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Morphological and molecular characterization of fungal entomopathogen *Isaria fumosorosea* (Wize) Brown and Smith as bioinsecticide against cucurbit leafworm, *Diaphania indica* Saunders (Lepidoptera: Pyralidae), in cucumber

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Abstract

This study aimed to: (1) isolate entomopathogenic fungi from infected host insects; (2) conduct bioassay of different entomopathogenic fungi (EPF) isolates against the cucurbit leafworm, *Diaphania indica*.; and (3) identify the most potential EPF isolate using morphological and molecular characterization. Four entomopathogenic fungi isolates that showed positive infection to *D. indica* larvae were evaluated. The most virulent isolate was subjected to morphological and molecular characterization. Results showed that EPF Isolate 1 is highly virulent to *D. indica* larvae with 97.5-100% mortality in the 2nd to 4th instar larvae. EPF Isolate 1 was first to show infection 1.38±0.41 days after inoculation, and larvae were entirely covered with white mycelia 5.25±0.41 days. Molecular identification revealed the isolate as *Isaria fumosorosea* as indicated by molecular marker internal transcribed space region of 5.8s (ITS-5.8s) ribosomal DNA. EPF Isolate 1 was identified as *Isaria fomosorosea.* It is the most virulent and potential isolate against cucurbit leafworm, *D. indica*.

Keywords: Entomopathogenic fungi (EPF), *Isaria fumosorosea*, *Diaphania indica*, bioassay

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is grown for its immature fruits used in salads (slicing type) or soaked in brine for pickles. Cucumber total production area in the country has 1,587 hectares, of which 140 ha are found in Region 2, where 40 ha are in Nueva Vizcaya (Narciso, 2005). Cucurbit leafworm, *Diaphania indica* Saunders (Lepidoptera: Pyralidae), also known as the cotton caterpillar and pumpkin caterpillar, is the major insect pest of cucumber, a polyphagous pest that is particularly serious on cucurbits. Larvae mainly attack leaves but also infest flowers and fruits that cause considerable yield loss during the outbreak. The use of synthetic pesticides is the only control method employed by farmers to suppress this pest. This practice causes some unfortunate consequences like environmental pollution, pest resistance, and toxicity to non-target organisms. To alleviate the fear of the hazardous effect of chemical residues on human and animal health and to address food safety, the use of entomopathogenic microorganisms such as bacteria, viruses, and fungi is of considerable importance. Entomopathogenic fungi (EPF) directly infect through insect cuticle and do not require ingestion to cause infection. Among the most intensively investigated genera are *Metarhizium*, *Beauveria*, *Paecilomyces*, *Isaria*, *Aspergillus*, *Hirsutella*, *Nomuraea*, *Culicinomyces*, *Tolypocladium*, and *Verticillium* (Stark and Banks, 2003). According to Jiang et al. (2007), *Beauveria bassiana* is considered an essential entomopathogenic fungi due to its broad host range and pathogenicity route to infect various host insects. However, *Isaria fumosorosea* is considered as an emerging entomopathogen in terms of its utilization in biological control. Hence, this study aimed to isolate entomopathogenic fungi from infected host insects, to conduct bioassay of different entomopathogenic fungi (EPF) isolates against the cucurbit leafworm, *D. indica*.; and to identify the most potential EPF isolate using morphological and molecular characterization.

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MATERIALS AND METHODS

Collection and Isolation of Entomopathogenic Fungi (EPF)

Lepidopterous larvae and other insects showing white mycelium suspected as entomopathogenic fungi were collected from Science City of Muñoz. Nueva Ecija and Bayombong, Nueva Vizcaya, Philippines. These fungi were brought to the laboratory for isolation. Infected insects were rinsed separately with sterile distilled water before placing them inside the laminar flow hood. Bits of mycelia from infected insects' surface were transferred aseptically into previously plated potato dextrose agar (PDA:Merck). Plates were incubated at room temperature for one week. Series of transfers to attain a pure culture of the desired organism was done.

Pathogenicity Test of Isolated Entomopathogenic Fungi (EPF)

A pathogenicity test to determine infection was done in each of the isolates on cucurbit leafworm larvae. Ten 2nd instar larvae were allowed to crawl inside a 15-day old PDA plate culture of each isolate for 45 minutes. After that, these were picked using flame-sterilized forceps and placed individually in a transparent plastic container lined with tissue paper moistened with 3-4 drops of sterile water. Fresh cucumber leaf with moistened cotton placed at the leaf's stalk was provided in each plastic container. The plastic cover was provided with small holes for aeration.

