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Pathogenicity Assessment of *Isaria javanica* (Frider. & Bally) Samson & Hywel - Jones isolates against *Spodoptera litura* Fabr.

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ABSTRACT: Eighteen *Isaria javanica* were collected in Pu Mat National Park, Nghe An province, Vietnam. Eighteen *Isaria javanica* isolates were used to develop a novel screening method aimed at selecting strains with the highest biocontrol potential. Based on five parameters: percent mortality, percent extrusion mycelium, average survival time, fungal life cycle duration and spore production. Only four strains merited further study: VN1472, VN1487, VN1801, VN1802. The life cycle of *Isaria javanica* on *Spodoptera litura* larvae includes pathogenesis phase and saprogenesis phase and has five steps. The pathogenesis phases begins with the formation of a germ tube, cuticle penetration and invasion throughout the insect body followed by death and has one step; the saprogenesis phase begins after the insect has died until spores begin to discharge and has four steps. They pathogenicity against *Isaria javanica* show that this fungus is a promising biocontrol agent to control of *Spodoptera litura*.

INTRODUCTION

Fungus kills insects are a common phenomenon in nature. Along with other groups of natural enemies, entomopathogenic fungi have an important role in regulating the amount of this class of animals everywhere. Despite the focused, but the study and use of entomopathogenic fungi have not achieved desired results, the efficiency is low pathogenic and unstable. There are many reasons for this situation, the most visible problem is studied entomopathogenic fungi have only paid attention to the production and use of surface preparations without due regard to the basic study as infection, pathogenic of fungi parasitic on insects, the relationship between an entomopathogenic fungi and its host in relation to the relevant factors. There are the essential knowledge, science as a basis for the evaluation and selection of potential isolates as well as proposed measures to improve impact efficiency for entomopathogenic fungi.

In the world, research on insect pathogenic fungi have gained certain achievements as the basis for selection of potential of entomopathogenic fungi isolates (Butt and Goettel, 2000; Posada and Vega, 2005; Tian *et al*., 2008; Vega and Posada, 2008; Vijayavani *et al*., 2009, Joseph *et al*., 2010). So far in Vietnam, only a few entomopathogenic fungi were researched and application of biological control agents as *Beauveria bassiana*, *Metarhizium anisopliae* (Pham *et al*., 2004, 2005; Dam *et al*., 2007); the basic research on insect pathogenic fungi has received little attention. In particular, fungus *Isaria javanica* collected in Pu Mat National Park, Nghe An province were evaluated to be promising in biocontrol, but so far there has been little studied of biocontrol insect (Nguyen *et al*., 2011; Nguyen *et al*., 2016).

Spodoptera litura (Fabr.) is dangerous insect pest to agricultural crop which is a polyphagous pest of many economically important crops and has been recorded from over 40 plant families. It is distributed most countries in the world. In Vietnam *S. litura* is one of the most important insect pests of many crops and chemical method of control are not effective. Towards using biological pesticides by entomopathogenic fungi is useful solutions and are increasingly interested. In this paper we present a method to evaluate candidate fungal entomopathogens based on five parameters including: percent insect mortality, percent extrusion mycelium, average survival time, fungal life cycle duration and spore production.

Based on these studies of inoculation and pathogenesis ability of *I. javanica* on *S. podoptera litura* in the relevant factors related, which evaluate, choose the potential specimen as well as proposals the impact of measures to improve the efficiency of application of biological products from fungal entomopathogens in plant protection.

MATERIALS AND METHODS

A. Fungi isolates

Fungus *Isaria javanica* (Frider. & Bally) Samson & Hywel-Jones (Clavicipitacea: Hypocrales) were collected in Pu Mat National Park, Nghe An province, Vietnam including 18 isolates: VN1359, VN1362, VN1366, VN1472, VN1477, VN1482, VN1487, VN1491, VN1493, VN1636, VN1701, VN1801, VN1802, VN1803, VN1911, VN1912, VN2002, VN2009. Single spore cultures for each isolate of *I. javanica* isolates were initiated on potato dextrose agar (PDA) and stored on slopes in Entomopathogenic fungi Laboratory, Faculty of Agriculture Forestry and Fisheries, Vinh University, Nghe An province, Vietnam. Spores sources of fungus *I. javanica* were cultured on potato dextrose liquid medium for 13 to 15 days mass produce of spore on mixture 150g brown rice and 3g silkworm pupae powdered solid medium into a 500ml plastic box for about 15-20 days at 25±2^{o}C, 70±5% humidity. The concentration of the spore suspension was adjusted using sterilized distilled water to 10^7 spores/ml and added with 0.2 ml of 0.02% Tween 20 solution to uniformly disperse the spore suspension (Posada and Vega, 2005; Vega *et al*., 2008; Alexandre, 2009; Barta, 2010).

