by mixing 50 ml of olive oil and 50 ml of gum acacia (10% w/v, Hi Media) in the ratio 1:1. The reaction mixture for lipase assay contained 5 ml of substrate emulsion, 2 ml of 50 mM phosphate buffer (pH 6.8) and 1 ml of crude enzyme extract. The reaction mixture was incubated at 37 °C for 1 h with intermittent shaking, followed by the addition of 4 ml acetone-ethanol (1:1 v/v), containing 0.09% phenolphthalein as an indicator to terminate the reaction. Enzyme activity was determined by titration of the above reaction mixture against 50 mM sodium hydroxide solution for the estimation of the free fatty acids released during the reaction. One unit of lipase activity was expressed as 1 μmol of fatty acids released per min per ml. All enzyme assays were carried out in duplicates at alternate days.

Statistical analysis

The data was subjected to one-way analysis of variance (ANOVA) in SPSS 16.0 statistical software, and means were compared (at 0.05 level of significance) by Tukey's post hoc test. Means with $P < 0.05$ were considered to be significantly different from each other. The transformed mean values were obtained by applying one-way ANOVA under CRD in CPCS1 software.

Results

Pathogenicity of *M. rileyi* against *S. litura* larvae

Three days post-treatment, a relatively low percent of mortality was detected, ranged from 3.33 to 16.67% (Table 1). After 5 days of treatment, the maximum cumulative mortality of 30% was recorded by *M. rileyi* NIPHM (12 g/l), followed by 26.67% mortality at *M. rileyi* MTCC 4254 and MTCC 10395 at the same

Table 1 Bio-efficacy of *Metarhizium rileyi* formulations against *Spodoptera litura*

Treatments	Concentrations (g/l)	Percent mortality			
		Days of observation			
		3rd	5th	7th	10th
***M. rileyi* NIPHM**	8	8.33^{cba} (16.59)	16.67^{ba} (24.03)	21.67^{dcb} (27.51)	33.33^{dc} (35.20)
	10	10.00^{cba} (18.42)	23.33^{a} (28.77)	33.33^{cba} (35.15)	45.00^{cba} (42.10)
	12	16.67^{a} (23.84)	30.00^{a} (33.02)	45.00^{a} (42.10)	63.33^{a} (52.77)
***M. rileyi* MTCC 4254**	8	6.67^{cba} (12.28)	15.00^{ba} (22.58)	21.67^{dcb} (27.58)	30.00^{dc} (32.98)
	10	8.33^{cba} (16.59)	21.67^{ba} (27.02)	28.33^{cba} (31.73)	40.00^{dcb} (39.13)
	12	13.33^{ba} (21.13)	26.67^{a} (31.05)	38.33^{ba} (38.17)	58.33^{ba} (48.81)
***M. rileyi* MTCC 10395**	8	3.33^{cb} (6.14)	13.33^{ba} (21.32)	16.67^{dc} (23.84)	23.33^{ed} (28.77)
	10	6.67^{cba} (12.28)	18.33^{ba} (24.98)	25.00^{dcba} (29.91)	36.67^{dc} (37.24)
	12	10.00^{cba} (18.04)	26.67^{a} (30.98)	33.33^{cba} (35.20)	43.33^{cb} (41.14)
Control		0.00^{c} (0.00)	5.00^{b} (10.44)	6.67^{d} (12.28)	10.00^{e} (18.42)
***CD (5%)**		(11.14)	(8.96)	(9.69)	(6.89)

Note: Values in parenthesis are arcsine transformation. Mean values followed by the same letter (a, b, c, d, e) in the vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test
*Critical difference at 5%

Table 2 Chitinase activity of *Metarhizium rileyi* isolates on different days after inoculation

Treatments	Chitinase activity on alternate days (U/ml)* (mean ± S.E.)**					
	2nd day	4th day	6th day	8th day	10th day	Mean
***M. rileyi* NIPHM**	0.26 ± 0.001^{Ab}	0.48 ± 0.001^{Aa}	0.48 ± 0.090^{Aa}	0.55 ± 0.019^{Aa}	0.45 ± 0.022^{Aa}	0.44
***M. rileyi* MTCC 4254**	0.25 ± 0.032^{Ac}	0.42 ± 0.027^{Bba}	0.46 ± 0.028^{Aba}	0.52 ± 0.003^{BAa}	0.35 ± 0.047^{BAcb}	0.40
***M. rileyi* MTCC 10395**	0.23 ± 0.039^{Ab}	0.26 ± 0.009^{Cba}	0.31 ± 0.019^{Aa}	0.33 ± 0.002^{Ca}	0.29 ± 0.008^{Bba}	0.28
Mean	0.24	0.38	0.42	0.46	0.37	–