Symptoms of infection were observed daily for seven days. When symptom like cessation of feeding or appearance of brown spots on the larvae was observed, the isolate was marked positive, otherwise marked negative. Isolates with positive results and high percent mortality were further evaluated. Reisolation of the EPF from infected larvae was done.

Establishment of Cucumber Plants and Mass Rearing of Test Insect

A 6m x 5m netted house was constructed for the mass rearing of *D. indica*. The netted house was enclosed with a mesh screen net (32x32 mesh) to ensure that the test insect was not attacked by any natural enemies that would limit mass production. Larvae of *D. indica* gathered from a cucumber field were introduced to enclosed plants to start mass rearing. Staggered planting of cucumber at the bi-weekly interval was done to ensure a continuous food supply for the test insects.

Larvae collected from cucumber vines in the netted house were reared in plastic containers (15cm x 25cm x 8cm) provided with net cover for proper ventilation. Twenty same instar larvae were placed inside containers. Larvae were provided with fresh young leaves, and rearing containers were cleaned daily. When larvae pupated, they were transferred to a bigger plastic container until they emerged in the adult stage. Male and female moths were paired and allowed to mate. Moths were fed with a 20% sugar solution on moistened cotton wool until they laid eggs. Adults were maintained inside the container until they died. Eggs were allowed to hatch inside a plastic container.

Bioassay of Four Potential EPF Isolates

Four EPF isolates showing positive results against *D. indica* larvae were further tested under laboratory conditions. The experiment was laid out in Randomized Complete Block Design with five treatments replicated four times. The treatments were as follows: T1 – control, T2 – EPF Isolate 1, T3 – EPF Isolate 2, T4 – EPF Isolate 3, and T5 – EPF Isolate 6.

Following the procedure of Yazdi et al. (2011), ten 2nd instar larvae of *D. indica* were allowed to crawl inside 15-day old plate culture of each isolate for 45 minutes. After that, these ten larvae were carefully picked using flame-sterilized forceps and placed individually in a round, transparent 8 cm diameter plastic container lined with tissue paper and moistened with 2-3 drops of sterile water. Fresh cucumber leaf with moistened cotton placed at the stalk was provided in each plastic container. The plastic cover was provided with small holes for aeration. Bioassay for 3rd and 4th instar larvae was done separately following the procedure as mentioned above.

Set up was left undisturbed until signs and symptoms of infection were noticed. Development of symptoms such as feeding cessation, changes in color, and other unusual behavior of treated larvae and infection signs were noted daily. Days to the appearance of infection and mortality of the larvae were also recorded.

Identification of Potential EPF

Morphological Characteristics.

EPF Isolate 1 was subjected to morphological characterization. Slide culture of the organism was prepared to examine the formation of conidiophore and conidia. Four slides and coverslips were placed inside Petri plates lined with tissue paper. A triangular-shaped plastic straw was also included inside the plates. These were sterilized in a pressure cooker at 15 psi for 20 minutes.

Using the sterilized razor blade, previously plated PDA was cut into 1 cm^2 blocks. One agar block was aseptically transferred at the center of the glass slide inside the Petri plate. Using a transfer needle, bits of mycelia from a pure culture of EPF Isolate 1 were carefully transferred into the side of the agar block. The tissue paper lining of Petri plates was moistened with four drops of sterilized water. The set-up was incubated for two weeks to develop conidiophore and conidia, then growth was observed under the photomicroscope.

Molecular Characteristics.

Molecular identification of EPF Isolate 1 followed the standard procedure for fungi. One week-old pure culture of isolation was brought to the Insect Pathology Laboratory, Crop Protection Cluster, University of the Philippines, Los Baños, and Laguna. This was allowed to grow in potato dextrose broth for 5 to 7 days.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) followed by comparing means of different treatments using Least Significant Difference (LSD) at 5% level. Correlation and regression analyses were calculated between concentration and larval mortality. Likewise, correlation analysis was calculated between relative humidity and percent mortality.

Data Gathered

Data such as suspected host insect, host plant, and place of collection of infected larvae were recorded for field collection of

isolates. The pathogenicity testing of the collected isolates included the reaction to larval infection by recording the positive and negative reaction of larvae to infection by each isolate and percent larval mortality by counting the number of dead larvae divided by the total number of test larvae multiplied by 100 taken daily after immunization.

