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., $3 \times 10^8$, $2 \times 10^8$, $1 \times 10^8$, $1 \times 10^7$, and $1 \times 10^6$ spores/ml. The lowest LC$_{50}$ value ($1.3 \times 10^7$ 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 $1.5 \times 10^7$, $5.3 \times 10^7$, and $1.02 \times 10^8$ 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...
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 10–15 s in one of the following concentrations of \( B. \) bassiana, i.e., 3×10⁶, 2×10⁶, 1×10⁶, 5×10⁵, and 1×10⁵ 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.

**Results**

**Virulence of \( B. \) bassiana against \( R. \) ferrugineus**

The virulence of \( B. \) bassiana on larvae of \( R. \) ferrugineus and the LC₅₀ values were estimated for populations of the weevil collected from 4 provinces in Pakistan. The lowest LC₅₀ value (1.3×10⁷ spores/ml) was recorded at the KPK population, followed by those populations from Punjab, Sindh, and Baluchistan, which had LC₅₀ values of 1.5×10⁷, 5.3×10⁷, and 1.02×10⁸ 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 (P) | LC₅₀ (spores/ml) | (95% FL) | Slope ± SE | χ² | df | P  | N  |
|---------------|-----------------|----------|------------|----|----|----|----|
| Punjab        | 1.5×10⁷         | 3.1×10⁶–4.0×10⁶ | 0.55±0.11 | 1.495 | 4  | 0.82753 | 480 |
| Sindh         | 5.3×10⁷         | 9.4×10⁶–1.8×10⁸ | 0.27±0.05 | 1.784 | 4  | 0.77541 | 480 |
| Baluchistan   | 1.02×10⁸        | 1.71×10⁶–5.06×10⁶ | 0.27±0.05 | 2.774 | 4  | 0.59633 | 480 |
| KPK           | 1.3×10⁷         | 2.6×10⁶–3.5×10⁷ | 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.
potential and less toxicity to non-target species (Freed et al. 2012).

*Beauveria 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 applied. The highest concentration tested, 7.85 × 10^8 spores/ml, resulted in the highest mortality of 75% against weevil larvae than the adults. The high efficacy of *B. bassiana* against larval mortality against *R. ferrugineus* 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*.

### 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 ab     | 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

P-value: 0.0007

LSD: 12.633

The values sharing same letters in columns are not significantly different (P<0.05)

### 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

P-value: 0.0007

LSD: 20.040

The values sharing same letters in columns are not significantly different (P<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

P-value: 0.0007

LSD: 21.961

The values sharing same letters in columns are not significantly different (P<0.05)
Abbreviations
EPF: Entomopathogenic fungi; RH: Relative humidity; FL: Fiducial limits; IPM: Integrated pest management

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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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The authors declare that they have no competing interests.

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