Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com
JEZS
Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 1972-1976
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Received: 05-07-2018
Accepted: 06-08-2018

Efficacy of different entomopathogenic fungi on biological parameters of pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract

Pulses are important stored grain products having a rich source of protein and of great value for developing countries like Pakistan who hardly afford animal protein in adequate quantities. The pulse seeds during storage are prone to insect attack and due to residual effect of pesticides biocontrol agents especially entomopathogenic pathogenic fungi are encouraged for control of *Callosobruchus chinensis*. In the current study, *Metarhizium anisopliae* showed 92.50%, *Beauveria bassiana* 87.50% and *Isaria fumosorosea* 80.0% mortality of *Callosobruchus chinensis*. The sublethal dose of fungus negatively affected the surviving adults irrespective of their sex difference. A considerable decrease in egg hatching (68.19%), reduced adult emergence (65.20%) and prolonged larval-pupal period (23.40) (days) was observed.

Keywords: Biocontrol, *Callosobruchus chinensis*, entomopathogenic fungi, house hold, mortality, sublethal dose

Introduction

Pulses are important stored grain products having a rich source of protein and of great value for the people of developing countries like Pakistan who hardly afford animal protein in adequate quantities. Grains that are selectively attacked by the insects will cause greater loss in germination weight, quality, and commercial value than others. The pulse seeds during storage are very much prone to insect attack [1]. According to an estimate, the overall damage caused by stored-grain insect pests accounts for 10-40% worldwide annually [2]. Pulse beetle, *Callosobruchus chinensis* L. is a serious pest of stored food grains and causes damage to various pulses i.e. chickpea (*Cicer arietinum* L.), cowpea (*Vignaungui culata* L.), black gram, *Phaseolus bengalensis* L. [3]. The grubs of *C. chinensis* damage the grains by eating out the whole grain content and leaving behind the empty shell or the seed coat.

Primarily, stored grain products insect pests are controlled by the use of fumigants, dust and residual chemical insecticides [4]. However, chronic exposure to synthetic insecticides exhibits greater environmental hazards besides various biochemical and behavioral changes in non-target animals [5, 6]. There is a need to develop safe alternatives to non-target organisms and entomopathogenic fungi proofs to an environmentally friendly substitute of chemical insecticides due to low mammalian toxicity. Insect pathogenic fungi are an important section of integrated pest management systems and effective as biological control agents for coleopteran pests [7, 8]. These fungi are low-cost, easy to formulate and competent in a wide array of humidity and temperature with commercial availability [9, 10]. Entomopathogenic fungi i.e. *Beauveria bassiana* (Bals.) Vuill., and *Metarhizium anisopliae* (Metsch.) Sorok. with a wide host range are previously reported for bruchid pests [11, 12]. Literature review emphasizes less resistance development in insects against entomopathogenic fungi [13]. Fungi infect the hosts primarily via the external insect cuticle although infection also occurs through the digestive tract in some insects [14]. Considering the economic damage caused by *C. chinensis* and potential of entomopathogenic fungi against insect pests of stored grains current study was planned to check the efficacy of three different entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *Isaria fumosorosea* on this insect pest.

Material and Methods

Insect Rearing

Insect rearing was done in the laboratory of insect microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan. In 2015 Adults of *C. chinensis* collected from infested *V. unguiculata* grains were reared on healthy *V. unguiculata* grains. The insects were kept in glass pots (20×10cm) covered with a mesh cloth under the prevailing environmental conditions of 28 ± 2 ˚C, 70 ± 5% RH and 14:10 L: D period. The freshly emerged adults (male & female) of *C. chinensis* were used in the bioassay.

Fungal Isolates

Three isolates of entomopathogenic fungi *B. bassiana* (Bb-08), *M. anisopliae* (Ma-2.3) and *I. fumosorosea* (If-2.3) were used in the experiment obtained from the stock cultures in the Laboratory of the Insect Microbiology and Biotechnology, Department of Entomology Bahauddin Zakariya University, Multan, Pakistan. Isolates were cultured on PDA (potato dextrose agar) media for 14 days at 28 °C. The fungi were stored at 4 °C until needed for the bioassay experiments.