B. Insect host

Spodoptera litura Fabr. generation of parents were collected on the peanut fields in Nghi Loc district, Nghe An province, Vietnam. All adult insects were cultured at 25±2^{o}C temperature, 70±5% humidity for progeny experiments. *S. litura* used in the bioassays were about 2-3th larval instar and randomly selected to layout used for experiment.

C. Experimental Design

The experiment included 18 isolates of *I. javanica* was conducted as a completely randomized design with 3 replicates at 25±2^{o}C temperature, 70±5% humidity, 10 larvae of 2-3th instar/plastic box (25 × 15cm); and spray solution with 3 ml dose of *I. javanica* 10^7 spores/ml into a plastic box; controlled spray of distilled water. Dead insects from different box experiment, are kept separately in each petri dishes that contained a piece of filter paper kept moist; indicators tracked daily until to complete life cycle of fungi (Posada and Vega, 2005; Vega *et al*., 2008; Alexandre, 2009; Barta, 2010).

D. Statistical analyses

The data were subjected to ANOVA using IRRISTAT version 5.0.

RESULTS

Spore germination at 24 h, and all isolates had germinated at 48 h. Spore suspensions of 1×10^7 spores ml^{-1} *I. javanica* caused high *S. litura* mortality. *S. litura* mortality was ranged between 45.40-81.48%, with two of the isolates reaching up to 80% mortality (VN1472 and VN 1487) and four isolates causing up to 70% mortality (VN1491, VN1801, VN1802 and VN2009). In eighteen of the isolates tested there was a high mortality within 3 days after the first insect died. There were significant differences in mortality due to isolates reached (p=0.05). Average survival time ranged between 3.43-8.50 days. Four isolates caused 76,60-81,48% *S. litura* mortality within 3,30 and 3,97 days, while five isolates took longer than 7 days to kill the insects (Table 1, Table 2).

Spodoptera litura extrusion mycelium was ranged between 37,03-87,11%, with four of the isolates reaching over 80% extrusion (VN1472, VN 1487, VN1801 and VN1802), while six of the isolates reaching less than 50% extrusion (Table 1). There were significant differences in extrusion mycelium due to isolates reached (p= 0.05). In eighteen of the isolates tested there was a high extrusion mycelium within 3 and 4 days after the insect died.

The life cycle of *I. javanica* isolates on *S. litura* which includes pathogenesis phase and saprogenesis phase, was completed in 12.46-17.42 days, and 44,44% of the isolates completed their life cycle in less than 15 days. The pathogenesis phase ranged between 2.08 to 3.42 days. The saprogenesis life cycle ranged between 10.30 to 14.07 days (Table 2, Fig. 1). The pathogenesis phase has 1st step: (1) Spray to death begins with the formation of a germ tube, cuticle penetration and invasion throughout the insect followed by death 2.08-3.42 days. The saprogenesis phase begins after the insect has died with has 2-5th step: (2) Death to mycelium start to appear through the cuticle 2.92-4.26 days; (3) Start mycelium to spore formation 2.48-3.67 days; (4) Spore formation to totally mycelium covered 2.37-3.69 days; and (5) Mycelium covered to spore discharge 2.16-3.22 days (Table 2, Fig. 1).

Most of the study isolates 2nd stage from death to start mycelium and 4th stage from spore formation to totally mycelium covered are longer of growth duration than 3th stages; 5st stage from mycelium covered to spore discharge is the shortest of growth duration. Duration of the life cycle of *I. javanica* isolates on *S. litura* is also different of the isolates. In particular, three isolates of VN1487, VN1801 and VN1802 were the shortest of their life cycle duration on *S. litura* ((Table 1, Fig. 1).

Fourteen isolates *I. javanica* produced more than 1×10^7 spores per *S. litura*, and four *I. javanica* isolates produced between 1×10^6 and 1×10^7 spores per *S. litura*. There were significant differences in extrusion mycelium due to isolates reached (p= 0.05) (Table 1).

