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Use of the entomopathogenic fungi *Beauveria bassiana* (Hyphomycetes: Moniliales) and *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) to control *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) under laboratory and semi-field conditions

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of the pathogen, *Candidatus* Liberibacter asiaticus that causes Huanglongbing (HLB) or citrus greening disease, the serious threats to citrus industry worldwide. The objective of this study was to evaluate the role of the entomopathogenic fungi (EPF) *Beauveria bassiana* (Hyphomycetes: Moniliales) and *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) in controlling the adult citrus psyllid of *D. citri* under laboratory and semi-field conditions. Bioassays were performed by exposing the adults to the EPF at the concentration of (1×10^8 conidia/ml). The results showed that both EPF had the potential to control the adult citrus psyllid, giving 64–74% mortality rate in the laboratory and 61–72% under semi-filed conditions. These results support the use of both EPF as effective biopesticides for integrated management of the Asian citrus psyllid, *D. citri*.

Keywords: *Diaphorina citri*, Citrus, Entomopathogenic fungi, Microbial control

Background

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an economically important insect pest of citrus that disperses throughout the citrus growing regions, including tropical and subtropical Asia, Middle East, Central America, South America, Mexico, and Brazil (Grafton-Cardwell et al. 2006; Hall et al. 2012). Primarily, it attacks young flushes of citrus and can also attack the stressed citrus trees, if the population is high (Hussain et al. 2018). Both nymphs and adult psyllid feed directly on phloem of tissues causing the distorted growth of plants as well as shoot dieback. It is also a vector of the phloem-limited bacteria, *Candidatus* Liberibacter asiaticus and the causal agent of citrus greening disease known as Huanglongbing (HLB) (Halbert et al. 2000; Bové 2006). HLB is the most deadly disease of *Citrus* spp. (Sapindales: Rutaceae) because the infected trees usually survive for 5–8 years with a poor fruit quality in both taste and color (Kamble et al. 2017). Citrus psyllids also excrete honeydews on leaf surface that promotes the sooty mold development and impairs the photosynthetic rate of plants (Reynolds 1999). Husain and Nath (1927) recorded the infestation of ACP in Pakistan's citrus orchards, and now, it has become the most destructive and well-established pest to citrus crop

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in the country (Mahmood et al. 2014). To date, application of synthetic insecticides is one of the tactics being engaged in psyllid management programs (Naeem et al. 2016), but it requires the repeated applications throughout the crop season (Tiwari et al. 2011).

However, the insecticides' applications are expected to increase the selection pressure for resistant psyllid population; residual effects on fruits and disruption of the natural enemies associated with the pest (Tiwari et al. 2011). To minimize these risks, there is a need to develop a sustainable management technique for both the vector and HLB disease. Entomopathogenic fungi (EPF) are noticeable and promising tools for effective management of insect pests due to easily mass production as well infecting various life stages of insect host though its cuticle (Subandiyah et al. 2000). To mitigate the resistance problem in ACP and its effective control, the EPF are considered one of the best options as alternatives to synthetic chemicals. The EPF *Isaria javanica* (Friederichs & Bally) Samson & Hywel-Jones, *I. fumosorosea* (Wize), *Hirsutella citriformis* Speare, *Lecanicillium lecanii* Zimm., *Beauveria bassiana* (Bals.-Criv.), *Cladosporium* sp. nr. *oxysporum*, and *Capnodium citri* Berk. and Desm. (Subandiyah et al. 2000; Meyer et al. 2007; Stauderman et al. 2012) have been reported earlier as effective bioagents against ACP. Similarly, *Metarhizium anisopliae* (Metschn.) is also very effective that has a wide range of hosts (de la Cruz et al. 2013).

The objective of this study was to evaluate the efficacy of the EPF, *Beauveria bassiana* and *Isaria fumosorosea*, against adult citrus psyllid, *D. citri*, under laboratory and semi-field conditions.

Materials and methods

Sampling of *D. citri*

Stem tap samplings of the psyllid population were obtained from two mature *Citrus reticulata* orchards: the first (5 ha) was selected from Citrus Research Institute (CRI), 32°06′45.0″N 72°40′53.2″E, and the second (2 ha) from research area at College of Agriculture (CA), 32°07′48.5″N 72°41′08.1″E, University of Sargodha, Pakistan. Each orchard was further divided into three blocks, and ten trees were randomly selected from each block. Four shoots (1 from each cardinal direction) were selected from each tree for tap sampling. A white metal pan (20 × 20 × 10 cm) was held under each selected branch and forcefully tapping the branch three times, using PVC pipe. All adult psyllids fallen on the white pan were counted immediately, and the population of ACP was recorded at weekly intervals during March 2018.

