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Research Article
Field Bioassay Assessment of Entomopathogenic Fungi on *Aphis gossypii* (Glover)

[1,3]Ghulam Ali Bugti, [1]Cao Na, [2]Lin Hua Feng and [1]Wang Bin

[1]Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, China
[2]School of Plant Protection, Anhui Agricultural University, Hefei, China
[3]Department of Entomology, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, Pakistan

Abstract

Background and Objectives: The entomopathogenic fungi are convenient and safe source for the controlling of plant sap sucking insects. So, this study aimed to investigate the field control efficiency of entomopathogenic fungi against *Aphis gossypii* Glover (Hemiptera: Aphididae) and to assess effects of environmental factors such as relative humidity and temperature on the pathogenicity of fungi. **Material and Methods:** Based on previous studies an optimum concentration of 1×10^{7} conidia mL^{-1} was sprayed on *Pittosporum tobira* plants. The declining percentages of insects were calculated by the online percentage change calculator. Whereas, level of significance among treatments were obtained by Tukey test using Minitab software at a significance level of $p\leq0.05$. **Results:** Both fungi *Isaria fumosorosea* strain (Ifu-13a) and *Beauveria bassiana* strain (Bb-202) showed high pathogenicity against *Aphis gossypii*. The maximum reduction of 95 and 91% in insect population was noticed in Ifu-13a and in the Bb-202 treatment, respectively, after 6 days of application of fungal conidia. **Conclusion:** A good field control indicates that the entomopathogenic fungi have a strong ability to induce an epizootic fungal disease in targeted insects and provide efficient control under favorable environmental conditions.

Key words: Entomopathogenic fungi, *Isaria fumosorosea*, *Beauveria bassiana*, sucking insect, *Aphis gossypii*

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Corresponding Author: Wang Bin, Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aphis gossypii Glover (Hemiptera: Aphididae) is one of the most destructive insect pests of vegetables, agricultural field crops and forest trees worldwide[1-4]. Nearly 1,100 aphid species, belonging to 268 genera, are known in China[5]. Their short lifecycle and ability to produce many generations through asexual reproduction is another cause of high aphid populations[6]. For example, *A. citricola* can produce 15-18 generations/year and it causes severe losses to host plants in the spring and autumn, while *A. gossypii* can produce up to 60 generations/year under favourable conditions. Aphids attack more than 400 host plants and may contribute to up to 90% of crop losses, depending on the infestation rate and crop stage[7-9]. Damage occurs either through the sucking of sap from the host plant leaves or by transmission of various viral diseases[10]. Both, adults and nymphs, feed on plant tissues by the insertion of a needle-like stylet and sucking the phloem content from the vascular system of the host plant. Toxins present in the saliva can cause concurrent thickening, crumbling and downward curling of leaves[11,12].

In recent decays entomopathogenic fungi are being considered as promising biocontrol agents of many insect[13]. These fungi can be found naturally in most of the regions worldwide either below or above the ground level[14]. And used in inset control as alternatives to synthetic insecticides, these insect pathogenic fungi are relatively safe to human, non-toxic to non-target insects and environment[15,16]. It is estimated that about 1,000 entomopathogenic fungal species are known worldwide[17,18]. They represent a large portion of current bio-pesticide in the market worldwide[19].

More than 100 conidia based insecticides are registered in the market[17]. And being used against various insect control associated with agricultural crops and forest trees, also control some other parasitic microbes[20,21]. These insecticide kill's targeted insects by coming into contact with the fungal conidia either by fine spray droplets or by moving on a sprayed area. The virulent spores attached to host insect's cuticle and penetrate into the insect's body, it takes few days to infect susceptible host till its death. The germinated spores on the infected host cadaver acts as a secondary source of the fungus in the field[22,23]. Pathogenicity and infection efficiency of fungi depends on many factors such as the host species, life stage, environmental conditions[24,25].

Generally farmers rely on chemical insecticide for insect control to minimise crop losses[26,27]. Of these chemicals pose severe adverse effects not only on human beings but also on non-target organisms[28-30]. Beside that their frequent use also cause insect resistance against certain insecticidal formulations[31,32].