Table 1: Parameters used for scoring of eighteen *Isaria javanica* isolates.

Isolates code	Parameters used for scoring (TB ± SD)				
	Mortality (%)	Extrusion mycelium (%)	Average survival time (days)	Fungal life cycle duration (days)	Spore production (spores insect-1)
VN1359	55.56±3.78	44.44±4.40	7.37±0.40	17.08±0.48	$5.9x10^6$±$4.5x10^5$
VN1362	65.76±4.47	58.20±5.61	8.50±0.46	17.16±0.48	$3.1x10^7$±2.4 $x10^6$
VN1366	51.85±3.53	37.03±3.67	6.67±0.36	15.24±0.43	$4.0x10^7$±3.1 $x10^6$
VN1472	80.43±5.47	78.11±7.73	3.50±0.19	13.65±0.38	$6.8x10^7$±$5.2x10^6$
VN1477	48.15±3.27	40.74±4.03	6.60±0.36	16.27±0.46	$4.3x10^7$±$3.3x10^6$
VN1482	66.67±4.53	59.26±5.87	6.13±0.33	14.56±0.41	$5.2x10^7$±4.0 $x10^6$
VN1487	81.48±5.54	74.08±7.33	3.43±0.19	12.79±0.36	$7.1x10^7$±$5.5x10^6$
VN1491	74.08±5.04	62.97±6.23	5.57±0.30	13.04±0.37	$6.5x10^7$±$5.0x10^6$
VN1493	55.50±3.77	51.85±5.13	7.43±0.40	16.90±0.47	$6.1x10^6$±$4.7x10^5$
VN1636	45.40±3.09	39.36±3.90	8.20±0.44	17.32±0.49	$6.7x10^6$±5.2 $x10^5$
VN1701	54.06±2.88	52.53±5.20	7.50±0.41	17.42±0.49	7.3 $x10^6$±$5.6x10^5$
VN1801	77.78±5.29	75.67±7.49	3.30±0.18	12.46±0.35	$6.7x10^7$±$5.1x10^6$
VN1802	76.60±5.21	70.68±7.69	3.97±0.21	12.80±0.31	$6.3x10^7$±$4.9x10^6$
VN1803	68.73±5.79	55.56±5.50	4.70±0.25	13.69±0.38	$4.2x10^7$±$3.2x10^6$
VN1911	50.53±3.44	42.03±4.16	5.70±0.31	15.59±0.44	$6.7x10^7$±$5.2x10^6$
VN1912	53.55±3.64	45.06±5.45	5.43±0.29	15.53±0.43	$4.4x10^7$±$3.4x10^6$
VN2002	64.60±4.39	52.61±5.21	4.60±0.25	15.78±0.44	$4.1x10^7$±$3.2x10^6$
VN2009	70.37±4.79	57.23±5.67	4.83±0.26	14.78±0.41	$4.5x10^7$±$3.4x10^6$
$LSD_{0.05}$	7.20	9.28	0.51	0.70	0.55
CV%	6.80	9.90	5.40	2.80	7.70

X: Mean values, SD: Standard deviation; $LSD_{0.05}$: The least significant difference (95%); CV: Coefficient of variation (%)

Table 2: Duration of the life cycle of *Isaria javanica* isolates on *Spodoptera litura.*

