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Virulence of *Beauveria bassiana* Balsamo to red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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Abstract

Background: The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is a cosmopolitan pest of palms. Current management strategies largely depend on insecticides, and use of insect pathogenic fungi is needed in integrated pest management (IPM) programs.

Results: The effectiveness of *Beauveria bassiana* was determined against 3rd instar *R. ferrugineus* larvae by using the following concentrations, i.e., 3×10^8, 2×10^8, 1×10^8, 1×10^7, and 1×10^6 spores/ml. The lowest LC_{50} value (1.3×10^7 spores/ml) was recorded at the Khyber Pakhtunkhwa (KPK) population, followed by those populations from Punjab, Sindh, and Baluchistan, which had LC_{50} values of 1.5×10^7, 5.3×10^7, and 1.02×10^8 spores/ml, respectively, on the 7th day post-treatment; the highest mortality rates were 90.0, 85.0, 77.5, and 75.0% for the KPK, Punjab, Sindh, and Baluchistan populations, respectively, at the highest tested concentration of *B. bassiana*.

Conclusion: The findings of this study indicate the potential use of *B. bassiana* in IPM programs for *R. ferrugineus* that are more ecologically sound than those programs that are heavily reliant on insecticides.

Keywords: Biocontrol, *Rhynchophorus ferrugineus*, Date palm, Insect pathogenic fungi

Background

Phoenix dactylifera L., date palm, is an ancient crop that is cultivated worldwide mostly in tropical regions and can be found as an important part of both large- and small-scale farming (Khushk et al. 2009). The production of dates has been decreasing in Pakistan due to the effect of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), which is a major pest of date palm all over the world (Abbas 2013). In the early stage of infestation, it is difficult to detect *R. ferrugineus* because it is concealed within the tree. In severe infestations, damaged trees can harbor multiple life stages at the same time (Faleiro 2006).

Synthetic insecticides are usually used to control insect pests that may cause resistance development, kill beneficial insects, pollute the atmosphere, and cause human health hazards (Thomas and Read 2007). To mitigate these problems, integrated pest management needs to be adopted such as the use of safer insecticides in combination with biological control agents, for example, entomopathogenic fungi (EPF) (Wraight et al. 2001). The insect pathogenic fungi have significant importance due to their infectious ability to insects (Mustafa and Kaur 2009). Around the globe, *Beauveria bassiana* Balsamo, *Metarhizium anisopliae* Sorokin, *Isaria fumosorosea* Wize, and *Beauveria brongniartii* Petch are commonly used EPF (de Faria and Wraight 2007; Dembilio et al. 2010) . Fungi are used for the management of various insect orders, e.g., Coleoptera including *R. ferrugineus* (Hussain et al. 2015).

B. bassiana is eco-friendly, safe in use, and a well-known biological control agent with a wide host range (Devi and Rao 2006). *B. bassiana* is a potential tool, and its efficacy differs among adult and larval instars of *R. ferrugineus* (Lo Verde et al. 2015). The present study was conducted to assess the potential of *B. bassiana* in

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managing populations of *R. ferrugineus* under laboratory conditions from 4 provinces of Pakistan.

Methods

Insect collection and rearing

Rhynchophorus ferrugineus larvae and adults were collected from 4 provinces of Pakistan, i.e., Punjab (30°11′52″N: 71°28′11″E), Baluchistan (28°22′37″N: 68°21′2″E), Sindh (27°32′N: 68°46′E), and Khyber Pakhtunkhwa (KPK) (31°49′53″N: 70°54′7″E). The field-collected *R. ferrugineus* were transferred to sterile plastic cages (30×60×60 cm) covered with the muslin cloth. Adults of *R. ferrugineus* were reared on clean, fresh, and uninfected *Saccharum officinarum* stems (refreshed every 2 days). The larvae were reared on prepared diet following the method described by Hussain et al. (2015). The insects were reared under the laboratory conditions of 27±2°C, 70±5% RH, and 12/12 h L/D photoperiod.

Beauveria bassiana

The local soil-extracted isolate of *B. bassiana* (Bb-01) was obtained from (30°05′11.65″N 71°39′15.65″E) Multan, Punjab, Pakistan, and maintained in the Laboratory of the Insect Microbiology and Biotechnology and was used for the experiments.

