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Control of Plant Sap-Sucking Insects using Entomopathogenic Fungi *Isaria fumosorosea* Strain (Ifu13a)

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Abstract

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We determined the virulence of the *Isaria fumosorosea* strain (Ifu13a) against different plant sap-sucking insects such as *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), *Aphis gossypii* Glover (Hemiptera: Aphididae), *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and *Stephanitis nashi* Esaki et Takeya (Hemiptera: Tingidae) in laboratory condition at 21 ± 1°C temperature and 78 ± 5% relative humidity. We found that the *Ifu13a* strain had excellent potential to control the target insects. The mortality of the tested insect species ranged from 81 to 100% in the concentration of 1×10^8 conidia/ml. However, the lowest mortality of 33% was observed in the concentration of 1×10^5 conidia/ml against the *S. nashi* population. Median lethal times (LT_{50}) were obtained from a regression-probit value which indicated 4.1, 4.1, 4.8, and 7.3 days at a concentration of 1×10^8 conidia/ml, whereas, median lethal concentration dosages (LC_{50}) were calculated as 3.9×10^3, 6.8×10^4, 3.0×10^4, and 6.9×10^5 conidia/ml against *J. formosana*, *A. gossypii*, *B. tabaci*, and *S. nashi*, respectively. The present study showed that the Ifu13a fungal strain is highly pathogenic to the target insects, and it can be used as a biocontrol agent against plant sap-sucking insect species under favourable weather conditions.

Keywords: microbial control; entomopathogen; *Jacobiasca formosana*; *Aphis gossypii*; *Bemisia tabaci*; *Stephanitis nashi*

Nearly 10 000 insect species damage plants worldwide. Of these, 10% identified insect species are considered harmful and cause large economic losses (Dhaliwal *et al.* 2010). Growers mainly depend on chemicals for insect pest control (Brück *et al.* 2009). In addition, extensive application of chemical pesticides causes target-insect resistance (Salim-Abadi *et al.* 2016), harmful effects to beneficial insects, adverse effect on human health and hazards to natural environment (Kontsedalov *et al.* 2012; Haider & Suhail 2013; Antwi & Reddy 2015; Baffourawuah *et al.* 2016).

Therefore, the development of an alternative to chemical insecticides is needed; biological control is an alternative to chemicals and is safe for the environment (Faria & Wraight 2001). Among several groups of biocontrol agents, microorganisms are the most effective and used worldwide against plant sap-sucking insects. It is estimated that 1000 entomopathogenic fungal species are known worldwide (Shang *et al.* 2015). More than 100 mycoinsecticides are commercially available worldwide and are being used as biocontrol agents (Jaronski 2010). They represent a large portion of the current biopesticide market worldwide (Muñiz-Paredes *et al.* 2017).

The entomopathogenic fungus *Isaria fumosorosea* Wize (Deuteromycotina: Hyphomycetes) is not as popular as *Metarhizium anisopliae* and *Beauveria*

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bassiana although it has potential as a biocontrol agent against many insect species (Zimmermann 2008). Several studies have confirmed the potential of *I. fumosorosea* such as *Bemisia tabaci* (Boopathi *et al.* 2015a; Gao *et al.* 2017), *Empoasca vitis* (Chen *et al.* 2014), *Aphis gossypii* (Jandricic *et al.* 2014) and *Corythucha ciliata* (Say) (Sevim *et al.* 2013). Entomopathogenic fungi have unique characteristics whereby they directly infect their host through integuments (Lacey *et al.* 1996; Ali *et al.* 2012). The infected insect host takes 3–5 days to die and the conidia generated cadaver plays a role in the secondary spread of the fungus (Long *et al.* 2000; Fytrou *et al.* 2006; Sevim *et al.* 2012). The efficiency of a fungal strain depends on temperature, relative humidity, host species, host life stage, and duration of incubation (Fransen *et al.* 1987; Chandler *et al.* 1994; Sosa-Gómez & Alves 2000; Baiyun *et al.* 2016).

In this study, we investigated the potential of *I. fumosorosea,* strain Ifu13a, against four plant sap-sucking insect species to determine its control efficiency under laboratory conditions. The results will be helpful for researchers of plant sap-sucking insect biological control programmes.