Insect colony

Adult citrus psyllids were collected from *C. reticulata* orchards, using aspirator, and were reared on orange jasmine, *Murraya paniculata* (L.) seedlings inside ventilated Plexiglas cages (40 × 40 × 40) under the environmental conditions of 23–28 °C, 55–70% RH, and a 14:10 h (L:D) photoperiod. *M. paniculata* was used as the preferred host for oviposition and feeding of ACP (Chow et al. 2016).

Entomopathogenic fungi

The commercial formulations of EPF *Beauveria bassiana* NCIM 1216 ATCC 26851 (AgriLife, India) and *Isaria fumosorosea* IF-171201 (AgriLife, India) were used against ACP in this study. Both EPF were tested at a feasible conidial concentration of (1×10^8 conidia/ml) (Dal Bello et al. 2018). The quality control of the conidia was determined by counting the conidial concentration in a Neubauer chamber, hemacytometer. The conidial germination was determined on PDA, and the conidial viability was measured based on the counts of 200 random conidia per plate 18 h post incubation at 25 ± 2 °C (Ayala-Zermeño et al. 2015).

Laboratory bioassay

Adult psyllids ($n = 20$; sex ratio 1:1) were collected from the rearing culture, and each treatment was sprayed with 400 μl. The experimental arena was glass vials, containing young seedling of *M. paniculata*. The control group was treated by distilled water. The mortality data were recorded after 10 days of incubation at 25 ± 2 °C, 65–80% RH, and 14-h photo period. The experiment was performed as a randomized experimental design, with three replications, and the test was repeated three times on different dates.

Semi-field bioassay

The efficacy of EPF against ACP, reared on *C. reticulata* plants, was assessed under a greenhouse (as semi-field conditions) (Ribeiro et al. 2015). The plants measuring, approximately 60 cm were placed on white plastic trays. Two branches from each plant were covered by mesh cages (net-like fabric bags), and 20 adults of ACP were released on each branch. The infested plants were placed in a greenhouse. Five plants were selected for each treatment, and the experiment was performed under a randomized design, with three replications and the whole trial was repeated three times on different dates. Fungal suspensions of both fungi were prepared as in the laboratory bioassay and were sprayed, at the rate of 15 ml per plant, using a handheld sprayer. The distilled water was applied in the control treatments. Ten days post branches were collected into bags and transferred to the laboratory to record the mortality data. The cadavers were separated and placed in an incubator (25 °C and 90% RH) for 3 days to check the fungal development and sporulation.

Statistical analysis

To determine the status of ACP population, data from tap sampling were analyzed by keeping the block as the main factor for each location. Similarly, the mortality data were analyzed, using one-way ANOVA technique by keeping the treatments as the main factor. Means were separated by the Least Significance Difference (LSD) test. All the analyses were performed using SPSS 20.0 software.

Results and discussion

Stem tap sampling was used to follow the population of ACP in the two different experimental locations of Sargodha district. The results showed that there was a significant ($F = 7.72$, $P = 0.0007$) difference in ACP populations sampled from the three blocks in CRI. However, in the second location CA, insignificant difference ($F = 1.24$, $P = 0.294$) was found among the three blocks. The highest ACP population averaged 5.42 adults/tap per sample in CRI and 3.85 in CA location (Fig. 1). Survey results from the research sites, carried out during March 2018, clearly indicated the developed population of ACP on *C. reticulata*, the most growing and economically important cultivar in Pakistan. A large number of young flushes with abundant number of psyllids at both sampled locations were found. There was a significant difference in ACP population in the three blocks of orchard in Citrus Research Institute; however, no difference was found in the orchard at College of Agriculture. It could be due to non-uniformity of young flushes across a block of trees having higher numbers of adult psyllids. The average number of adults per tap sample was (3–5.5 adults/trap) in both sampling sites, during March 2018, indicating the well-established population of the psyllids in these locations. The psyllids' population has increased in Sargodha region since the last couple of years, and the increase in population was expected with increasing the availability of young flushes for psyllid development and oviposition. Flush growth quality, especially in term of physical characteristics and its nutritional value, had an important influence on the fitness of citrus psyllids (Nava et al. 2007; Tsagkarakis and Rogers 2010; Alves et al. 2014). *Citrus mandarin*'s fitness effects were more significant for the development of citrus psyllids compared to Rangpur lime (*Citrus limonia* Osbeck), sour orange (*C. aurantium* L.), or orange jasmine (*Murraya exotica* L.) (Nava et al. 2007).

