Bulletin of Biological and Allied Sciences Research
***ISSN:* 2521-0092**
www.bbasr.org
DOI: https://doi.org/10.54112/bbasr.v2023i1.52
*Bull. Biol. All. Sci. Res., Volume, **8**: 52*

Original Research Article

EVALUATION OF DIFFERENT STRAINS OF ENTMOPATHOGENIC FUNGI AS POTENTIAL AGENTS FOR THE MANAGEMENT OF *TRIBOLIUM CASTANEUM*

BHATTI MHT, AHMAD S, BILAL S, IQBAL M

Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab, P.O BOX. 54590, Lahore, Pakistan
**Correspondence author email address:* htb.hamza1@gmail.com

(Received, 15th February 2023, Revised 12th November 2023, Published 17th November 2023)

Abstract *The global economic significance of wheat (Triticum aestivum) is undeniable, as it serves as a primary food source for 40% of the human population. T. castaneum, a notable economic pest, particularly impacts stored wheat grains and flour. In the pursuit of sustainable pest control, entomopathogenic fungi (EPF) have emerged as advanced microorganisms, providing viable alternatives to harmful synthetic chemical insecticides. This study employed four fungal strains—Beauveria bassiana, Isaria cateniannulata, Trichoderma harzianum, and Metarhizium attenuatum—against mixed adult populations of T. castaneum under controlled laboratory conditions. Different concentrations (1×10^2, 1×10^4, 1×10^6, 1×10^8, 1×10^{10} cfu/ml) for each fungus were employed, and mortality data, LC50, and LT50 were recorded at post-exposure intervals of 4, 6, 8, and 10 days. The application method involved using the fungus through a filter paper dip, and red flour beetle adults were introduced to wheat grains. The highest mortality, observed at the concentration of 1×10^{10} cfu/ml, was 80% for both M. attenuatum and B. bassiana. Conversely, the lowest mortality, recorded at the concentration of 1×10^2 cfu/ml, was 64% for I. cateniannulata, while T. harzianum demonstrated 70% mortality at 1×10^{10} cfu/ml. The utilization of entomopathogenic fungi for insect control represents an emerging strategy. B. bassiana and M. attenuatum emerge as promising alternatives for managing T. castaneum in stored grains.*

*[Citation: Bhatti, M.H.T., Ahamd, S., Bilal, S., Iqbal, M. (2023). evaluation of different strains of entmopathogenic fungi as potential agents for the management of Tribolium castaneum. Bull. Biol. All. Sci. Res. **8**: 52. doi: https://doi.org/10.54112/bbasr.v2023i1.52]*
Keywords*:* *Entomoathogenic fungus, Tribolium castaneum, wheat grains, stored grains*

Introduction

Throughout centuries, people have relied on cereal crops and stored grains as a primary source of sustenance (Molina et al., 2011). The International Irrigation Research Institute (IIRI) highlights the pivotal significance of global primary cereal grains—maize, rice, and wheat—in shaping future food systems. These grains have played a crucial role in bolstering global food security in the past fifty years. Through dedicated endeavors, there has been a substantial improvement in the yields of these crops, both in terms of quality and quantity. These concerted efforts have rendered these cereals more resilient and tolerant of challenges such as drought, flood, pests, and diseases.

Wheat cultivation in Pakistan spans 8976 hectares with an average yield of 26394 K Metric Tons during the Rabi season, solidifying its position as the fourth most crucial cereal in modern agriculture. Globally, wheat harvests reached an impressive 755 million metric tons (Outlook, 2018). Despite a 2.7% decrease from the record production of 2017, the Food and Agriculture Organization (FAO) forecasted a global wheat production of 736.1 million tons in 2018, contributing to worldwide production of 722 MMT (Nardi et al., 2018).