Mean values followed by the same letter (a, b, c) in the horizontal column are not significantly different at 0.05% level of probability using Tukey's post hoc test. Mean values followed by the same letter (A, B, C) in the vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test
**Values are mean ± standard error of two replicates
*1 U/ml corresponds to 1 μg of N-acetylglucosamine produced min^{-1} ml^{-1}

concentration. However, the minimum mortality rate (13.33%) was recorded by *M. rileyi* MTCC 10395 (8 g/l). Seven days post-treatment, the maximum mortality (45%) was recorded by *M. rileyi* NIPHM (12 g/l) and was found to be significantly better than the other treatments. This was followed by 38.33% mortality by *M. rileyi* MTCC 4254 at the same concentration. Ten days post-treatment, the percent mortality ranged from 23.33 to 63.33%. Of the 3 isolates, the maximum cumulative mortality (63.33%) was recorded by *M. rileyi* NIPHM (12 g/l) and it was significantly better than other treatments. This was followed by 58.33% mortality rate, recorded by *M. rileyi* MTCC 4254 (12 g/l). The minimum percent mortality (23.33%) was recorded at the lowest concentration by *M. rileyi* MTCC 10395. However, all treatments were significantly better than the untreated control.

Chitinase assay

Chitinase activity of the fungal isolates was recorded to increase till the 8th day and then decreased slightly on the 10th day. Maximum mean chitinase activity (0.44 U/ml) was recorded by *M. rileyi* NIPHM, followed by MTCC 4254 (0.40 U/ml), whereas MTCC 10395 recorded the minimum chitinase activity (0.28 U/ml) (Table 2). Variation in chitinase activity was observed on alternate days. The highest chitinase activity of 0.55 U/ml was recorded by *M. rileyi* NIPHM on the 8th day after inoculation, followed by an activity of 0.52 U/ml by MTCC 4254 on the same day. However, the minimum chitinase activity (0.23 U/ml) was recorded by MTCC 10395 after 2 days of inoculation. Chitinase activity of the fungal isolates was observed to decrease slightly on the 10th day.

Protease assay

Maximum mean protease activity (1.58 U/ml) was recorded by *M. rileyi* NIPHM, which was at par with the MTCC 4254 (1.57 U/ml) (Table 3). *M. rileyi* MTCC 10395 recorded the minimum mean protease activity (1.34 U/ml). Among the individual protease activities in different isolates on various days, the maximum enzyme activity was recorded by *M. rileyi* MTCC 4254 (2.39 U/ml) on the 8th day, which was at par with the activity by *M. rileyi* NIPHM (2.30 U/ml) and *M. rileyi* MTCC 10395 (2.14 U/ml) on the same day. The minimum protease activity (0.53 U/ml) was recorded on the 2nd day after inoculation by *M. rileyi* MTCC 10395. The protease activity was observed to increase up to the 8th day after inoculation and slightly decreased thereafter.

Lipase assay

The mean lipase activity was recorded to be the highest on the 6th and 8th day after inoculation and decreased on the 10th day (Table 4). The maximum mean lipase activity (2.95 U/ml) among the isolates was recorded by

Table 3 Protease activity of *Metarhizium rileyi* isolates on different days after inoculation

Treatments	Protease activity on alternate days (U/ml)* (mean ± S.E.)**					
	2nd day	4th day	6th day	8th day	10th day	Mean
***M. rileyi* NIPHM**	0.72 ± 0.03^{Ad}	0.98 ± 0.03^{Ac}	1.67 ± 0.01^{Ab}	2.30 ± 0.07^{Aa}	2.20 ± 0.02^{Aa}	1.58
***M. rileyi* MTCC 4254**	0.69 ± 0.02^{Ad}	0.94 ± 0.02^{Ad}	1.71 ± 0.03^{Ac}	2.39 ± 0.10^{Aa}	2.11 ± 0.02^{BAb}	1.57
***M. rileyi* MTCC 10395**	0.53 ± 0.01^{Bd}	0.84 ± 0.03^{Ac}	1.20 ± 0.07^{Bb}	2.14 ± 0.06^{Aa}	2.01 ± 0.03^{Ba}	1.34
Mean	0.65	0.92	1.53	2.28	2.10	–

