Research Article

# Entomopathogenicity and Modeling Aptness of Fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) against Pulse Beetle, *Callosobruchus chinensis* L. (Bruchidae: Coleoptera)

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**Abstract** | Aptness of entomopathogenic fungi (EPF), *Beauveria bassiana* and *Metarhizium anisopliae* was evaluated to manage pulse beetle (PB), *Callosobruchus chinensis* by using different fungal concentrations of $1\times10^6$, $1\times10^7$ and $1\times10^8$ spores/ml in stored chickpea grains. Highest mortality (90%) of PB was observed in 72 hours through application of $1\times10^8$spores/ml of *M. anisopliae* compared to *B. bassiana* with 60% mortality. On 24 hours of exposure, minimum mortality (4%) in PB was recorded in grains treated with $1\times10^6$ spores/ml of *B. bassiana*. In *M. anisopliae,* the concentrations of $1\times10^8$ after 24 hours, $1\times10^7$ after 48 hours and $1\times10^7$ after 72 hours showed significantly similar results with mortality around 55%. Results proved *M. anisopliae* as more virulent against PB than *B. bassiana* and fungal effectiveness against this pest was directly proportional to its concentration as well as exposure time. For effective management of *C. chinensis*, $1\times10^8$ spores/ml of *M. anisopliae* and *B. bassiana* is recommended for safer storage of chickpea, pulses and grains.

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## Introduction

Chickpea (*Cicer arietinum*) ranks third most important pulse crop followed by peas and soybean and it contributes 15% of total pulse production in world (FAO, 2012). In Pakistan, it is cultivated on more than 945000 hectares and covers about 76% of the total land under pulses with production of 312000 tons in 2016 (GOP, 2016). Punjab Province contributes around 80% of chickpea production in the country where 90% region of chickpea is under rain-fed conditions (Hussain et al., 2015).

The grains of chickpea are severely damaged under storages causing huge economic losses. Due to heavy insect infestation, grains lose their germination capability and become unfit for human consumption. Pulse beetle (*C. chinensis*) is known as one of the most devastating insect pests of stored chickpea grains. *C. chinensis* has been distributed in different countries of the world with a severe destruction records to pulses in India and Bangladesh. It is cosmopolitan in distribution and has ability to damage stored grains and as field cultivated host plants (Fahad, 2011). It is reported that infestation caused by this beetle results in severe losses in seed weight and protein content of pulses that are 55-60% and 46-66%, respectively (Faruk et al., 2011).

Synthetic pesticides and fumigants are commonly used to control PB infestation, yet injudicious use of these pesticides have resulted in creating issues of genetic resistance among insect pests. These chemicals are also known to cause persistent toxicity, environmental pollution, pest resurgence and economical unfeasibility (Elhag, 2000). Currently, botanicals and biological control agents like pathogenic microorganisms; predators and parasitoids are efficiently used as alternative source of synthetic pesticides in integrated pest management (IPM) due to risks to human health as well as ecosystem (Isman, 2006). Many biotic organisms, particularly entomopathogenic nematodes, bacteria and fungi are playing important roles to manage stored grain insect infestations for the last many years.

Entomopathogenic fungi (EPF) are widely distributed pathogens with broad host series that shows effective control against insects. These microorganisms are well known for their efficient bio-control approach particularly against arthropods (Huxham et al., 1989). Generally, these entomopathogenic fungi are host-specific and thus reduce probabilities of adverse effects on non-target or beneficial organisms. EPFs cause infection in their host insects with the help of conidia/spores that are produced asexually and germinate and then penetrate in exoskeleton of host in suitable environmental conditions and germinate inside host cuticle (Bateman et al., 1996). Penetration in the host cuticle is accomplished with the help of mechanical and enzymatic degradation that permits germ tube to propagate inside haemocoel, where EPFs produce endotoxins resulting in death of host insect. Afterwards, fungus develops out from the cadaver and with development of spores/conidia outside the cadaver, completes its lifecycle. As the fungal spores disperse in air and land on another host, its infection cycle starts again and further spreads (Inglis et al., 2001).

Keeping in view the potential of EPFs it was planned to evaluate efficacy of entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) against *C. chinensis* in stored chickpea grains.