MATERIAL AND METHODS

Preparation of the entomopathogenic fungus. The entomopathogenic fungi *Isaria fumosorosea*, strain Ifu13a, used in this experiment, originated from the Aleyrodidae (Hemiptera) species, and was obtained from the Research Center on Entomogenous Fungi (RCEF) Anhui Agricultural University, Hefei, China (Latitude 31°N and Longitude 117°E). The strain was preserved at –70°C prior to use. Two hundred microlitres of conidial suspension was inoculated to Sabouraud dextrose agar in 9 cm diameter Petri dishes, comprising agar (20 g), peptone (10 g) and dextrose (40 g), and kept at 24 ± 1°C in an incubation chamber for 12 days. Prior to plating, potassium (0.5 mg), cycloheximide (40 g), and penicillin/streptomycin (2.5 ml/l) were added to the medium. Fully-grown conidia were harvested from the upper surface of the culture by scraping and diluted in a 200 ml conical flask containing 100 ml 0.05% Tween®80. The flask containing the conidia was homogenised in a vortex for 5 minutes. The diluted conidia were filtered through a sterile 30 ml syringe with cotton into a small sterile beaker. Suspensions were adjusted to defined concentrations using a hemocytometer and a microscope. conidial suspensions were standardised at 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml.

Insect collection and bioassay procedures. Cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), Tea green leafhopper *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), sweet potato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and Japanese pear lace bug *Stephanitis nashi* (Hemiptera: Tingidae) were collected from gardens at the Anhui Agricultural University (Hefei, China) from *Pittosporum tobira*, *Prunus cerasifera*, *Gardenia jasmiodides*, and *Malus halliana*, respectively. *B. tabaci* and *J. formosana* were collected using an aspirator. *A. gossypii* and *S. nashi* were manually picked from the host plants' infested leaves. About 700 individuals of each species were collected. Insects were brought to the laboratory for the fungal bioassay. *B. tabaci* and *J. formosana* were cooled for 3 min at 5°C to reduce activity prior to inoculation.

Conidial suspensions of four different concentrations (1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml) were used in the bioassay. The conidial suspension was sprayed onto the insects to be tested by a Potter Precision Laboratory Spray Tower (UK), with a droplet spray nozzle (0.7 mm internal diameter). The control insects were sprayed with 0.05% Tween 80, transferred into plastic boxes (17 × 12 × 5.5 cm) containing host-plant twigs and placed in an insect rearing chamber at 21 ± 1°C and relative humidity 78 ± 5%. Old twigs were daily exchanged for fresh twigs through a 6 cm diameter hole covered with nylon fabric on the top of the boxes to avoid the adults escaping. Each treatment was repeated four times (20 insects each replicate). Daily observations were continued for up to 10 days. Dead insects were removed from the boxes and placed in Petri dishes with wet filter paper for mycelial growth.

Data analysis. Insect mortality percentages were corrected by Abbott's formula: $1 - (nt/nc) \times 100$; where: n – insect number; t – treatment; c – control (Abbott 1925).

The data was analysed using one-way analysis of variance (ANOVA), and the effects of the fungi were compared using a post hoc Tukey's test to determine significant differences in the concentrations on the different insect pests. The grouped data were analysed via regression-probitfor median lethal time (LT_{50}) and median lethal concentration dosage (LC_{50}) (Finney 1971). All the statistical analyses were performed using SPSS v21 (Crop 2012) at a significance level of $P \leq 0.05$

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RESULTS

The experimental results showed that the entomopathogenic fungus *I. fumosorosea* exhibited excellent pathogenicity against all targeted insect pests. This was even stronger than a control, *B. bassiana* strain 202 (not reported in this paper). The cumulative mortality percentages of *J. formosana* Paoli, *A. gossypii* Glover, *B. tabaci* Gennadius, and *S. nashi* are shown in (Figure 1), and corrected mortality percentages are shown in (Figure 2). Not more than 10% mortality was observed in the control treatment of all target insect pest populations. Dosage-response parameters such as median lethal time (LT_{50}) and median lethal concentration dosage (LC_{50}) for all insect species are shown in Tables 1 and 2, respectively.

Jacobiasca formosana Paoli. *I. fumosorosea* exhibited excellent control against *J. formosana*. Initial fungal infection was observed after three days of application of the fungal conidia and rapidly increased from the 5th day (Figure 1), whereas no significant difference was observed in mortality rate of the targeted insect populations between the concentrations of 1×10^8–1×10^7 and 1×10^5–1×10^6 conidia/ml. The final corrected mortality reached 100% in concentrations 1×10^7, 1×10^8 and 1×10^5 conidia/ml after the 8th and 9th days, respectively. Concentration 1×10^6 conidia/ml caused 96% mortality after 10 days (Figure 2), and 7.5% mortality was observed in the control treatment (Figure 1). There was no significant difference in mortality among the concentrations (df = 4, F = 4.37, P = 0.002). The least LT_{50} was found in the highest concentration at 4.1 days (Table 1) and the LC_{50} obtained was 3.9×10^3 conidia/ml (Table 2).