In regions facing harsh weather conditions, stored products become a vital food source. However, despite their critical importance, funding for cereal grains management, acknowledged to be less than 5% to address post-harvest losses, remains negligible on the world stage (Shiferaw et al., 2013), (Alexandratos and Bruinsma, 2012). The wheat crop encounters significant threats from the moment it matures in the field to its storage and consumption. Cereal grains, utilized by animals and humans for millennia, face substantial losses due to mishandling during storage, harvesting, poor storage structures, and various physical factors. In Pakistan, wheat grains are susceptible to attacks by over 23 insect species, including sucking and stored grain insects (Tadesse et al., 2022).

As challenges intensify, especially in developing countries with burgeoning populations, issues like hunger, food security, climate change, rapid urbanization, invasive pest infections, and massive

post-harvest losses have become prominent. Countries are increasingly focusing on improving agricultural crop production, wise land use, and population control to overcome these challenges (Greeley, 1986); (Kitinoja et al., 2011), (Pantenius, 1988).

Among the array of pests, beetles (Coleoptera) emerge as highly invasive and cause significant infestations in stored grains compared to moths (Lepidoptera). Beetles, both in their larval (grub) and adult stages, wreak havoc on stored products. The presence of these insects, along with their remains and debris, can deteriorate the quality and quantity of stored grains, encompassing species like termites, cockroaches, and flies (Upadhyay and Ahmad, 2011). Classification of stored grain pests is based on their mode of damage, life biology, and feeding habits, including major pests, minor pests, and external and internal feeders (Srivastava and Subramanian, 2016).

The red flour beetle, scientifically identified as *T.castaneum* and categorized within the family Tenebrionidae under the order Coleoptera, stands out as a highly damaging pest to stored grains. An insightful laboratory examination delved into the biology of *T.castaneum*, uncovering intriguing details. Conducted in a controlled room setting with conditions maintained at 29°C and 59% relative humidity from January to July 2013, the study unveiled that female beetles displayed a daily fecundity, laying approximately 24 eggs on wheat flour. The incubation period for the eggs ranged from 4 to 5 days, with larvae undergoing development through seven instars. The overall developmental period for immature stages spanned from 70 to 83 days, averaging at 76.5 days. Pupation transpired within the wheat flour throughout 6 to 9 days, leading to the emergence of fully developed adults in approximately 7.5 days. Unmated male and female adults showcased activity periods ranging from 45 to 67 days and 75 to 89 days, respectively. The complete life cycle of the beetle under the specified conditions unfolded over a period ranging from 164 to 194 days (Devi and Devi, 2015).

The red flour beetle utilizes prothoracic and abdominal glands to generate and release specific compounds, serving as defense mechanisms that either repel predators or induce irritations. Among these compounds are Methyl-1,4-benzoquinone (MBQ), allomones, 1-pentadecene (C15:1), and ethyl-1,4-benzoquinone Pathogens (EBQ), classified as volatile organic compounds emitted by beetles in defensive responses (Villaverde et al., 2007). *T.castaneum*, belonging to Coleoptera and Tenebrionidae, exerts both direct and indirect effects on the quality and quantity of stored products, showcasing its significance as a pest (Villaverde et al., 2007).

Tribolium castaneum, commonly known as the red flour beetle, is a prevalent pest that infests wheat flour and stored grains. Flourishing at an ideal habitat temperature of 30 degrees Celsius (Nakakita and Winks, 1981), these insects can cause considerable damage to seed embryos, leading to disruptions in germination capabilities and the deterioration of growth habits. As a result, the imperative need arises to control stored grain pests, ensuring the availability of high-quality staple and safe food at affordable rates for everyone (Nadeem et al., 2012); (Jahromi et al., 2014).

Various methods exist for controlling insect pests, ranging from cultural and physical controls to mechanical and biological methods. Cultural control emphasizes proper sanitation of storage facilities, handling materials, and filling cracks and crevices. Physical control involves manipulating temperature, humidity, and pressure to manage pests, either by raising temperatures to 550-650 degrees Celsius for 8-10 hours to eliminate most insects or by lowering temperatures to restrict insect growth and development in storage houses (Upadhyay and Ahmad, 2011).