As a consequence, safe alternative strategies are required for the control of insects to minimize the crop losses and reduce the environmental pollution caused by synthetic pesticides. These adverse stress emphases the need of efficient and safer insect control strategies, in current era of insect control rather than using inorganic insecticides to manage insect pest in field crops and in forest ecosystem. Therefore, considering the above problems the present study was aimed to investigate the pathogenicity of fungi, *Isaria fumosorosea* Ifu-313a and *Beauveria bassiana* strain Bb-202 against *Aphis gossypii* in open field condition to obtain a highly virulent fungal stain to control pest population and minimise the use of chemical pesticide.

MATERIALS AND METHODS

The experimental trail was carried out in the garden of Anhui Agricultural University, Hefei, China, in September, 2017 to investigate the pathogenicity of entomopathogenic fungal species *Isaria fumosorosea* Ifu-13a and *Beauveria bassiana* strain Bb-202 against *Aphis gossypii* Glover.

Preparation formulation of Insect pathogenic fungi: The entomopathogenic fungal species *I. fumosorosea* Ifu-13a and *B. bassiana* strain Bb-202 were selected after screening initial lab bioassay test published data[33,34], which were obtained from the Research Center on Entomogenous Fungi (RCEF) Anhui Agricultural University, Hefei, China. The fungal strains were originated from the Aleyrodidae (Hemiptera) and Cerambycidae (Coleoptera) species respectively and preserved at -70°C prior to use. About 200 µL of the conidial suspension were 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. Fully-grown conidia were harvested from the upper surface of the culture by scraping and diluted in a 500 mL conical flask containing 300 mL 0.05% Tween 80. The flask containing the conidia were homogenised a vortex for 15 min. The diluted conidia were filtered through a sterile muslin cloth into a sterile beaker. A suspension was adjusted to defined concentrations using a hemocytometer and a microscope. Conidial suspension was standardised at 1×10^7 conidia mL^{-1}.

Fig. 1(a-c): (a-b) Experimental field

Site selection and fungal inoculation procedure: To evaluate the pathogenicity of 2 fungal isolates *I. fumosorosea* and *B. bassiana* for this purpose the infested *Pittosporum tobira* plants were chosen which were grown in the garden of Anhui Agricultural University, Hefei, China. Overall 3 treatments were conducted. For each fungal treatment, the 10 plants were selected and same numbers of plants were selected for control. And each treatment was 10 times replicated About 300 replications were done in this experiment. The selected plant branches were checked for aphid infestation and selected for the treatment. To make sure easy count of aphid population the only the 50 aphids were left on one treatment while other aphids were removed from the branch of plant with help of a small chines soft brush. To avoid the attack of predator the branches covered with plastic bags and close with help of stapler punch machine. To avoid the suffocation of the insect's the tiny holes were made on the tope of plastic bags for air crossing with help needle. An optimum rate of 1×10^{7} conidia mL^{-1} was selected for target insect control. The conidial spores were sprayed on treated plants while control was treated with distal water. The data were recorded at 3 days interval (Fig. 1).

Statistical analysis: The declining percentage in insect population was calculated by online percentage change calculator by using equation:

$$\frac{nn - on}{on}\times100$$

Where:

- First: Work out to compute the difference (increase) between the numbers as:
- Increase = New number-original number
- Then: Increase number is divided by the original number and answer multiplied by 100:

$$\text{Increase (\%)} = \frac{\text{Increase}}{\text{Original number}}\times100$$

https://www.skillsyouneed.com/num/percent-change.html. Data further analysed by performing one way-ANOVA, followed by Tukey test to compare the means of the treatments using Minitab software version 18.0 at $p\leq0.05$.