The bioassay of four isolates included: Symptoms and days to the appearance of infection were done by daily observation to develop symptoms after inoculation. Days to the appearance of infection were noted by counting the number of days from inoculation until the infection's appearance. Infection refers to the period from inoculation to the appearance of visible symptoms. Percent larval mortality was done by counting the number of dead *D. indica* larvae after inoculation with EPF divided by the total number of larvae in each plate multiplied by 100. This was transformed to corrected mortality as follows:

$$\text{Corrected Mortality} \quad \frac{X_1 - X_0}{n} \quad X \; 100$$

Where:

X_1 = number of dead larvae in the treatment
X_0 = number of dead larvae in the control
n = Total Number of larvae from the control

In identifying potential EPF evaluated: Morphological characteristics were done through a slide culture of the EPF. Arrangement of conidia and formation of conidiophore were noted under a microscope. Molecular characteristics were done by analyzing the DNA sequence of the organism to determine its genetic identity.

RESULTS AND DISCUSSION

Field Collection of Isolates

Eight EPF were isolated from infected insects collected from the field (Table 1). EPF Isolate 1 was isolated from infected cucurbit leafworm larva in bitter gourd. EPF Isolate 2 was isolated from pod borer of pole sitao, EPF Isolate 3 from rice bug, EPF Isolate 4 from citrus larva, EPF Isolate 5 cutworm in eggplant, EPF Isolate 6 from citrus larva, EPF Isolate 7 from tomato fruitworm, and Isolate eight from green leafhopper. Three isolates were collected from the Science City of Muñoz, Nueva Ecija and Bayombong, Nueva Vizcaya, Philippines.

Table 1. Possible host insect, host plant, place of collection, and date of collection of the eight entomopathogenic fungal isolates

EPF* Isolates	Possible Host Insect	Host Plant	Date of Collection
EPF Isolate 1	Cucurbit leafworm	Bitter gourd	01.19.2014
EPF Isolate 2	Pod borer	Pole sitao	08.16.2013
EPF Isolate 3	Rice bug	Rice	02.26.2014
EPF Isolate 4	Citrus larva	Citrus	12.16.2013
EPF Isolate 5	Cutworm	Eggplant	08.16.2013
EPF Isolate 6	Citrus larva	Citrus	12.16.2013
EPF Isolate 7	Tomato fruitworm	Tomato	02.26.2014
EPF Isolate 8	Green leafhopper	Eggplant	01.12.2014

*EPF – Entomopathogenic fungi,

Pathogenicity Tests of EPF Isolates

Of the eight entomopathogenic fungi isolated, five have a positive reaction to the second instar larvae of *D. indica* (Table 2). These include EPF Isolate 1, EPF Isolate 2, EPF Isolate 3, EPF Isolate 4 and EPF Isolate 6. EPF Isolate 1 took five days to infect the larvae while seven days for EPF Isolate 2, EPF Isolate 3, and EPF Isolate 6. Death of larvae was observed ten days after inoculation with EPF Isolate 4. The rest of the isolates did not cause any symptoms of infection.

Table 2. Pathogenicity test of 8 entomopathogenic fungi isolates using 2nd instar larvae of *D. indica*

EPF Isolates	Reaction	Days to Mortality	Larval Mortality (%)
EPF Isolate 1	+	5	95.00[a]
EPF Isolate 2	+	7	60.00[c]
EPF Isolate 3	+	7	72.50[b]
EPF Isolate 4	+	10	32.50[d]
EPF Isolate 5	-	-	0.00[e]
EPF Isolate 6	+	7	90.00[a]
EPF Isolate 7	-	-	0.00e
EPF Isolate 8	-	-	0.00e

EPF – Entomopathogenic fungi; + infected, - not infected
Means in a column followed by the same letter did not differ significantly using LSD at 5% level

Of the five isolates, EPF Isolate 1 was the most efficient with 95% larval mortality. Statistically, EPF Isolate 1 was comparable with EPF Isolate 6 but differed from EPF Isolate 2, EPF Isolate 3, and EPF Isolate 4. EPF Isolate 5, EPF Isolate 7, and EPF Isolate 8 did not cause infection on larvae. EPF Isolate 4 showed a slow infection process and low mortality rate.