Materials and methods

Insect collection and rearing

Adults of T. castaneum were sourced from wheat flour infested with pests in Okara, Punjab, Pakistan. A healthy culture was isolated using sterilized sieves and camel brushes, and 100 insects were introduced into a glass jar filled to one-third capacity with wheat grains. The opening of the jar was covered with muslin cloth. The insect-rearing process occurred at the Entomology laboratory in the Institute of Agriculture Sciences (IAGS) at the University of Punjab, Lahore. Temperature conditions were controlled at 28 ± 2 degrees Celsius during winter, using heaters, and in summer, using air conditioners. The relative humidity was maintained at 65 ± 5.

Entomopathogenic culture

Fungal strains, namely Beauveria, Isaria cateniannulata, Metarhizium, and Trichoderma harzianum, were procured from the University of Agriculture, Faisalabad. The growth medium was formulated by combining Potato Dextrose Agar (PDA) with 1000 ml of distilled water, followed by autoclaving for 2 hours.

Pouring and inoculation

After extraction from the autoclave, cool the media to room temperature and pour it into sterilized Petri plates. Add 25 ml of media to each plate, ensuring the surface is fully covered. Allow approximately 30 minutes for the media to cool. Inoculate the fungus onto the Petri plates by scraping a few spores from the fungal culture. Fill around 25 Petri plates with the inoculated media. Place the plates into an incubator for three days at 25±2 degrees Celsius. Carry out the inoculation process in a laminar flow environment to prevent contamination.

Preparation of fungal concentrations

To collect fungal conidia, a two-week-old fungal culture is needed. Utilize sterile needles to scrape the topmost layer of the colony, transferring it into Twin-20 at a ratio of 1 ml per liter (polyoxyethylene sorbitan monooleate). Employ a magnetic shaker to agitate the mixture for 10 minutes continuously. Use double-distilled water for both the mother solution and stock solution, as well as for subsequent serial dilutions. Once the fungus is dissolved in the Twin-20 solution, create concentrations through serial dilution.

Spore counting

For spore counting, utilize the mother solution with a hemocytometer. Count the number of spores per milliliter of the fungal solution. Take 5 microliters of solution from the stock solution and place it on the hemocytometer. Form and adjust concentrations accordingly. The process is repeated for each of the four fungal strains until harvesting:

- B. bassiana
- B. cateniannulata
- T. harzianum
- M. attenuatum

Prepare concentrations measured in colony-forming units per milliliter (cfu/ml) for each strain: B. bassiana *(102, 104, 106, 108, 1010),* I. cateniannulata *(102, 104, 106, 108, 1010),* T. harzianum *(102, 104, 106, 108, 1010),* M. attenuatum *(102, 104, 106, 108, 1010), including a control group.*

The experimental design adheres to a completely randomized design (CRD) with three replicates and four treatments for each fungus, alongside an overall control group. Insert a filter paper into every Petri plate to cover the surface. Sprinkle 2.5 ml of the solution from each concentration into Petri plates T1, T2, T3, T4, and T5, each with replicates R1, R2, and R3. Sprinkle a small quantity of water in the control group. Introduce some wheat grains into each plate and place 20 adults of T. castaneum into every Petri plate. Cover the plates with muslin cloth and secure them with a rubber band.

Data collection

The mortality rate was documented after intervals of 4, 6, 8, and 10 days for each fungal strain. These values were then transformed into percentage corrected mortality using the Abbot Formula.

$$\text{Percentage mortality} = \frac{\text{Number of dead adults}}{\text{Total number of adults treated}} \text{X } 100$$

Statistical analysis

The data underwent analysis employing a completely randomized design (CRD) approach. LC50 and LC90 values were computed using Minitab software, while Statistic 8.1 was utilized to analyze variance. The Least Significant Difference (LSD) test was applied for all pairwise comparison tests, facilitating a statistical comparison of the data results with each other at a 5% significance level.