Strains code	Growth stages duration (X±SD) (days)					
	Spray to death	Death to start mycelium	Start mycelium to spore formation	Spore formation to totally mycelium covered	Mycelium covered to spore discharge	Life cycle duration
VN1359	3.39±0.16	4.15±0.27	3.52±0.19	3.67±0.19	2.35±0.15	17.08±0.48
VN1362	3.26±0.15	4.17±0.27	3.62±0.20	3.62±0.19	2.49±0.16	17.16±0.48
VN1366	3.04±0.14	3.69±0.24	3.12±0.17	3.24±0.17	2.16±0.14	15.24±0.43
VN1472	2.18±0.11	3.15±0.22	3.02±0.17	2.73±0.14	2.37±0.15	13.45±0.38
VN1477	3.11±0.14	3.87±0.25	3.56±0.20	3.29±0.17	2.43±0.16	16.27±0.46
VN1482	2.16±0.10	3.28±0.21	3.17±0.17	3.17±0.16	2.79±0.18	14.56±0.41
VN1487	2.08±0.09	2.92±0.19	2.82±0.15	2.63±0.14	2.38±0.15	12.79±0.36
VN1491	2.33±0.11	2.79±0.18	2.70±0.15	2.80±0.15	2.42±0.15	13.04±0.37
VN1493	3.14±0.14	3.88±0.25	3.34±0.18	3.59±0.19	2.95±0.19	16.90±0.47
VN1636	3.24±0.15	4.26±0.28	3.67±0.20	3.66±0.19	2.50±0.16	17.32±0.49
VN1701	3.42±0.16	4.20±0.27	3.55±0.20	3.71±0.19	2.55±0.16	17.42±0.49
VN1801	2.13±0.10	2.89±0.19	2.48±0.14	2.55±0.13	2.41±0.15	12.46±0.35
VN1802	2.24±0.10	2.97±0.19	2.75±0.16	2.37±0.12	2.47±0.1	12.80±0.31
VN1803	2.50±0.12	3.04±0.20	2.81±0.15	2.88±0.15	2.46±0.16	13.69±0.38
VN1911	3.09±0.14	3.76±0.24	2.74±0.15	3.23±0.17	2.77±0.18	15.59±0.44
VN1912	3.07±0.14	3.37±0.22	2.71±0.15	3.29±0.17	3.09±0.20	15.53±0.43
VN2002	3.15±1.15	3.11±0.20	2.99±0.16	3.31±0.17	3.22±0.21	15.78±0.44
VN2009	2.59±0.12	3.33±0.22	2.83±0.16	3.14±0.16	2.89±0.18	14.78±0.41
$LSD_{0.05}$	0.21	0.38	0.28	0.27	0.27	0.70
CV%	4.60	6.50	5.50	5.20	6.40	2.80

X: Mean values, SD: Standard deviation; $LSD_{0.05}$: The least significant difference (95%); CV: Coefficient of variation (%)