Mean values followed by the same letter (a, b, c) in the horizontal column are not significantly different at 0.05% level of probability using Tukey's post hoc test. Mean values followed by the same letter (A, B, C) in the vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test
**Values are mean ± standard error of two replicates
*1 U/ml corresponds to 1 μg of tyrosine produced min^{-1} ml^{-1}

Table 4 Lipase activity of *Metarhizium rileyi* isolates on different days after inoculation

Treatments	Lipase activity on alternate days (U/ml)* (mean ± S.E.)**					
	2nd day	4th day	6th day	8th day	10th day	Mean
***M. rileyi* NIPHM**	1.57 ± 0.12^{Ab}	2.95 ± 0.25^{Aa}	3.57 ± 0.12^{Aa}	3.45 ± 0.25^{Aa}	3.20 ± 0.25^{Aa}	2.95
***M. rileyi* MTCC 4254**	1.20 ± 0.25^{BAb}	2.57 ± 0.12^{Aa}	3.20 ± 0.00^{BAa}	3.32 ± 0.12^{Aa}	2.95 ± 0.25^{Aa}	2.65
***M. rileyi* MTCC 10395**	0.45 ± 0.25^{Bb}	1.95 ± 0.25^{Aba}	2.45 ± 0.25^{Ba}	2.32 ± 0.37^{Aa}	2.07 ± 0.37^{Aa}	1.85
Mean	1.07	2.49	3.07	3.03	2.74	-

Mean values followed by the same letter (a, b, c) in the horizontal column are not significantly different at 0.05% level of probability using Tukey's post hoc test.
Mean values followed by the same letter (A, B, C) in the vertical column are not significantly different at 0.05% level of probability using Tukey's post hoc test
**Values are mean ± standard error of two replicates
*1 U/ml corresponds to 1 μmol of fatty acids produced min^{-1} ml^{-1}

M. rileyi NIPHM, while the minimum (1.85 U/ml) was recorded by *M. rileyi* MTCC 10395. Among individual activities on various days, the highest lipase activity was recorded by *M. rileyi* NIPHM after 6 days of inoculation (3.57 U/ml), followed by the same isolate on the 8th day (3.45 U/ml), which was statistically at par with *M. rileyi* MTCC 4254 (3.32 U/ml) on the same day. However, the minimum lipase activity (0.45 U/ml) was recorded by *M. rileyi* MTCC 10395 on the 2nd day after inoculation.

Correlation between mortality rates and enzyme activities

The larval mortality rate of *S. litura* was correlated with the enzymatic activity of *M. rileyi* (Table 5). The correlation coefficient (*r*) obtained between mortality and enzyme activities, i.e. chitinase, protease and lipase, showed positive correlations for all isolates.

Discussion

A variation in mortality was recorded among different isolates of *M. rileyi*. This could be due to the process of infection and development of disease in larvae being directly related to the specificity of fungal isolates along with the tolerance possessed by the host species (Fronza et al. 2017). The invasion of host by pathogen and germination success could be affected by the absence of certain characteristics in the fungal isolates pertaining to penetration mechanisms as well as the characteristics of host integument (Ignoffo and Garcia 1985). Virulence of EPF against a particular host depends upon the biological properties of the specific isolate-host combination along with other factors, and a variation in virulence among isolates is determined on quantitative differences in fungal growth kinetics (Anderson et al. 2011). Krutmuang and Thungrabeab (2017) screened and tested 8 different strains of the fungus *M. rileyi* for the control of *S. litura* that showed varying degrees of pathogenicity. The mortality on the 7th day post-inoculation ranged from 2.5 to 92.5%, suggesting that *M. rileyi* was overall pathogenic to *S. litura* though individual strains could be divided into those possessing high, moderate and low virulence.