Spore suspension

Conidial suspensions of these fungal isolates were made by adding sterile 0.05% tween 80 into the fully overgrown Petri dishes. A sterile bent glass rod was used to remove the conidia from the surface of the agar plates after gently scraping. The obtained conidial suspensions were stirred on a magnetic stirrer for 5 min for homogenization. Finally, the spore concentration was determined with a Neubauer hemocytometer and prepared desired concentrations 1×10^{5}, 1×10^{6}, 1×10^{7}, 1×10^{8}, 2×10^{8} and 3×10^{8} spores/ml of each isolate by the serial dilution method.

Bioassay

Pathogenicity of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* and their sublethal effects on *C. chinensis* was studied by the immersion method. Five pairs of freshly emerged (1 day old) adults per replication with six treatments including control and each treatment was replicated four times. The adults of *C. chinensis* were individually immersed in the concentrations for about 8-10 seconds while in the control adults were treated with Tween 80 (0.05%). After treatment, the adults were then shifted in Petri dishes provided with 10 grams of *V. unguiculata* grains for food and oviposition. Observations of mortality were recorded in each treatment. The adult mortality was recorded daily for consecutive seven days after the release and all the remaining adults were removed after seven days of release and number of eggs laid was recorded.

Effect of sublethal dose of entomopathogenic fungi on biological parameters of *C. chinensis*

The effect of sublethal dose (1×10^{5} spores/ml) of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* on the fecundity and longevity of *C. chinensis*. A total of 20 pairs (sex ratio, 1:1) were used for each treatment with four replications. A similar procedure was adopted for bioassay as explained earlier. Data regarding biological parameters including longevity, fecundity, hatching, larval-pupal period, adult emergence and sex ratio were calculated as by Varma and Anandhi (2010).

Statistical Analysis

Data on adult's mortality were corrected by using Abbott's formula [15] and percentages of mycosed cadavers were calculated. The data regarding biological parameters i.e., longevity, fecundity, hatching, larval-pupal period, adult emergence and sex ratio were analyzed by analysis of variance (ANOVA) with LSD test (0.05) for mean separation.

Results

The results showed that *M. anisopliae* (Ma-2.3) has least with LC_{50} of 2.3×10^{5} (spores/mL) followed by *B. bassiana* (Bb-08) 3.3×10^{5}(spores/mL) and *I. fumosorosea* (If-2.3) 2.1×10^{6}(spores/mL). Cumulative percentage mortality of fungi on *C. chinensis* was *M. anisopliae* (Ma-2.3) 92.50%, *B. bassiana* (Bb-08) 87.50% and *I. fumosorosea* (If-2.3) 80.0% are represented in (Fig a, b and c). *M. anisopliae* (Ma-2.3) showed maximum mortality with LT_{50} of 5.05 (3.49-7.45) days concluded as most venomous isolate against *C. chinensis*; while other fungi (Table 2) *B. bassiana* (Bb-08) and *I. fumosorosea* (If-2.3) represents the cumulative mortality with LT_{50} of 5.19 (4.82-5.58) and 5.53(5.10-6.00) days respectively.

Effect of sublethal dose of entomopathogenic fungi on biological parameters of *C. chinensis*

The fungal infection had a negative effect on surviving adults of *C. chinensis* as longevity tended to decrease regardless of sex difference. The mean longevity of male *C. chinensis* decreased from 14.00 to 8.10 (days) (F=12.45, P=0.027) while 12.50 to 7.10 (days) (F=11.47, P=0.031) for female *C. chinensis* after application of a sublethal dose of entomopathogenic fungi. The mean of number due to entomopathogenic fungi ranged 49.10-73.40 as compared to control (95.0) (F=23.67, P=5.79). A considerable decrease in egg hatching (68.19%) was observed in *C. chinensis* as compared to control (94.73) (F=45.81, P=6.89). The larval-pupal period was significantly increased as a sublethal dose of *M. anisopliae* (Ma-2.3) showed maximum value (23.40) (days) in comparison to control group (19.0) (days) (F= 3.78, P=0.042). In addition, a significant decrease in adult emergence was observed as Ma 2.3 showed least 65.20% emergence in comparison to other treatments including control (F=18.32, P=0.001). Moreover, a significant change in the sex ratio of *C. chinensis* was observed.