Once larvae were infected, they ceased feeding and stopped moving or sometimes sluggish. At this stage, spores of entomopathogenic fungi might have already penetrated the cuticle of the larva through enzymatic actions of protease, lipase, and chitinase that degrade corresponding chemical components of the cuticle, as was noted by St. Leger et al. (1994). According to Luangsa-ard et al. (2009), cessation of feeding is the effect of infection when the hemolymph's blastospores produce beauvericin toxin that paralyzes the larvae and subsequently death. This result agrees with the findings of Dunlap et al. (2007) where *D. indica* larvae exposed to blastospores and conidia have declined growth and had high mortality levels.

Bioassay of Four Isolates

Symptoms and signs and days to appearance of infection.

Infection with the four isolates started with feeding cessation followed by the appearance of brown or black spot color of the integument of larvae, becoming brown or black, then white. These symptoms and signs were observed in all infected larvae, regardless of an isolate.

Table 3. Days to the appearance of symptoms and days to be fully covered with white mycelia in 2nd instar larvae of *D. indica*

Epf Isolates	Days to Appearance of Symptom		Days to Fully Covered with White Mycelia	
	Range	Mean ± Sd	Range	Mean ± Sd
EPF Isolate 1	1-2	1.38±0.41[a]	4-6	5.25±0.41[a]
EPF Isolate 2	4-5	4.75±0.50[c]	8-10	9.10±0.50[c]
EPF Isolate 3	4-5	4.80±0.59[c]	9-11	9.82±0.59[c]
EPF Isolate 6	2-4	3.10±0.35[b]	7-8	7.25±0.35[b]

EPF – Entomopathogenic fungi

Means in a column followed by the same letter did not differ significantly using LSD at 5% level

Second instar larvae

When second instar larvae of *D. indica* were allowed to crawl in plated isolates, a variation on days to the appearance of symptoms and signs of infection were observed. Larvae infected with EPF Isolate 1 were first to show symptoms and signs of infection. In 1-2 days, symptoms were observed, a mean of 1.38±0.41 days after larvae were allowed to crawl on the isolates (Table 3). Infected larvae were entirely covered with white mycelia 5.25±0.41 days after they were allowed access to EPF.

On the other hand, larvae allowed access to EPF Isolate 6 showed symptoms and signs of infection in 3.10±0.35 days and were entirely covered with white mycelia in 7.25±0.35 days. Larvae that crawled in EPF Isolate 2 showed symptoms and signs of infection in 4.75±0.50 days. These larvae were entirely covered with white mycelia in 9.10±0.50 days. Larvae that crawled in EPF Isolate 3 showed symptoms and signs of infection in 4.80±0.35 days and were entirely covered with white mycelia in 9.82±0.59 days.

Statistically, larvae allowed access to EPF 1 were earliest to show symptoms and signs of infection, followed by those that crawled in EPF Isolate 6, EPF Isolate 2, and EPF Isolate 3. Larvae that crawled in EPF Isolate 1 were significantly earliest to be fully covered with white mycelium, followed by those with access to EPF Isolate 6, EPF Isolate 2, and EPF Isolate 3. This finding conforms with Prasad and Syed (2010) observations where infection establishes within 1–2 days while infected insects may live for three to five days after hyphal penetration.

Infected larvae were soft when black spots were observed on the integument. Probably infection by blastospores reproducing inside the host had already invaded the hemocoel of insects. Infection develops in the hemocoel to produce beauvericin to paralyze the host insect within 24 hours (Pick et al., 2012). Beauvericin kills the host by inciting progressive degeneration of host tissues due to loss of the structural integrity of membranes and dehydration of cells by fluid loss (Ali et al., 2010).

Third instar larvae.

Three EPF isolates caused an infection on the 3rd instar larvae of *D. indica* (Table 4). Larvae allowed to access plated EPF Isolate 1 significantly showed symptoms and signs of infection in 2.48±0.41 days. The whole larva was entirely covered with white mycelium in 6.12±0.60 days with a range of 5-7 days. Larvae that crawled in EPF isolate six significantly showed infection symptoms in 3.33±0.70 days, followed by those that were allowed access to EPF Isolate 2 (5.20±0.10 days). Infected larvae were entirely covered with white mycelia of EPF Isolate 2 and EPF Isolate 6 in 9.09±0.51 and 8.58±1.40 days. No symptom of infection was observed in larvae that crawled in EPF Isolate 3.