The results showed that both *B. bassiana* and *I. fumosorosea* were significantly ($F = 172$, $P < 0.001$) effective against the adult psyllids by direct contact, resulting in higher mortality reaching 64.4 and 73.8%, respectively, compared to the control (8.3%) under laboratory conditions. A total number of psyllid cadavers, showing fungal development and sporulation, was insignificantly different ($F = 0.06$, $P = 0.834$) after *B. bassiana* and *I. fumosorosea* applications. *B. bassiana* caused 56.1% sporulation, while *I. fumosorosea* caused 57.6% under laboratory conditions (Fig. 2). Similarly at semi-field conditions, both EPF showed significant ($F = 1189$, $P < 0.001$) mortality rates of adult psyllids. *I. fumosorosea* caused higher mortality was 72.1%, followed by *B. bassiana* that showed 61.2%. In the control treatment, the mortality rate was 5.6%. Similarly, sporulation percentage was also significantly ($F = 34.9$, $P < 0.001$) different

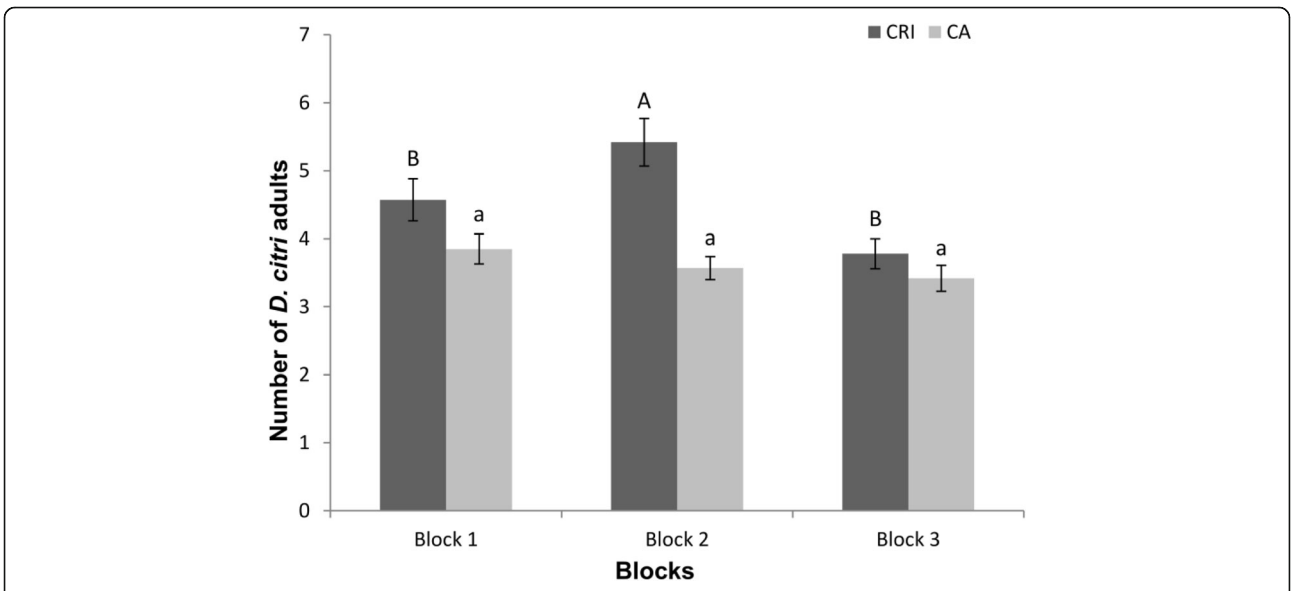

Fig. 1 Number (means ± SE) of adult psyllids per tap sample per location: CRI (Citrus Research Institute) and CA (College of Agriculture). Means sharing similar letters for each location are insignificantly different at ($P > 0.05$)