Bemisia tabaci Gennadius. All concentrations of *I. fumosorosea* were pathogenic to the targeted insect populations. The fungal infection was found to be dosage-dependent. A 15% cumulative mortality was observed in the treatment of 1×10^8 conidia/ml after 3 days post inoculation. On the 5th day, 3.75% mortality was found in the treatment of the 1×10^5 conidia/ml (Figure 1), whereas 100% mortality was recorded in all concentrations except for 1×10^5 conidia/ml at the end of the experiment. Only the concentration of 1×10^5 conidia/ml caused 88% mortality (Figure 2). No significant differences were observed among the concentrations (df = 4, F = 5.00, P = 0.001). Mortality of 8.8% was observed in the control treatment. An LT_{50} of 4.8 days was calculated for the highest concentration (Table 1). The least lethal concentration was calculated as 3.1×10^4 conidia/ml (Table 2).

Aphis gossypii Glover. The target insects were highly susceptible to the entomopathogenic fungi, which caused 100% mortality at concentrations of 1×10^6, 1×10^7, and 1×10^8 conidia/ml, and 68% mortality was recorded in the concentration of 1×10^5 conidia/ml after 10 days of fungal inoculation (Figure 2). The results showed that the concentration 1×10^8 exhib-

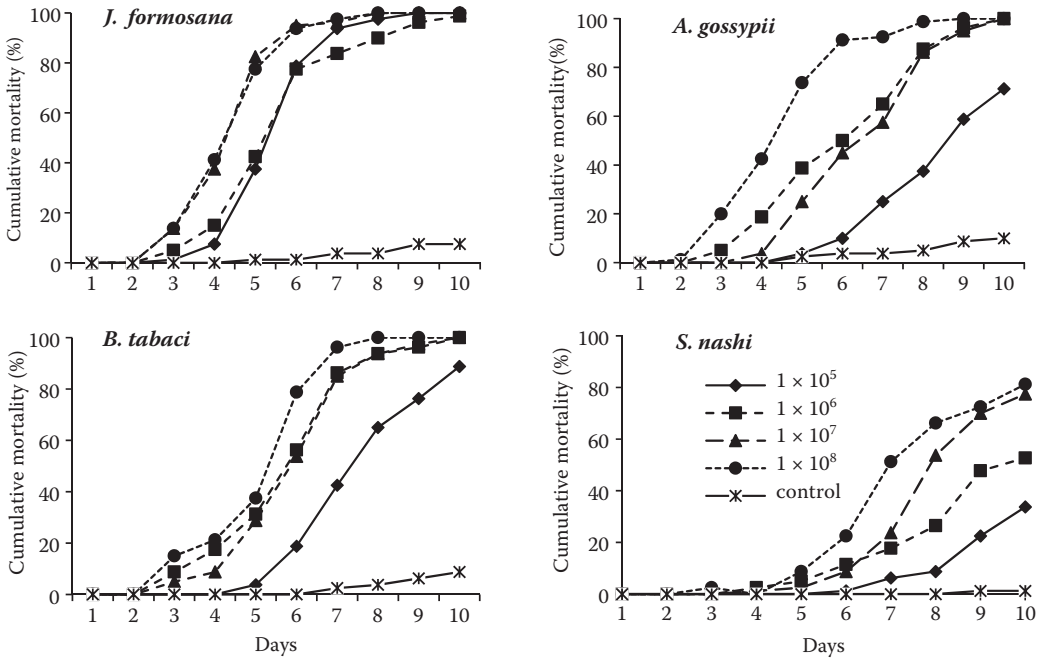

Figure 1. Cumulative mortality of target insect pests after application of different spore concentrations of *I. fumosorosea* (Ifu13a)

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Table 1. Median lethal times (LT_{50}) for target insect pests caused by various concentrations of *I. fumosorosea* strain (Ifu13a)