Table 1: LC_{50} (spores/ml) values of *B. bassiana, M. anisopliae* and *I. fumosorosea* against adults of *C. chinensis*by immersion method.

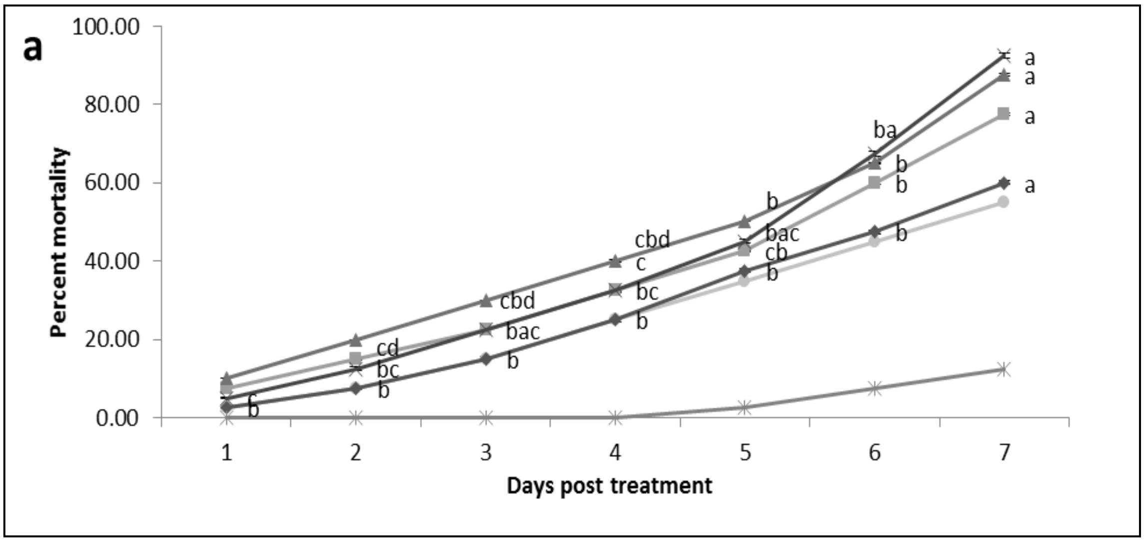
Fungi	Isolate	LC_{50} (spores/ml)	FD	Slope	DF	Chi
B. bassiana	Bb-08	3.3×10^{5}	5.5×10^{4}- 2.0×10^{6}	0.33±0.08	3	0.98
M. anisopliae	Ma-2.3	2.3×10^{5}	5.0×10^{4}- 1.0×10^{6}	0.42 ±0.08	3	1.26
I. fumosorosea	If-2.3	2.1×10^{6}	6.3×10^{5} – 7.4×10^{6}	0.36±0.08	3	1.79