## Materials and Methods

Samples of stored chickpea grains infested with *C. chinensis* were collected from National Agriculture Research Centre (NARC), Islamabad and different flour mills and storages/go downs of the Potohar region. *C. chinensis* culture was maintained in an incubator set at 30±2°C temperature and 70±5 % RH in 'Stored Product Entomology Laboratory' of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

Cultures of *B. bassiana* and *M. anisopliae* were maintained in 'Fungal Plant Pathology Laboratory' of the university. Initially culture was grown on Potato Dextrose Agar (PDA) at 25°C and 200 rpm for two weeks, and then it was multiplied in Potato Dextrose Broth medium to count number of conidia/spores per unit volume. The conidia/spores were grown on PDA medium. Later on spores/conidia were counted by haemocytometer at 24 hours' interval (Tuan et al., 2009). The concentrations of $8\times10^7$and $5.6\times10^7$ spores/ml of *B. bassiana* and *M. anisopliae*, respectively were obtained by counting spores/conidia using haemocytometer. Distilled water was added to get the required concentrations of $1\times10^6$, $1\times10^7$ and $1\times10^8$ spores/ml for insect bioassays. For mass culturing of fungi, petri plates were kept in the incubator at 28 ± 1°C and 75 ± 5% R.H. Finally, culture was oven dried and conidia were harvested from culture with the help of rubber scalpel.

Mortality of *C. chinensis* was determined in treated and untreated 10g of stored chickpea grains in petri plates of 7cm diameter (38.5 $cm^2$), having Whatman No. 1 filter paper. Ten 1-3 days old beetles were released in each petri plate. Different concentrations ($1\times10^6$, $1\times10^7$ and $1\times10^8$ spores/ml) of both fungi were applied to grains at 2 ml rate of fungal concentration in each petri plate. Each treatment had 3 replications. Mortality of *C. chinensis* was observed after 24, 48 and 72 hours. Petri-plates were placed in the incubator.

For studying modeling of aptness, regression model was used to compare relationship of treatment impacts on insect parameters. The model equation was y = a + bx, where y = insect parameters and x = treatments of fungal concentrations. Firstly, simple linear regression was used to check individual effect of each treatment on insect parameters. Afterwards, an impact of all treatments on mortality was modeled using multiple linear regression approach and supported with stepwise forward regression. The performance of model was evaluated by $R^2$.

The data recorded was subjected to statistical analysis using appropriate statistical package i.e. SPSS 22.0 for Windows and Microsoft Excel programs.

## Results and Discussion

*Entomopathogenicity of B. bassiana against C. chinensis*

As evident from the Figure 1, highest mortality (33%) of *C. chinensis* adults after 24 hours' interval was observed in jars treated with fungal concentration of $1\times10^8$ spores/ml followed by concentration of $1\times10^7$ spores/ml with 16% mortality. On the other hand, minimum mortality (3%) was observed in lowest concentration ($1\times10^6$ spores/ml) of *B. bassiana* contrary to no mortality in the control petri plates. The concentration of $1\times10^6$ and the control showed statistically similar results and were significantly different from those of $1\times10^7$ and $1\times10^8$ spores/ml, being statistically alike with each other. Mortality of PB was seen to increase with increase in time interval and concentration of entomopathogenic fungus. After 48 hours, higher mortality (50%) at fungal concentration of $1\times10^8$ spores/ml was recorded whereas 3%, 7% and 23% mortality was observed in control and fungal concentrations of $1\times10^6$ and $1\times10^7$ spores/ml, respectively. All the three fungal concentrations were significantly different from one another; however, concentration of $1\times10^6$ was not significantly different from control.

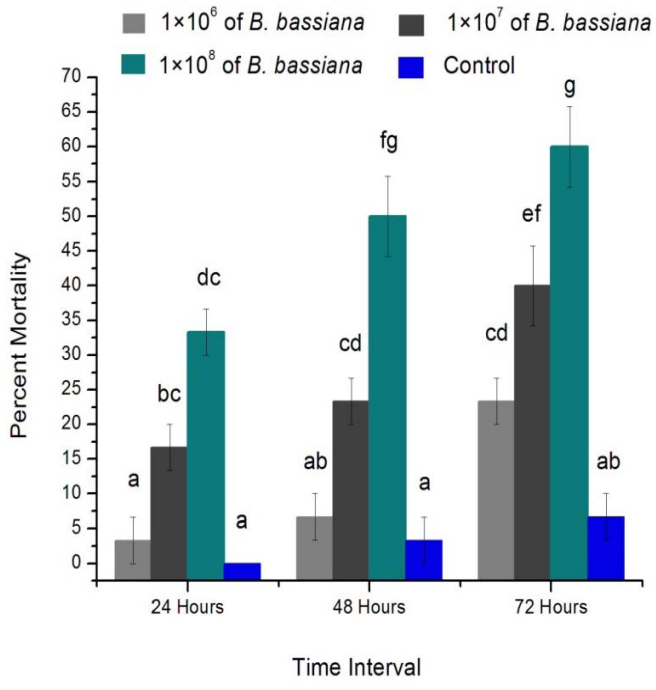


**Figure 1:** *Mortality (%) of C. chinensis after application of different concentrations of B. bassiana at different time intervals.*