Table 4. Days to the appearance of symptoms and days to be fully covered with white mycelia in 3rd instar larvae of *D. indica*

EPF Isolates	Days to Appearance of Symptom		Days to Fully Covered with White Mycelia	
	Range	Mean ± Sd	Range	Mean ± Sd
EPF Isolate 1	2-3	2.48±0.41[c]	5-7	6.12±0.60[b]
EPF Isolate 2	4-6	5.20±0.60[a]	8-10	9.09±0.51[a]
EPF Isolate 3	0	0.00[d]	0	0.00[c]
EPF Isolate 6	2-4	3.33±0.70[b]	7-11	8.58±1.40[a]

EPF – Entomopathogenic fungi
Means in column followed by the same letter did not differ significantly using LSD at 5% level

Third instar larvae that were allowed access to EPF Isolate 1 showed symptoms of infection one day later than 2nd instar larvae. This indicates that 3rd instar larvae have tougher cuticle making it difficult to degrade by enzymes present in the entomopathogen (Pick et al., 2012).

Fourth instar larvae

Fourth instar larvae allowed access to plated EPF Isolate 1 (Table 5) had symptoms of infection in 2.35±0.47 days, 3.50±0.58 days in EPF Isolate 6 and 5.30±0.60 days in EPF Isolate 2. No symptom of infection was observed in EPF Isolate 3. Statistically, 4th instar larvae that were allowed access to EPF Isolate 1 were significantly earliest to show symptoms of infection, followed by those that crawled in EPF Isolate 6 and EPF Isolate 2. Infected larvae that crawled in EPF Isolate 1 were significantly earliest to be fully covered with white mycelium with 6.25±0.54 days, 8.70±1.07 days in EPF Isolate 6 and 9.15±0.88 days in EPF Isolate 2.

Table 5. Days to appearance of symptoms and days to be fully covered with white mycelia in 4th instar larvae of *D. indica*

EPF Isolates	Days to Appearance of Symptom		Days to Fully Covered with White Mycelia	
	Range	Mean ± Sd	Range	Mean ± Sd
EPF Isolate 1	2-3	2.35±0.47[c]	5-7	6.25±0.54[b]
EPF Isolate 2	5-6	5.30±0.60[a]	8-11	9.15±0.88[a]
EPF Isolate 3	0	0.00[d]	0	0.00[c]
EPF Isolate 6	3-4	3.50±0.58[b]	8-11	8.70±1.07[a]

EPF – Entomopathogenic fungi
Means in column followed by the same letter did not differ significantly using LSD at 5% level

Second instar larvae of *D. indica* were found more susceptible to infection than 3rd and 4th instar larvae. This indicates that cuticle of the second instar larvae can be easily degraded by enzymes of entomopathogen. Specifically, lipase enzyme easily degrades the lipid content of the host insect's epicuticle, making it susceptible for penetration to the inner layer of the cuticle. Afterwards, protease enzyme is activated to degrade the proteinaceous exocuticle and endocuticle then the chitinase enzyme to degrade the chitin content. Production of blastospores follow after penetration (de Carolina Sanchez-Perez et al., 2014).

In infection process, EPF isolate synthesizes metabolite which acts as toxins, playing important role in infection process and provoking series of symptoms in the insect such as convulsions, lack of coordination, behavior alteration, cessation of feeding and paralysis (Arboleda-Valencia et al., 2011). Death results by severe damage in the tissues, toxicosis, cell dehydration, and loss of nutrient intake (Tellez et al., 2009) and finally, the hypha emerges from the insect body, sporulates and starts a new infection cycle. Besides, enzyme nutrients to the fungus, facilitating its proliferation inside the insect (Hasan et al., 2013; Yang et al., 2007).

On the other hand, *D. indica* larvae can possibly escape infection during molting process. Molting influences penetration by the fungus because infection could be started when it penetrated the hemocoel and the hyphae reached the hypodermis. If infection is more advanced at ecdysis, the new cuticle is wounded during ecdysis because of adhesion of the two cuticles by the mycelium. However, if penetration into old integument is only superficial, it can escape infection by casting away the infectious inoculum (Pick et al., 2012).

Percent larval mortality.