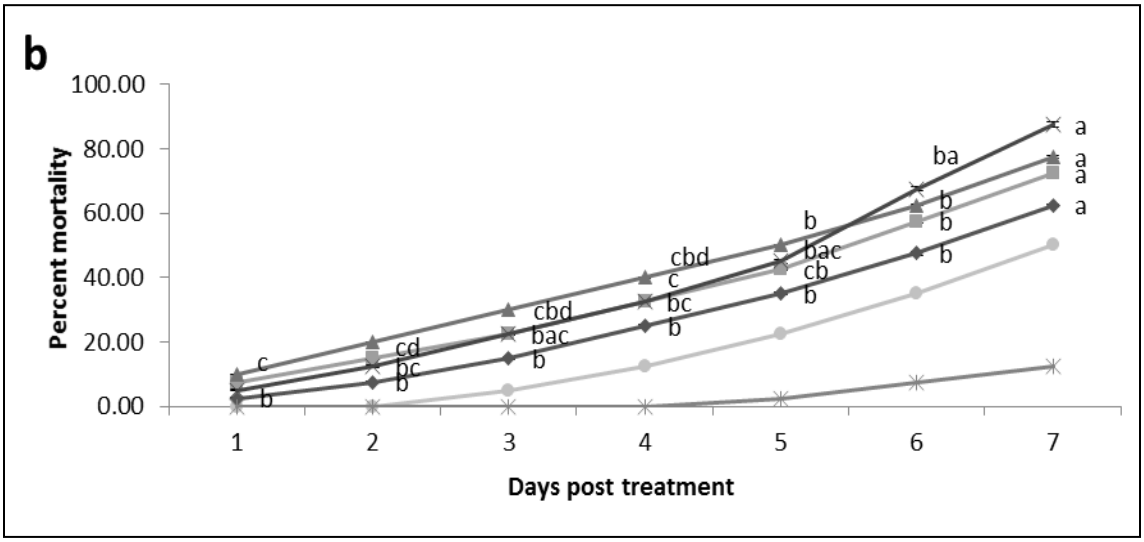
Table 2: LT_{50} values of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* against adults of *C. chinensis*by immersion method.

Fungi	Isolate	Concentration	LT_{50}	FD	Slope	Chi
B. bassiana	Bb-08	2×10^8	5.10	4.82-5.58	6.14±0.79	10.8
		1×10^8	5.28	4.62-5.82	3.68±0.51	1.63
		1×10^7	5.80	5.19-6.47	4.36±0.64	0.94
		1×10^6	6.56	5.87-7.34	5.33±0.87	6.37
M. anisopliae	Ma-2.3	2×10^8	4.89	3.49-7.45	6.53±1.91	27.38
		1×10^8	5.05	4.42-5.40	4.16±0.54	5.59
		1×10^7	5.59	5.06-6.17	4.68±0.66	2.07
I. fumosorosea	If-2.3	2×10^8	5.53	5.10-6.00	5.72±0.78	5.08
		1×10^8	5.61	4.94-6.37	3.60±0.52	0.77
		1×10^7	6.03	5.43-6.70	4.84±0.73	1.27

Table 3: Effect of sublethal dose (1×10^5 spores/mL) of entomopathogenic fungi on the biological parameters of *C. chinensis*

	Male longevity (days)	Female longevity (days)	Fecundity (Eggs/ ♀)	Egg Hatching (%)	Larval-pupal period (days)	Adult emergence (%)	Sex ratio
Bb-08	9.53±0.31c	8.70±0.40b	65.30±2.30c	73.53±4.10bc	22.48±0.41ab	74.40±3.2c	1.5: 1
Ma-2.3	8.10±0.54d	7.10±0.30c	49.10±4.15d	68.19±3.47c	23.40±0.75b	65.20±3.72d	1.3:0.8
If-2.3	11.30±0.23b	10.40±0.60ab	73.40±2.60b	79.00±2.51b	21.5±0.32a	82.50±4.82b	01:01.3
Control	14.00±0.40a	12.50±0.32a	95.00±3.00a	94.73±4.00a	19.0±0.80a	91.93±2.56a	01:01.5
F-value	12.45	11.47	23.67	45.81	3.78	18.32	
P-value	0.027	0.031	0.03	0.007	0.042	0.001	
Lsd-value	2.3	2.7	5.79	6.89	1.47	6.7	