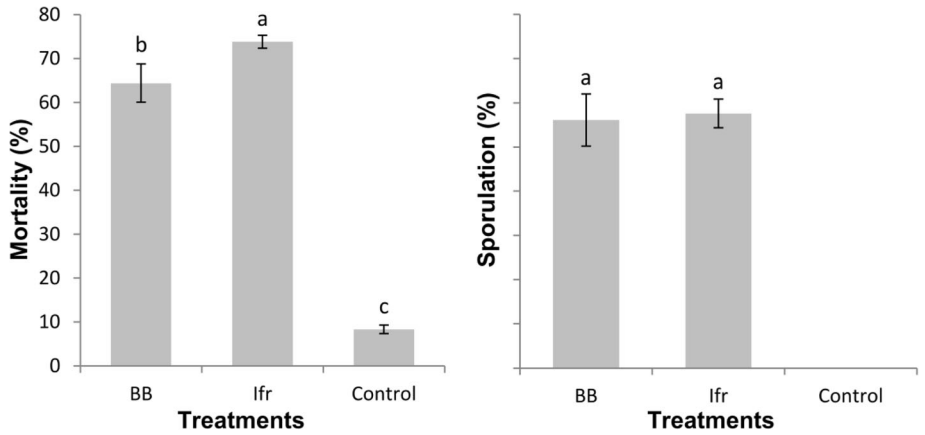

Fig. 2 Percent (means ± SE) mortality of adult psyllids and sporulation from cadavers after application of *Isaria fumosorosea* (Ifr) and *Beauveria bassiana* (BB) under laboratory conditions. Means sharing similar letters are insignificantly different ($P > 0.05$)

between *B. bassiana* and *I. fumosorosea*. The sporulation percentage was 52.6% in case of *I. fumosorosea* and 38.4% in *B. bassiana* application (Fig. 3).

Both microbial insecticides have shown encouraging results in controlling adult psyllids. Less number of sporulation under semi-filed conditions could be strongly affected by climatic conditions, temperature, and RH, but interestingly, the mortality level was satisfactory. *I. fumosorosea* induced (9.4–10.9%) higher mortality rate of psyllid adults under laboratory and semi-field conditions. The efficacy of fungal species against ACP has been reported earlier (Hoy et al. 2010; Ferreira Pinto et al. 2012; Stauderman et al. 2012). The results are in accordance to those of Ferreira Pinto et al. (2012), Lezama-Gutiérrez et al. (2012), and Ausique et al. (2017). The entomopathogens cause the direct effect on insect pests by infection; however, Avery et al. (2011) reported that *I. fumosorosea* decreased the feeding activity of ACP before their death and led to a reduction in the transmission of the bacterial pathogen responsible for citrus greening disease in citrus. The efficacy of EPF in open field with respect to environmental conditions of Pakistan needs to be studied because the environmental factors have great influence on the development of diseases induced by entomopathogenic fungi. However, Ausique et al. (2017) reported that environmental factors, mainly temperature and RH, strongly affected the sporulation on the fungal-killed citrus psyllids but the mortality level was satisfactory even at the drier conditions of the year.

Conclusions

The microbial pesticides based on the fungi *I. fumosorosea* and *B. bassiana* had a potential in controlling the citrus psyllids' population. Yet, further studies are needed to check their efficacy and compatibility against

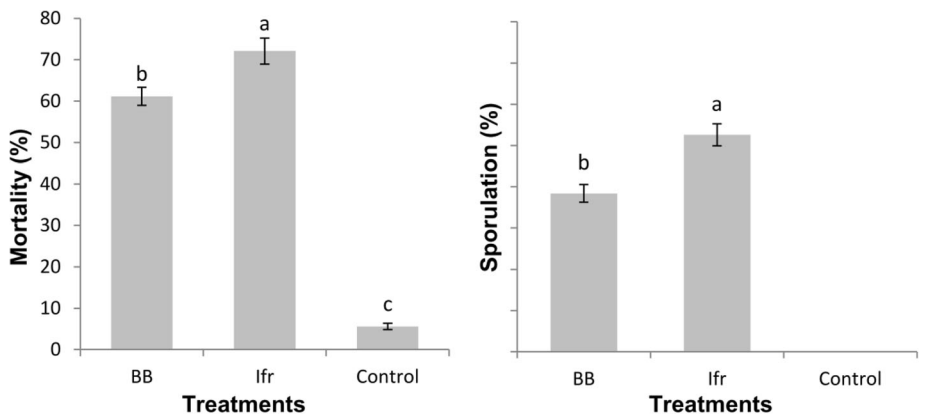

Fig. 3 Percent (means ± SE) mortality of adult psyllids and sporulation from cadavers after application of *Isaria fumosorosea* (Ifr) and *Beauveria bassiana* (BB) under semi-field conditions. Means sharing different letters are significantly different at ($P < 0.05$)

psyllids under different field climatic conditions of Pakistan.

Abbreviations
ACP: Asian citrus psyllid; CA: College of Agriculture; CRI: Citrus Research Institute; EPF: Entomopathogenic fungi; HLB: Huanglongbing disease

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Availability of data and materials
Data and materials will be shared if needed.

Authors' contributions
MIU and MA designed and conducted the study. SK and YI helped in reviewing, proof reading, and data analyses. AA and SMAZ helped in data collection and analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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