Sharmila and Manjula (2015) evaluated the efficacy of *M. rileyi* formulations against the larvae of *Spodoptera litura* and *Helicoverpa armigera* (Hubner). They reported 65.35% larval reduction at *S. litura* with talc-based formulation of *M. rileyi*. Similar results were obtained in the present study where the maximum larval mortality (63.33%) was recorded with *M. rileyi* NIPHM isolate. Mortality was also observed to be increased with rising concentration of formulation possibly due to great spore load. Patil et al. (2014) reported the percent mortality of 64.48% against 2nd instar larvae of *S. litura* when applied with 10^8 conidia/l of *M. rileyi*. However, the values of percent mortality observed by them on various days ranged from 33.68 to 87.50% after 5 and 8 days of applications.

The main barrier for the penetration of a fungus into the host insect is created by its cuticle. To dissolve this barrier, fungi produce different cuticle-degrading enzymes like chitinase, protease and lipase (de Moraes et al. 2003). Studies suggested that penetration and infection of the host was the result of a combined effect of enzymatic degradation and mechanical pressure. Endo- and exo-chitinases are the chitin hydrolases that have been detected in numerous EPF like *Beauveria bassiana*, *Metarhizium anisopliae* and *M. rileyi* (El-Sayed et al. 1989).

Table 5 Correlation between percent mortality and enzymatic activities of *Metarhizium rileyi* isolates

Mortality with fungal isolates	Chitinase	Protease	Lipase
M. rileyi NIPHM	$r = 0.87$, $p = 0.13$	$r = 0.99$, $p = 0.008$	$r = 0.85$, $p = 0.15$
M. rileyi MTCC 4254	$r = 0.92$, $p = 0.07$	$r = 0.98$, $p = 0.016$	$r = 0.88$, $p = 0.11$
M. rileyi MTCC 10395	$r = 0.96$, $p = 0.03$	$r = 0.92$, $p = 0.07$	$r = 0.92$, $p = 0.08$

r correlation coefficient, *p* *p*-value

Among exo-chitinases, N-acetylglucosaminidase was the most prominent one as it was known to release acetylglucosamine from the reducing ends of chitin chain (Ramanujam et al. 2011). El-Sayed et al. (1989) studied the chitinolytic activity of a native and 2 mutant isolates of *M. rileyi* and recorded endo- and exo-chitinase activities during different developmental stages of the fungus on various days. They observed the greatest difference in chitinolytic activity during the germination stage as the exo-chitinase activity in the virulent isolates was 15–18 times higher than that in a virulent isolate. In a study on chitinase and chitobiase activity in the extracellular fluids of *M. anisopliae*, a chitinase activity of 1.32 μmol NAG released $ml^{-1}\,h^{-1}$ was reported when colloidal chitin was used as substrate (St. Leger et al. 1991).

Extracellular proteases are produced in EPF during the host infection process to degrade the host cuticle and are also suggested to be determinants of virulence (Nunes et al. 2010). The production of cuticle-degrading proteases in *M. rileyi* and its virulence against *Anticarsia gemmatalis* was studied by Nunes et al. (2010), where they observed a protease activity of 1.40 U/ml after 192 h, when grown on minimal media containing casein, which decreased to an activity of 0.94 U/ml after 216 h. Upon analysing the correlation between the mortality percentage and Pr1-like protease activity, they suggested a positive correlation between them, which might indicate the importance of this enzyme in pathogenicity although it depends upon genetic variability among different strains. Similar observations were recorded during the present study with the maximum mean protease activity (2.28 U/ml) obtained on the 8th day, after which it decreased slightly. Saleem and Ibrahim (2019) reported the protease activity of *B. bassiana* (2.61 U/ml), *M. anisopliae* (2.97 U/ml) and *L. lecanii* (2.49 U/ml) was the highest on the 6th day after inoculation and incubation. During the infection of insects by *M. rileyi*, lipases hydrolyse the ester bonds of fats, waxes and lipoproteins and act as important cuticle-degrading enzymes. The lipase production on basal medium (containing olive oil) was observed to rise sharply up to 5 days further increasing gradually till 8 days and then decreased as it reached the 10th day. Maximum lipase production (20.8 U/ml) was recorded on the 8th day (Supakdamrongkul et al. 2010). It was also inferred from the present study that the maximum rise in lipase activity could be observed from 6th to 8th day after inoculation beyond which it decreased slightly till the 10th day in all the isolates. Further, correlation analysis between mortality of *S. litura* larvae and cuticle-degrading enzyme activities of *M. rileyi* isolates showed a positive correlation between them which suggests the possible role of these enzymes in virulence of *M. rileyi*.