EPF Isolate 1 significantly was the most virulent causing 100% mortality of 2nd and 4th *D. indica* larvae compared with other isolates (Table 6). Third instar larvae allowed to crawl in EPF Isolate 1 had 97.5% mortality. There was no mortality observed in 3rd and 4th instars larvae that were allowed to crawl in EPF Isolate 3.

Results showed that *D. indica* larvae are highly susceptible to EPF Isolate 1. This indicates that this isolate is highly virulent as compared with the other isolates.

Table 6. Percent larval mortality of 2nd, 3rd and 4th larval instars of *D. indica* 7 days after access to the EPF

Treatment	Percent Larval Mortality		
	2nd Instar	3rd Instar	4th Instar
EPF Isolate 1	100.00[a]	97.50[a]	100.00[a]
EPF Isolate 2	60.00[b]	37.50[b]	37.50[b]
EPF Isolate 3	27.500[c]	0.00[d]	0.00[c]
EPF Isolate 6	70.00[b]	27.50[c]	30.00[b]
CV (%)	13.02	8.43	13.89

EPF – Entomopathogenic fungi
Means in column followed by the same letter did not differ significantly using LSD at 5% level

EPF Isolate 1 was the most virulent against *D. indica* larvae. The virulence of isolate may be attributed to its protease that are considered important enzymes in infection process. Results conform to the study of Mustafa and Kaur (2009), where the isolate probably caused the break down of the epicuticle of the host by its lipase then produces great quantities of Pr1 protease, which degrades the proteinaceous material. This Pr1 is the key extracellular enzyme involved in cuticle penetration that without this enzyme infection process cannot be achieved. Solubilized proteins are degraded by amino peptidases and exopeptidases, serving as nutrients for entomopathogenic fungi (Wang et al., 2002). Proteases are secreted during first cuticle degradation stage and they are subject to signal transduction mechanism with the activation of protein kinase A (PKA) (Fang et al., 2009).

Identity of Potential EPF

Cultural and morphological characteristics.

EPF Isolate 1 appeared dirty white and cottony when grown in Potato Dextrose Agar (PDA, Metech). As described by Zimmermann (2008), *Isaria fumosorosea* has a basal felt with raised floccose overgrowth with dark white color especially when sporulating abundantly.

Microscopic observation showed that EPF Isolate 1 has chains of spores attached to conidiophore (Figure 2b). Individual spores are longitudinally arranged to form long chain. Conidial structures mostly complex, consisting of erect conidiophores arising from lateral hyphae. Conidiophores consist of verticillate branches bearing whorls of 3 to 6 phialides. Phialides have ellipsoidal basal portion which tapers into a long distinct neck (Zimmermann, 2008).

Molecular characteristics.

BLAST search revealed 99% nucleotide identity with *Isaria fumosorosea* isolate (Genebank Acc. No. FJ765017.1) from China (Freed et al., 2011). The deduced 559 sequences (Table 7) showed that the partial sequence of ITS1 showed no difference between EPF Isolate 1 and the known *I. fumosorosea*. The whole 5.8s region and ITS2 region showed exact nucleotide sequence between *I. fumosorosea* and EPF-Isolate 1 to show that they are the same organisms. The 5.8S region contains protein sequence of isolate that showed the same as the one stored in Gene Bank indicating their similarity. Thus, EPF Isolate 1 confirmed at the molecular level to be *Isaria fumosorosea*.

Table 7. Results of Basic Alignment Search Tool (BLAST) (Deduced ITS-5.8s nucleotide sequence amplified from EPF Isolate 1)

SIGNIFICANT BLAST HIT	
Query length:	559 nucleotide
Query Cover:	100
E-value:	0
% Identity:	99
Species:	*Isaria fumosorosea*
GenBank Accession Number:	FJ765017.1

CONCLUSIONS

Based on the results of the study, the following conclusions were drawn:

1. The EPF-Isolate 1 was the most virulent isolate against cucurbit leafworm, *D. indica*.
2. EPF-Isolate 1 was identified as *Isaria fumosorosea* based on morphological and molecular analysis.

RECOMMENDATIONS

Based on the result of the study, the following recommendations were given:

1. Further study on the preparation of the substrate will be conducted to improve the production of spores.
2. Research on the possible substrates for *I. fumosorosea* is needed to improve the fermentation process to produce blastospores that are more resistant to ultraviolet when applied in the field.

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CONFLICT OF INTEREST

Author declare no conflict of interest.

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