Insect	Concentration	*n*	Slope ± SE	LT_{50} (days)	95% CI	Chi-square
J. formosana	1×10^5	80	11.998 ± 1.096	5.246	5.052–5.421	3.401
	1×10^6	80	6.577 ± 6.577	5.434	5.034–5.775	7.286
	1×10^7	80	9.110 ± 1.077	4.112	3.719–4.413	4.413
	1×10^8	80	8.950 ± 1.036	4.118	3.727–4.421	2.066
A. gossypii	1×10^5	80	8.184 ± 1.131	8.548	8.129–8.947	1.012
	1×10^6	80	6.956 ± 0.868	5.581	5.070–5.956	13.786
	1×10^7	80	9.418 ± 0.934	6.184	5.824–6.479	9.442
	1×10^8	80	7.149 ± 0.610	4.080	3.818–4.311	4.086
B. tabaci	1×10^5	80	9.573 ± 0.873	7.421	7.082–7.730	1.444
	1×10^6	80	7.541 ± 0.478	5.315	4.916–5.704	19.147
	1×10^7	80	10.104 ± 0.915	5.691	5.445–5.905	10.006
	1×10^8	80	8.240 ± 0.538	4.757	4.246–5.251	37.075
S. nashi	1×10^5	80	5.347 ± 0.871	12.973	11.253–16.585	6.275
	1×10^6	80	5.658 ± 1.099	9.789	9.182–10.839	3.217
	1×10^7	80	8.912 ± 1.155	8.218	7.927–8.526	6.379
	1×10^8	80	8.088 ± 0.675	7.384	7.116–7.659	10.892

ited a high mortality rate with a cumulative mortality of 73.75% on the 5th day, whereas the concentration of 1×10^5 exhibited 2.5% mortality on the same day. This indicates that the higher concentration of fungal treatment resulted in faster control of the targeted insect pests (Figure 1). However, no significant differences were found among concentrations over 1×10^5 ($df = 4$, $F = 6.5$, $P = 0.001$). Mortality of 10% was observed in the control treatment. The shortest LT_{50}, 5 days, was found in the highest concentration (Table 1) and the least LC_{50} was determined as 6.8×10^4 conidia/ml against *A. gossypii* (Table 2).

Stephanitis nashi Esaki et Takeya. The strain Ifu13a showed relatively lower pathogenicity against *S. nashi* compared to *J. formosana*, *A. gossypii* and *B. tabaci*. Total corrected mortality of 33, 52, 77, and 81% was obtained at the concentrations of 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml, respectively (Figure 2). Development of the fungal infection in the *S. nashi* population showed an obvious dosage-dependence. This indicated that a high application dosage is required for effective control of *S. nashi*. Statistical analysis revealed that mortality in the concentration of 1×10^5 conidia/ml was significantly lower than the other concentrations ($df = 4$, $F = 6.3$, $P = 0.001$). However, 1.3% mortality was observed in the control treatment. Accordingly, the LT_{50} of 7.3 days was calculated for the highest concentration (Table 1) and a LC_{50} of 6.9×10^5 spore/ml was recorded as the lowest dose (Table 2).

Table 2. Median lethal concentration dosage (LC_{50}) values of concentrations against different target insect pests caused by various concentrations of *I. fumosorosea* strain (Ifu13a)

Insect	Intercept	Slope ± SE	95% LC (lower – upper)	Calculated LC_{50}	Chi-square
J. formosana	2.260 ± 390	1.609 ± 3.812	0.039	3.9×10^3	0.007
A. gossypii	561 ± 0.148	3.309 ± 3.804	0.677	6.8×10^4	0.004
A. gossypii	1.220 ± 0.185	2.354 ± 2.289	0.303	3.0×10^4	0.016
S. nashi	–0.385 ± 0.121	0.459 ± 0.069	6.879 (2.568–14.528)	6.9×10^5	1.340

The effect of concentration on 50% mortality of insects was analysed by a regression-probit method without control mortality, and the data was further manually calculated to obtain LC_{50} values (Finney 1971)

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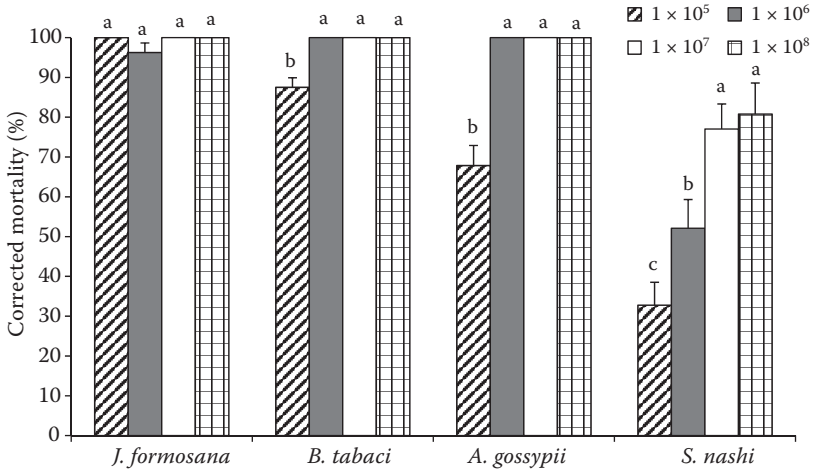

Figure 2. Corrected mortality (%) of target insects caused by different spore concentrations of *I. fumosorosea* (Ifu13a).