Conclusions

Obtained results showed that the EPF *M. rileyi* NIPHM formulation was the most pathogenic to *S. litura* larvae, when applied at 12 g/l. The extracellular enzyme activity was also recorded high in *M. rileyi* NIPHM. Further, the mortality and enzyme activity showed a positive correlation between them. The results presented in this study increase the knowledge about cuticle-degrading enzyme production in *M. rileyi* and open new avenues for studies regarding the role of these enzymes in virulence against *S. litura* larvae.

Abbreviations

SMAY: Sabouraud maltose agar media with yeast extract; cfu: Colony-forming unit; MTCC: Microbial Type Culture Collection and Gene Bank; ANOVA: Analysis of variance; CRD: Completely randomized design; CD: Critical difference

Acknowledgements

The authors are thankful to Head, Department of Entomology, Punjab Agricultural University, for providing the facility to conduct the present experiment.

Authors' contributions

GKG carried out the experiments, recorded the data, interpreted the results and wrote the manuscript. NJ designed and supervised the experiments, provided technical guidance and edited the manuscript. YS provided technical guidance. All authors read and approved the final manuscript during the present study.

Funding

Not applicable

Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 17 December 2020 Accepted: 23 March 2021
Published online: 02 April 2021

References

Ahmad M, Arif MI, Ahmad M (2007) Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Prot 26(6):809–817. https://doi.org/10.1016/j.cropro.2006.07.006

Anderson RD, Bell AS, Blanford S, Paaijmans KP, Thomas MB (2011) Comparative growth kinetics and virulence of four different isolates of entomopathogenic fungi in the house fly (*Musca domestica* L.). J Invertebr Pathol 107(3):179–184. https://doi.org/10.1016/j.jip.2011.04.004

Boucias DG, Schoborg EA, Allen GE (1982) The relative susceptibility of six noctuid species to infection by *Nomuraea rileyi* isolated from *Anticarsa gemmatalis*. J Invertebr Pathol 39(2):238–240. https://doi.org/10.1016/0022-2011(82)90017-9

de Moraes CK, Schrank A, Vainstein MH (2003) Regulation of extracellular chitinase and proteases in the entomopathogen and acaricide *Metarhizium anisopliae*. Curr Microbiol 46(3):205–210. https://doi.org/10.1007/s00284-002-3863-x

Devi PSV, Prasad YG, Chowdary DA, Rao LM, Balakrishnan K (2003) Identification of virulent isolates of the entomopathogenic fungus *Nomuraea rileyi* (F) Samson for the management of *Helicoverpa armigera* and *Spodoptera litura*. Mycopathologia 156(4):365–373. https://doi.org/10.1023/B:MYCO.0000003578.38483.34

Dhakal R, Singh DN (2019) Biopesticides: a key to sustainable agriculture. Int J Pure App Biosci 7(3):391–396

Dhawan M, Joshi N (2017) Enzymatic comparison and mortality of *Beauveria bassiana* against cabbage caterpillar *Pieris brassicae* LINN. Braz J Microbiol 48(3):522–529. https://doi.org/10.1016/j.bjm.2016.08.004

El-Sayed GN, Coudron TA, Ignoffo CM, Riba G (1989) Chitinolytic activity and virulence associated with native and mutant isolates of an entomopathogenic fungus, *Nomuraea rileyi*. J Invertebr Pathol 54(3):394–403. https://doi.org/10.1016/0022-2011(89)90124-9

Fronza E, Specht A, Heinzen H, Monteiro de Barros N (2017) *Metarhizium (Nomuraea) rileyi* as biological control agent. Biocontrol Sci Technol 27(11):1243–1264. https://doi.org/10.1080/09583157.2017.1391175

Ignoffo CM, Garcia C (1985) Host spectrum and relative virulence of an Ecuadoran and a Mississippian biotype of *Nomuraea rileyi*. J Invertebr Pathol 45(3):346–352. https://doi.org/10.1016/0022-2011(85)90113-2

Kaur A, Joshi N (2014) Conidial production of *Beauveria bassiana* on agricultural products and effect of storage temperature on its formulation. Afr J Microbiol Res 8(34):3164–3170. https://doi.org/10.5897/AJMR2013.6501

Kepler RM, Humber RA, Bischoff JF, Rehner SA (2014) Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia 106(4):811–829. https://doi.org/10.3852/13-319