RESULTS

Both myco-insecticides based on *B. bassiana* and *I. fumosorosea* showed a significant control in the open field condition Fig. 2. The environmental conditions played an important role in causing fungal infection, data shown in the (Table 1). A cloudy weather and rainfall increased the relative humidity, promote the fungal disease development and mycelial growth on dead insect cadavers (Fig. 3). The maximum reduction percentage in aphid population was

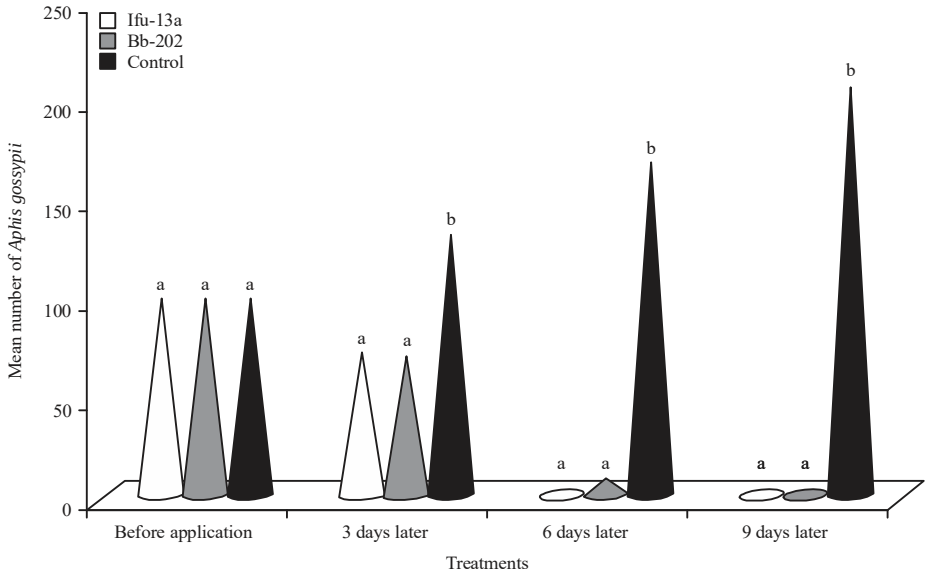

Fig. 2: Mean populations of *A. gossypii* on different treatments in open field
Means that do not share a letter are significantly different at 0.05 levels

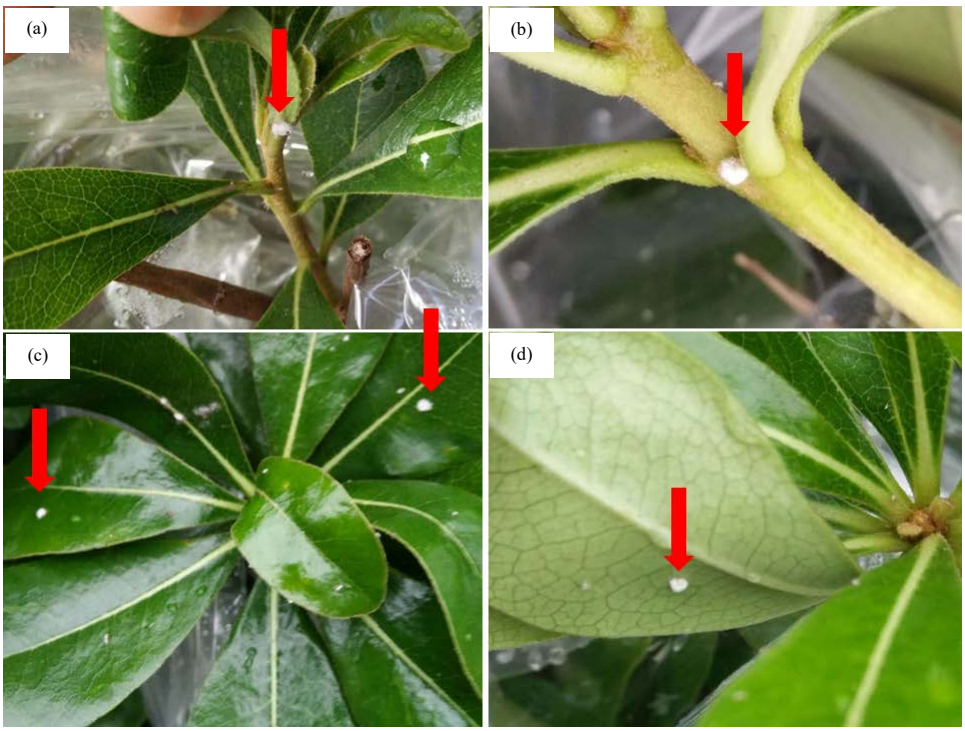

Fig. 3(a-d): Infected cadavers with mycelial growth found on host plant during data collection, (a-b) Mycelial growth on *A. gossypii* cadavers caused by *I. fumosorosea* and (c-d) Mycelial growth on *B. bassiana*