Mortality of 60% was reported after 72 hours' interval when using fungal concentration ($1\times10^8$spores/ml) as compared to control with only 7%. Mortality in control jars was significantly different from all fungal concentrations. Similarly, all fungal concentrations were also statistically dissimilar with one another. Concentrations of $1\times10^6$ and $1\times10^7$spores/ml showed mortality of 23% and 40%, respectively. Concentration of $1\times10^7$ spores/ml after 48 hours showed statistically similar results as that of $1\times10^6$ spores/ml after 72 hours. The efficacy of concentration ($1\times10^8$ spores/ml) after 48 hours was significant similar with concentrations of $1\times10^7$ and $1\times10^8$ spores/ml after 72 hours' interval.

Linear regression model was used to check effect of different concentration of *B. bassiana* on percent mortality after 24, 48 and 72 hours (Figure 2). The modeled equation (Y= 11.33x -15) of mortality percentage after 24 hours revealed that all fungal concentrations performed better in causing mortality with the coefficient of determination ($R^2$) 0.93. Whereas the regression equation (Y= 15.67x -18.33) of interval 48 hours showed that the intercept (a) value remained -18.33 but slope (b) was 15.67 presented direct relation of mortality between time interval and fungal concentration. On the other hand, the maximum mortality was observed after 72 hours where the modeled equation was (Y= 17.67x -11.65) with the coefficient of determination ($R^2$) 0.99, which exposed that the fungal concentrations (independent variable) showed 99% effect on the dependent variable.

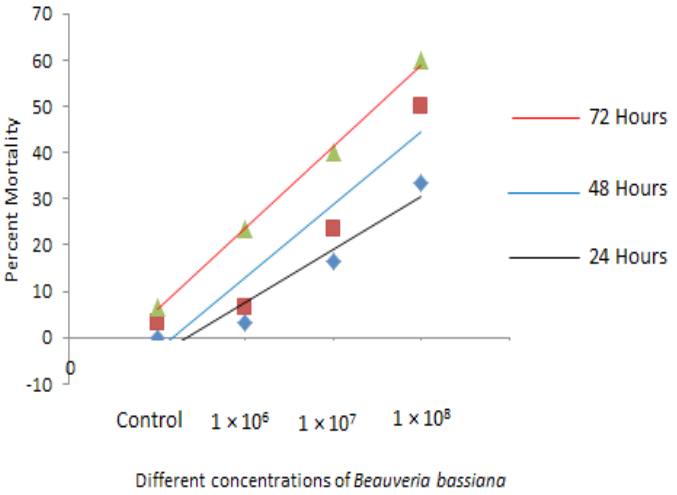


**Figure 2:** *Modeling trend of C. chinensis mortality in response to B. bassiana after 24, 48 and 72 hours.*

*Entomopathogenicity of M. anisopliae against C. chinensis*

Results revealed that fungal concentration is directly proportional to percent mortality and time interval (Figure 3). After 24 hours' exposure, 58% mortality of PB was recorded with concentration ($1\times10^8$ spores/

ml) of *M. anisopliae* and was significantly different from other two fungal concentrations and the control. Mortality of 7% and 30% was noted when treated with concentrations of $1\times10^6$ and $1\times10^7$ spores/ml, respectively depicting statistically different response with each other and also with control. However, all fungal concentrations were better than control for higher mortality.