Krutmuang P, Thungrabeab M (2017) Efficacy of the entomopathogenic fungus *Nomuraea rileyi* in the biological control of vegetable pest *Spodoptera litura* (Lepidoptera: Noctuidae). Int J Environ Rural Dev 8(2):8–12

Liu S, Xu Z, Wang X, Zhao L, Wang G, Li X, Zhang L (2019) Pathogenicity and in vivo development of *Metarhizium rileyi* against *Spodoptera litura* (Lepidoptera: Noctuidae) Larvae. J Econ Entomol 112(4):1598–1603. https://doi.org/10.1093/jee/toz098

Nahar P, Ghormade V, Deshpande MV (2004) The extracellular constitutive production of chitin deacetylase in *Metarhizium anisopliae*: possible edge to entomopathogenic fungi in biological control of insect pest. J Invertebr Pathol 85(2):80–88. https://doi.org/10.1016/j.jip.2003.11.006

Namasivayam SKR, Arvind BRS (2015) Biocontrol potential of entomopathogenic fungi *Nomuraea rileyi* (f.) Samson against major groundnut defoliator *Spodoptera litura* (Fab.) Lepidoptera; Noctuidae. Adv Plants Agric Res 2(5):221–225

Nunes ARF, Martins JN, Furlaneto MC, de Barros NM (2010) Production of cuticle-degrading proteases by *Nomuraea rileyi* and its virulence against *Anticarsia gemmatalis*. Ciênc Rural 40(9):1853–1859. https://doi.org/10.1590/S0103-84782010005000149

Patil RK, Bhagat YS, Halappa B, Bhat RS (2014) Evaluation of entomopathogenic fungus, Nomuraea rileyi (Farlow) Samson for the control of groundnut Spodoptera litura (F.) and its compatibility with synthetic and botanical pesticides. J Biopest 7(Supp.):106–115

Petrisor C, Stoian G (2017) The role of hydrolytic enzymes produced by entomopathogenic fungi in pathogenesis of insects mini review. Romanian J Plant Prot 10:66–72

Pignede G, Wang H, Fudalei F, Gaillardia C, Semon M, Nicaud JM (2000) Characterisation of an extra lipase encoded by L1P2 in *Yarrowia lipolytica*. J Bacteriol 182(10):2802–2810. https://doi.org/10.1128/JB.182.10.2802-2810.2000

Ramanujam B, Balachander M, Roopa G, Rangeshwaran R, Karmakar P (2011) Chitinase activity and virulence of different isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium* spp. J Biol Control 25(3):223–228

Saleem A, Ibrahim RA (2019) Assessment of the virulence and proteolytic activity of three native entomopathogenic fungi against the larvae of *Oryctes agamemnon* (Burmeister) (Coleoptera: Scarabaeidae). Egypt J Biol Pest Control 29(1):21. https://doi.org/10.1186/s41938-019-0120-1

Sharmila T, Manjula K (2015) Field evaluation of oil formulations of *Nomuraea rileyi* (Farlow) Samson against *Spodoptera litura* and *Helicoverpa armigera* in groundnut. Int J Plant Protec 8(1):142–147. https://doi.org/10.15740/HAS/IJPP/8.1/142-147

Singh H, Joshi N (2020) Management of the aphid, *Myzus persicae* (Sulzer) and the whitefly, *Bemisia tabaci* (Gennadius), using biorational on capsicum under protected cultivation in India. Egypt J Biol Pest Control 30(1):67. https://doi.org/10.1186/s41938-020-00266-5

Somogyi M (1952) Notes on sugar determination. J BiolChem 195:19–23

Srivastava K, Sharma D, Anal AKD, Sharma S (2018) Integrated management of *Spodoptera litura*: a review. Int J Life Sci Scienti Res 4(1):1536–1538

St. Leger RJ, Cooper RM, Charnley AK (1991) Characterization of chitinase and chitobiase produced by the entomopathogenic fungus *Metarhizium anisopliae*. J Invertebr Pathol 58(3):415–426. https://doi.org/10.1016/0022-2011(91)90188-V

Supakdamrongkul P, Bhumiratana A, Wiwat C (2010) Characterization of an extracellular lipase from the biocontrol fungus, *Nomuraea rileyi* MJ, and its toxicity toward *Spodoptera litura*. J Invertebr Pathol 105(3):228–235. https://doi.org/10.1016/j.jip.2010.06.011

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