DISCUSSION

To avoid detrimental over-reliance on chemical insecticides, multiple control tactics would seem valuable. Former studies have verified the potential of entomopathogenic fungi to control insect pests. Duarte *et al.* (2016) compared the efficacy of five species of entomopathogenic fungi with some registered pesticides against *Plutella xylostella* (L.) on cabbage crops and obtained 80–100% control. Boopathi *et al.* (2015b) compared virulence among *B. bassiana*, *M. anisopliae, Lecanicillium lecanii*, and *I. fumosorosea,* he reported that found *I. fumosorosea* and *L. lecanii* were more lethal to *Aleurodicus dispersus* causing 70% mortality. Similarly was found that *I. fumosorosea* were most toxic to *Cameraria ohridella* (Lepidoptera: Gracillariidae) as compared to ten other fungal isolates among *B. bassiana* and *M. anisopliae* (Robert *et al.* 2016). Majeed *et al.* (2017) reported that among three commercial isolates, *B. bassiana*, *M. anisopliae*, and *Isaria fumosorosea*, the isolate *I. fumosorosea* caused strong pathogenicity and observed mortality of 85% to adult and 92% to nymph of Asian citrus psyllid (Hemiptera; Psyllidae).

The current study also showed that the entomopathogenic fungus, *I. fumosorosea*, has potential to control multiple sap-sucking insects. The *I. fumosorosea* Ifu13a strain caused a final corrected mortality of nearly 100% in *A. gossypii*, *J. formosana*, and *B. tabaci* populations, while relatively low pathogenicity to *S. nashi*.

The results revealed host-specificity of entomopathogenic fungi to some degree. Host-specificity was also supported by (Potrich *et al.* 2011) *Isaria* sp. highly suppressed the target nymph population of *B. tabaci* (Genn.) with a mortality of 98.6%, followed by *B. bassiana* 84.1%, while *M. anisopliae* caused a minimum mortality of 23.2%. Similarly, Barrios *et al.* (2016) observed a large difference among *B. bassiana, I. fumosorosea* and *Purpureocillium lilacinum* on *Leptopharsa gibbicarina* (adult) and recorded pathogenicity levels of 74.3, 92.8, and 100%, respectively. Kavallieratos *et al.* (2014) compared the virulence of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* against *Sitophilus myzae* and calculated that *B. bassiana* and *M. anisopliae* were more pathogenic to *S. myzae* compared to *I. fumosorosea.* Similarly, Arthurs *et al.* (2013) found mortality rates of commercial entomopathogenic fungi (*B. bassiana* GHA, *Metarihzium brunneum* F52, and *I. fumosorosea* Apopka 97 on chili *Scirtothrips dorsalis* as 84–93, 81–94, and 62–66%, respectively.

The current study revealed dosage-dependency of the fungal infection, both on mortality and mortality rate. We recorded initial mortalities of 1.25 and 13.75% in *J. formosana*, 0.0 and 20% in *A. gossypii*, 0.0 and 15% in *B. tabaci* and 0.0 and 2.5% in *S. nashi* populations on the 3rd day for the lowest to highest conidial concentration levels, respectively (Figure. 1). Müller (2000) documented the infection and killing speed of *Locuslana pardalina* (Walker) treated with different conidial concentrations of *M.anisopliae* and observed the highest mortality 100 in 3–4 days in highest concentration of 1×10^8 conidia/ml, while mortality reported in concentration 1×10^7, 1×10^6, and 1×10^5 conidia/ml was 5–6, 6–10, and 12–14 days respectively. Which conform that the high dosages of conidia cause a rapid fungal infection to the target insect.

Dose-dependency in fungal disease normally indicates a higher density of conidia and often results in a faster control of targeted insects. Therefore, in the case of large insect pest populations, higher

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conidial concentrations are more effective than lower concentrations for pest control to avoid pest populations reaching economic injury levels. However, low conidial concentrations can be used for the initial development stage of pest populations to maintain them below economic thresholds.

It was concluded from the current study that *I. fumosorosea* has strong potential to cause pathogenicity against plant sap-sucking insect pests, and can be used as a biocontrol agent. Moreover, controlling of many insect species with a single fungal strain could save farmers' input costs and provide a wide range of insect control with a single dose application. Furthermore, it is suggested that the recommended dosage of mycoinsecticides should be used against targeted insect pests for effective control.

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