Fungal bioassay

Third instar larvae of *R. ferrugineus* were used in the assays. Each larva was dipped for 10–15s in one of the following concentrations of *B. bassiana*, i.e., 3×10^8, 2×10^8, 1×10^8, 1×10^7, and 1×10^6 spores/ml. All concentrations were prepared in an aqueous solution of 0.1% Tween 80 as defined by Alkhaibari et al. (2017). For each concentration and replicate, 80 larvae were exposed, at 4 replicates. A total of 480 larvae were tested in the bioassay including a control. For the control, only the aqueous solution containing Tween 80 was used. The treated larvae were placed in plastic Petri plates (2.5-cm diameter) with an artificial diet. The data on mortality was recorded on the 3rd, 5th, and 7th day post-treatment.

Statistical analysis

Fungal bioassay data were analyzed by the POLO Plus software (LeOra Software 2005) which yielded LC_{50} values, 95% confidence limits (FL), slope ± SE, and chi-square values (χ^2). The means for mortality were separated among the treatments by using least significant difference (LSD) test ($P \leq 0.05$). All analysis was performed by using Statistix 8.1.

Results

Virulence of *B. bassiana* against *R. ferrugineus*

The virulence of *B. bassiana* on larvae of *R. ferrugineus* and the LC_{50} values were estimated for populations of the weevil collected from 4 provinces in Pakistan. The lowest LC_{50} value (1.3×10^7 spores/ml) was recorded at the KPK population, followed by those populations from Punjab, Sindh, and Baluchistan, which had LC_{50} values of 1.5×10^7, 5.3×10^7, and 1.02×10^8 spores/ml, respectively (Table 1).

Virulence of *B. bassiana* against *R. ferrugineus* on different days post-treatment

The highest percent mortality of the weevil larvae were 57.5, 53.75, 52.5, and 50.0% on the 3rd day post-treatment, for the Punjab, KPK, Sindh, and Baluchistan populations, respectively (Table 2). On the 5th day post-treatment, correspondent percentages were 70.0, 67.5, 65.0, and 53.75% for the KPK, Punjab, Sindh, and Baluchistan populations, respectively (Table 3). On the 7th day post-treatment, respective mortality rates were 90.0, 85.0, 77.5, and 75.0% for the KPK, Punjab, Sindh, and Baluchistan populations, respectively (Table 4). These percent mortality rates occurred at the highest tested concentration of *B. bassiana*.

Discussion

The date growers mostly rely on the insecticides due to its rapid and spectacular results against *R. ferrugineus*. Development of an alternative strategy such as introduction of insect pathogenic fungi is necessary as a major component of IPM. EPF have proved to be an effective tool for the control of insect pests due to larvicidal

Table 1 Virulence of *Beauveria bassiana* against *Rhynchophorus ferrugineus* from Pakistan

Provinces	LC_{50} (spores/ml)	(95% FL)	Slope	χ^2	df	*P*	N
Punjab	1.5×10^7	3.1×10^6–4.0×10^7	0.55±0.11	1.495	4	0.82753	480
Sindh	5.3×10^7	9.4×10^6–1.8×10^8	0.27±0.05	1.784	4	0.77541	480
Baluchistan	1.02×10^8	1.71×10^7–5.06×10^8	0.27±0.05	2.774	4	0.59633	480
KPK	1.3×10^7	2.6×10^6–3.5×10^7	0.28±0.05	5.151	4	0.27215	480

P-values are based on Chi-square goodness of fit test. *P* values > 0.05 recommend goodness of fit of the model
FL Fiducial limits, *N* number of *R. ferrugineus* used in the bioassay, with the control

Table 2 Percent mortalities of *Rhynchophorus ferrugineus* on the 3rd day after treatment with *Beauveria bassiana*

Concentrations (spores/ml)	Baluchistan	Sindh	KPK	Punjab
1×10^6	25.0 cd	22.5 de	28.7 c	31.75 c
1×10^7	32.5 bc	30.0 cd	36.2 bc	40.0 bc
1×10^8	37.5 abc	40.0 bc	42.5 ab	43.7 b
2×10^8	40.0 ab	42.5 ab	46.2 ab	45.5 b
3×10^8	50.0 a	52.5 a	53.7 a	57.5 a
Control	13.75 d	15.0 e	12.5 d	18.7 d
F-value	20.1	26.3	32	36.6
P-value	0	0	0	0
LSD	12.633	12.137	11.545	9.9983

The values sharing same letters in columns are not significantly different ($P \leq 0.05$)

potential and less toxicity to non-target species (Freed et al. 2012).

B. bassiana is the most important EPF of Coleopterans (Cottrell and Shapiro-Ilan 2003) and showed promising results against the larvae of *R. ferrugineus* (Hajjar et al. 2015). In the present study, the pathogenicity of *B. bassiana* was evaluated against larvae of *R. ferrugineus* collected from 4 provinces of Pakistan. The highest percent mortality was found in the KPK population (90%), followed by the Punjab (85%), Sindh (77.5%), and Baluchistan (75%) populations at the highest concentration, 7th day post-treatment. These rates of mortality on day 7 suggest that the fungal spores successfully penetrated the cuticle of the larvae and were able to grow vegetatively inside the larvae, causing death due to a lack of nutrition (Inglis et al. 2001).