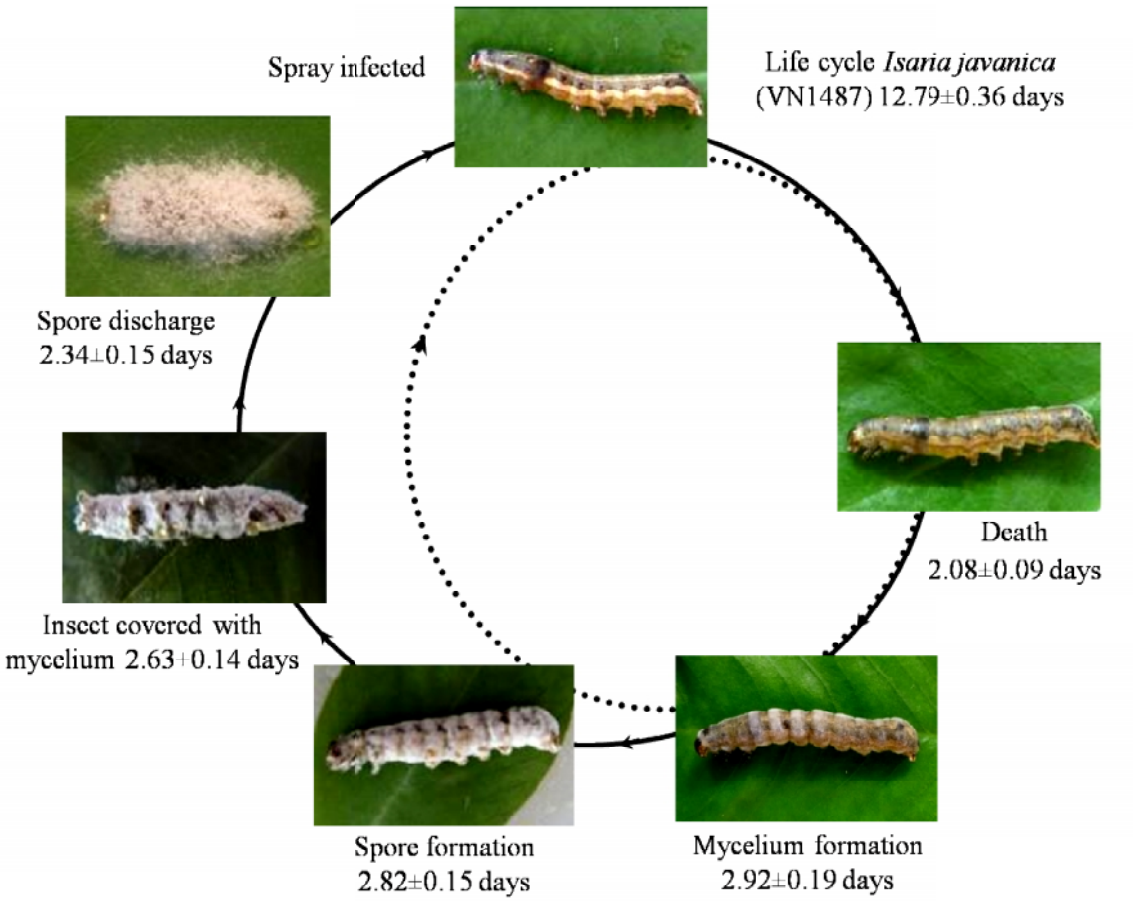

Fig. 1. Life cycle for *Isaria javanica* VN1487 strain on *Spodoptera litura.*

DISCUSSION

Eighteen *I. javanica* isolates were used to develop a novel screening method aimed at selecting strains with the highest biocontrol potential. When conducting *S. litura* bioassays with *I. javanica* can be used various spore application methods, such as dipping the insects in the spore suspension and spraying either the berries or leaves for subsequent spore pickup by the insect, or spraying the insects directly (Posada and Vega, 2005).

The life cycle of *I. javanica* on *S. litura* larvae includes pathogenesis phase and saprogenesis phase and has five steps. The pathogenesis phases has one step: (1) Spray to death begins with the formation of a germ tube, cuticle penetration and invasion throughout the insect followed by death. The saprogenesis phase begins after the insect has died until spores begin to discharge and has four steps: (2) Death to mycelium start to appear through the cuticle; (3) Start mycelium to spore formation; (4) Spore formation to totally mycelium covered; and (5) Mycelium covered to spore discharge. The *I. javianica* isolates with a fast kill are usually selected for subsequent assessment and possible field use even.

Some of the species caused rapid mortality shortly after exposure while others took much longer to kill the insect. The life cycle of *I. javanica* isolates on *S. litura* larvae were average leve that comparision with some other fungal entomopathogens. The life cycle of *Nomuraea rileyi* on *S. litura* larvae was completed in 8–9 days (Pornpoj *et al*., 2005); the life cycle of *Beauveria bassiana* on *Hypothenemus hampei* was completed in 8.4–12.4 days (Posada and Vega, 2005); while *Clonostachy rosea* took longer than 16.4 days to completed the life cycle on *Hypothenemus hampei* (Vega *et al*., 2008).

Spore germination rates are known to have very important consequences for insect infection (Posada and Vega, 2005). Spores formation of *I. javanica* was fast after the mycelium start to appear through the cuticle 2–3 days, and produced more than 1×10^7 spores per *S. litura*, and equivalent to spores formation of *B. bassiana* produced on *Hypothenemus hampei* (Posada và Vega, 2005), or spores formation of *M. anisopliae* produced on *Heterotermes tenuis* (Alcides *et. al.*, 2002).

I. javanica produced spores befor to totally mycelium covered *S. litura*. Spores production on the cadaver could provide a fresh source of fungal inoculum directly in the agroecosystem. The fungus is passing through the host to be necessary to maintain the virulence of the isolates.

Based on five parameters: percent mortality, percent extrusion mycelium, average survival time, fungal life cycle duration and spore production, of eighteen strains we evaluated, only four strains show some potential as biocontrol agents: VN1472, VN1487, VN1801, VN1802. Fungal entomopathogen isolates that provide a fast kill and produce a high number of spores in the insect cadaver can play an important role in causing natural epizootics, thus reducing *S. litura* populations and leading to a more sustainable agricultural system.

CONCLUSION

(1) *Isaria javanica* caused high *Spodoptera litura* mortality. *S. litura* mortality was ranged between 45.40-81.48%. Based on five parameters percent mortality, percent extrusion mycelium, average survival time, fungal life cycle duration and spore production there are four strains show some potential as biocontrol agents on *S. litura:* VN1472, VN1487, VN1801, VN1802.

(2) The life cycle of *I. javanica* isolates on *S. litura* which includes pathogenesis phase and saprogenesis phase, was completed in 12.46-17.42 days.

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