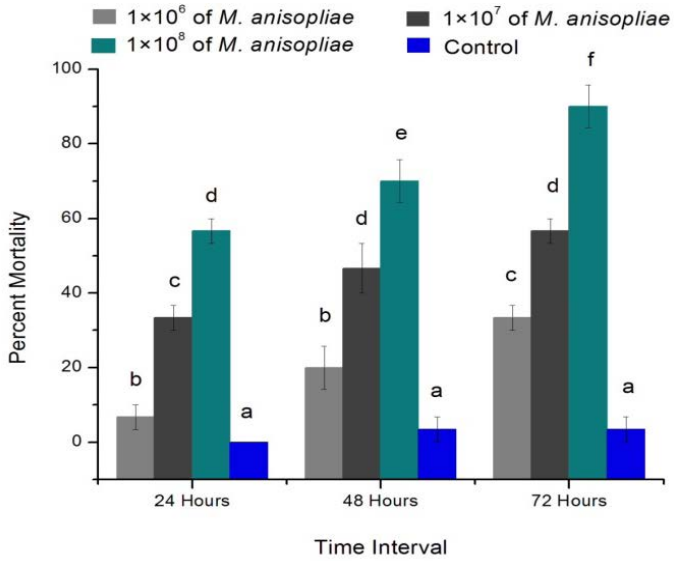


**Figure 3:** *Mortality (%) of C. chinensis after application of different concentrations of at different time intervals.*

After 48 hours, 70% mortality was caused by fungal concentration of $1\times10^8$ spores/ml that was significantly different from those of $1\times10^6$ and $1\times10^8$ spores/ml with 18% and 42% mortality, respectively that were also statistically not alike with each other. Highest mortality of 90% was observed after 72 hours when grains were treated with fungal concentration of $1\times10^8$ spores/ml while concentration ($1\times10^7$ spores/ml) caused 57% mortality and both were statistically different with each other. Mortality of 30% was reported when using $1\times10^6$ concentration and showing significantly higher mortality than in control plates. Concentration of $1\times10^8$ after 24 hours, $1\times10^7$ after 48 hours and $1\times10^7$ spores/ml after 72 hours showed significantly similar results with mortality around 55%. Similarly, statistically similar mortality was seen in grains treated with $1\times10^6$ spores/ml of fungal concentration after 24 and 48 hours of interval.

Linear regression model was applied to determine effectiveness of concentrations of *M. anisopliae* against PB. After the 24 hours' interval the modeled equation (Y= 19.67x-25) of mortality percentage depicted that all the fungal concentrations showed better results in causing mortality with the coefficient of determination ($R^2$) 0.95. After 72 hours, where the modeled equation was (Y= 28.33x -25) with the coefficient of determination ($R^2$) 0.99 exposed that the fungal concentrations (Independent variable) have 99% effect on the dependent variable (Figure 4).

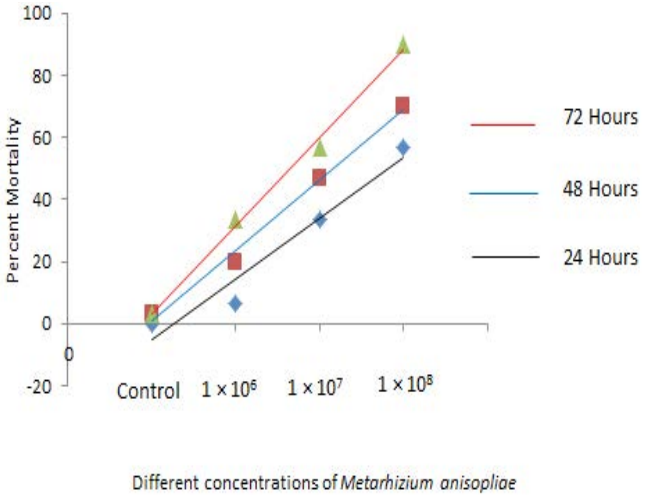


**Figure 4:** *Modeling trend of C. chinensis mortality in response to M. anisopliae after 24, 48 and 72 hours.*

Results of this research were similar to findings of Anitha et al. (2015), who observed that mortality rate was directly proportional to fungal concentration as well as exposure time. Riasat et al. (2011) revealed that highest mortality of beetles was resulted with highest dose and greater exposure time and similar trend was observed in current studies. Results depicted that mortality of adults was increased with increase in fungal concentration and time interval. However, entomopathogenic fungus *M. anisopliae* showed comparatively higher mortality of *C. chinensis* as compared to *B. bassiana*.

## Author's Conribution

**Wasim Javaid:** Conducted research and wrote manuscript.
**Farid Asif Shaheen:** Coceived and supervised research, wrote manuscript and is the corresponding author.
**Farah Naz:** Analyzed data and supervised pathological aspects of research.
**Muhammad Usman Raja:** Proof checking and supervised pathlogical aspects of research.

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