The outcomes of this study were consistent with Gindin et al. (2006) who examined the susceptibility of *R. ferrugineus* to *B. bassiana* at the concentration 2×10^7 spores/ml that caused 100% larval mortality within 6–7 days. Obtained results are also supported by the studies

Table 3 Percent mortalities of *Rhynchophorus ferrugineus* on the 5th day after treatment with *Beauveria bassiana*

Concentrations (spores/ml)	Baluchistan	Sindh	KPK	Punjab
1×10^6	32.5 cd	35.0 cd	35.0 cd	31.2 cd
1×10^7	38.7 bc	41.2 bc	41.2 bc	42.5 bc
1×10^8	45.0 ac	50.0 ac	47.5 bc	50.7 b
2×10^8	50.0 ab	55.0 ab	57.5 ab	55.0 ab
3×10^8	53.7 a	65.0 a	70.0 a	67.5 a
Control	21.25 d	18.7 d	21.2 d	21.2 d
F-value	7.28	19.3	17.8	34.1
P-value	0.0007	0	0	0
LSD	20.040	16.646	18.254	12.840

The values sharing same letters in columns are not significantly different ($P \leq 0.05$)

Table 4 Percent mortalities of *Rhynchophorus ferrugineus* on the 7th day after treatment with *Beauveria bassiana*

Concentrations (spores/ml)	Baluchistan	Sindh	KPK	Punjab
1×10^6	43.7 bc	45.0 c	50.0 d	45.0 d
1×10^7	52.5 b	52.5 bc	61.2 cd	62.5 c
1×10^8	57.5 ab	66.2 ab	70.5 bc	71.2 bc
2×10^8	65.0 ab	70.0 a	81.2 ab	80.0 ab
3×10^8	75.0 a	77.5 a	90.0 a	85.0 a
Control	25.0 c	26.2 d	27.5 e	26.2 e
F-value	12.7	28.9	45.1	70.4
P-value	0	0	0	0
LSD	21.961	15.781	15.099	11.992

The values sharing same letters in columns are not significantly different ($P \leq 0.05$)

of Hou et al. (2018) who found that percent mortality of adult *R. ferrugineus* increased with increased conidial concentration applied. The highest concentration tested, 7.85×10^8 spores/ml, resulted in the highest mortality of the adult weevils. The findings were also supported by Yasin et al. (2019), who reported >75% adult and >88% larval mortality against *R. ferrugineus* by using *B. bassiana.* Collectively, the results of these studies suggest that mortality of various life stages of *R. ferrugineus* increased with increasing time after exposure and concentration of *B. bassiana*. Francardi et al. (2012) found that treatment of both larvae and adults of *R. ferrugineus* with *B. bassiana* resulted in 100% larval and 90% adult mortality under laboratory conditions. Moreover, El Husseini (2019) reported *B. bassiana* to be pathogenic against the 3rd instar larvae of *R. ferrugineus* causing 100% mortality. However, Lo Verde et al. (2015) found higher efficacy of *B. bassiana* against weevil larvae than the adults. The high efficacy of *B. bassiana* against *R. ferrugineus* was demonstrated in field trials (Abbas 2013). In the present study, *B. bassiana* treatment resulted in 90% larval mortality, whereas in earlier studies, this treatment provided 45% mortality (Francardi et al. 2013). Findings of current research provided novel options to develop very effective biocontrol agents based on fungi. This research will facilitate productive date industry with the use of an efficient tactic for the management of *R. ferrugineus*.

Conclusion

In conclusion, the present study described *B. bassiana* pathogenicity in *R. ferrugineus*. *B. bassiana* with its novel mode of action properties is a good alternative to chemical control as it tremendously reduced the survival of *R. ferrugineus.* This research also opens novel options to develop mycoinsecticides that might provide date industry with an efficient technique for management of *R. ferrugineus*.

Abbreviations
EPF: Entomopathogenic fungi; RH: Relative humidity; FL: Fiducial limits; IPM: Integrated pest management

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Not applicable.

Studies involving plants must include a statement specifying the local, national, or international guidelines and legislation and the required or appropriate permissions and/or licences for the study (if applicable)
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Authors' contributions
RH performed the experiment, analysis of data, and manuscript writing; SF contributed to technical assistance, analysis of data, supervision, and manuscript writing. The authors read and approved the final manuscript.

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