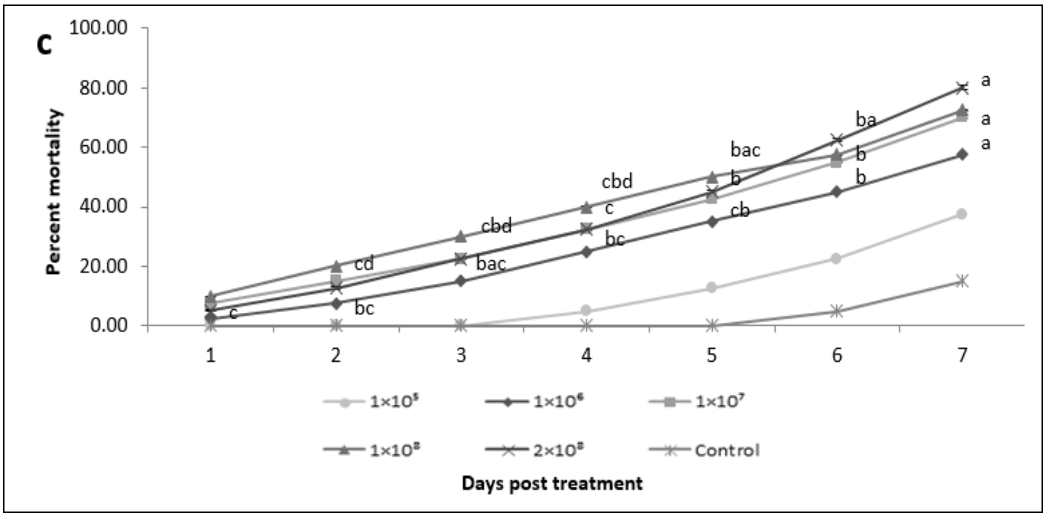


Fig 1: Percent mortality of *C. chinensis* after exposure to different concentrations of (a) *M. anisopliae* (b) *B. bassiana* (c) *I. fumosorosea*. For each day, the letters sharing similarity are not significant different ($p<0.05$)* by Duncan's Multiple Range Test (DMRT)*. The comparison was done on different days

Discussion

Despite the widespread use of chemical insecticides efforts have been made to find environmentally safe and nontoxic techniques for control of stored grain pests [16]. Insect pathogenic fungi are a unique group of microbial control agents as they play a vital part in integrated management systems against insect pests [17, 18]. Being capable of penetrating through insect cuticle by secreting cuticle-degrading enzymes it has been effective against several bruchids [19, 20]. The current study was planned to evaluate the *B. bassiana*, *M. anisopliae,* and *I. fumosorosea* against the *C. chinensis*. The results of the current study have highlighted the potential of insect pathogenic fungi by showing lethal and sublethal effects against *C. chinensis*. Similar results have been reported in which it is stated that desiccant dust synergized the effect of *B. bassiana* on stored grain beetles [21].

In current study, entomopathogenic fungi *B. bassiana* (Bb-08), *M. anisopliae* (Ma-2.3), *I. fumosorosea* (If-2.3) showed dose-dependent rapid mortality 92.50%, 87.50% and 80.0% respectively on $2×10^8$ spores/ml. A similar trend was observed when *B. bassiana* was used for control of *Lyguslineolaris* with rapid mortality and a large number of propagated conidia were present in small time [22]. In addition to lethal effects, sublethal effects include various physiological characteristics of which are being influenced by a fungal infection. It may include longevity, nutritional status, and feeding status. In previous studies decreased longevity and fecundity was observed with increasing doses of entomopathogenic fungi. In the present study, longevity (despite sex), fecundity, egg hatching and adult emergence was decreased due to fungal treatment in comparison to control conditions. A similar result was reported when B. *bassiana* was used which showed decrease in longevity and adult emergence of *Corcyra Cephalonica* [23]. Decreased longevity and fecundity was also reported in different insect pest after application of entomopathogenic fungi which goes in favored of the current study [24, 25]. The present investigation highlighted the importance of entomopathogenic fungi not only by causing mortality but also affecting the normal development period of *C. chinensis* by reducing the longevity, fecundity, egg hatching and adult emergence. Further, the dead cadavers can be a source of further fungal infection which ultimately led to the suppression of pest population. Therefore, the results of the current study provide bases for use of fungal pathogens to be an integral part of pest management strategies for stored grain pests. However, the practical value of these results requires an evaluation of fungal spores on large scale.

Conclusion

In conclusion, *B. bassiana* could be promising control of *C. chinensis* however, field trials are prerequisites.

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