Table 1: Weather conditions in the month of September in the year, 2017

Parameters	Values
Average rainfall (days)	8.8
Average rainfall (mm)	74.6
Average humidity	78%
Average max temperature	27.3°C
Average min temperature	19.3°C
Average UV light index	9.0
Sun shining	5.2 h
Daily light duration	12 h

Source: Weather data is obtained from https://www.weather-atlas.com/en/china/hefei-weather-September

noticed in both treatments. The initial decline of 27.2% was observed on 3rd day and 95% on 6th day in *I. fumosorosea* treatment. While *B. bassiana* showed 28.6% decline on 3rd day and 91.2% on 6th day, respectively. While over than 100% increase in insect population was observed in control treatment (Fig. 2). However, statistical results revealed that there was no significant difference between fungal treatments (Df = 2, F = 18.1, p = 0.001), while both treatments were significantly different from the control.

DISCUSSION

As we observed that both fungi have good control against targeted insect. The environmental condition especially high relative humidity and favourable temperature favoured the fungal infection process. The initial 27-28% decline in insect population on 3rd day in insect population indicated that the conidia based insecticide greatly effect on targeted insect. That provided a pathway for fungal infection and stopped further development and reproduction process of target insects.

Lin *et al.*[35] conducted an experiment on different environmental conditions and reported that infection process and spore germination of *B. bassiana* on the larvae of *Dendrolimus punctatus*, was highly influenced by environmental factors and found that temperature above then 10°C and over 92% relative humidity was suitable and ideal environmental condition for fungal infection. Similarly, Cui *et al.*[36] studied the pathogenicity of *B. bassiana* by placing *Spodoptera exigua* larvae at different relative humidity and temperature levels. Their results suggested that temperature ranged from 24~27 and relative humidity over than 90% were high infectious to target insect and provide a good control. Whereas, Mishra *et al.*[37] reported that *B. bassiana* infect both adult and larvae of house fly and caused 100% mortality at RH, 90 and 100% at temperature of 30°C.

During the field experiment the weather condition was cloudy after the 48 h application of fungal conidia it was a slow rained which increased the relative humidity and enhanced the fungal infection. The fewer decline percentage was recorded in aphids' population on 3rd day after application of conidia. However no dead insect with mycilial growth was found. A sudden sharp decline was noticed on 6th day after application of conidia. Whereas, Ali *et al.*[38] reported that fungal conidia need time to penetrate in host cuticle after in host cuticle the infection process need 3-5 days to kill the host and conidia were produced on the cadaver[39].

As we observed that high relative humidity favoured the fungal infection process and a decline of 27-28% was observed in ifu-13a and Bb-202, respectively in treated plots on 3rd day, while over 31% increase was observed in control treatment. The decline in insect population indicated that the conidia effect positively and at initial stage fungal infection stopped the growth and reproduction process of the target insects.

Similar results were reported by Tooyama *et al.*[40] that initial time 48-82 h are most important for fungal infection process. Nearly 90% conidial germination was observed in *B. tabaci* population exposing them in 99% relative humidity for 48-82 h. From the results it was concluded that the favourable environmental condition helped in fungal infection process and conidial germination. The both fungal strain showed strong control against target insect.

CONCLUSION

Both fungal strains showed strong pathogenicity to target insects. Relative humidity played key role in fungal infection and posed a great influence on the fungal pathogenicity and ensured a successful fungal disease development in targeted insect population.

SIGNIFICANCE STATEMENT

This study will help the researcher to understand the control efficacy of entomopathogenic fungi as a strong alternative candidate to chemical pesticide with least harmful effects on non-target organisms, as well as range of environmental condition. Furthermore the entomopathogenic fungi can be safely used in integrated pest management as microbial control agent against sucking insects.

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