Results

The mortality percentage of *T. castaneum* was documented at intervals of two, four, six, eight, and ten days post-exposure to four fungal strains (*B. bassiana, I. catenullata, T. harzianum, M. attenuatum*) at varying concentrations (10^2, 10^4, 10^6, 10^8, 10^10). The observed trends were as follows:

M. attenuatum

M. attenuatum significantly impacted the mortality of *T. castaneum* adults ($p < 0.6$). The application of different concentrations resulted in varying levels of virulence, with mean mortality percentages ranging from 47% to 80%. The maximum mortality percentage was observed at 10^{10} cfu/ml, reaching 80%, while other concentrations showed mortality percentages of 47%, 48%, 53%, and 65% at 10^2, 10^4, 10^6, 10^8, respectively (Fig 1).

B. bassiana

Beauveria bassiana substantially affected the mortality of *T. castaneum* adults ($p < 0.9$). The application of different concentrations resulted in varying levels of virulence, with mean mortality percentages ranging from 47% to 80%. The maximum mortality percentage was observed at 10^{10} cfu/ml, reaching 80%, while other concentrations showed mortality percentages of 47%, 58%, 62%, and 76% at 10^2, 10^4, 10^6, 10^8, respectively (Fig 2).

T. harzianum

T. harzianum significantly affected the mortality of *T. castaneum* adults ($p < 0.4$). The application of different concentrations resulted in varying levels of virulence, with mean mortality percentages ranging from 25% to 70%. The maximum mortality percentage was observed at 10^{10} cfu/ml, reaching 70%, while other concentrations showed 25%, 39%, 50%, and 55% at 10^2, 10^4, 10^6, 10^8, respectively (Fig 3).

I. cateniannulata

I. cateniannulata significantly affected the mortality of *T. castaneum* adults ($p < 0.5$). The application of different concentrations resulted in varying levels of virulence, with mean mortality percentages ranging from 30% to 64%. The maximum mortality percentage was observed at 10^{10} cfu/ml, reaching 64%, while other concentrations showed mortality percentages of 30%, 42%, 50%, and 62% at 10^2, 10^4, 10^6, 10^8, respectively (Fig 4).

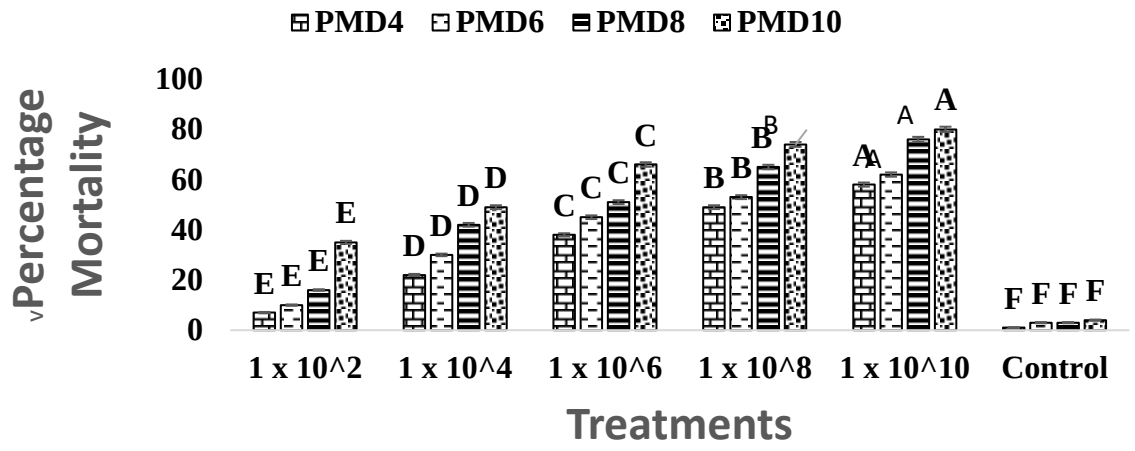

Figure 1: Percent mortality of *Tribolium castaneum* against etomopathogenic fungi *Metarhizium attenuatum* at different intervals after exposure of 4,6,8 and 10 days

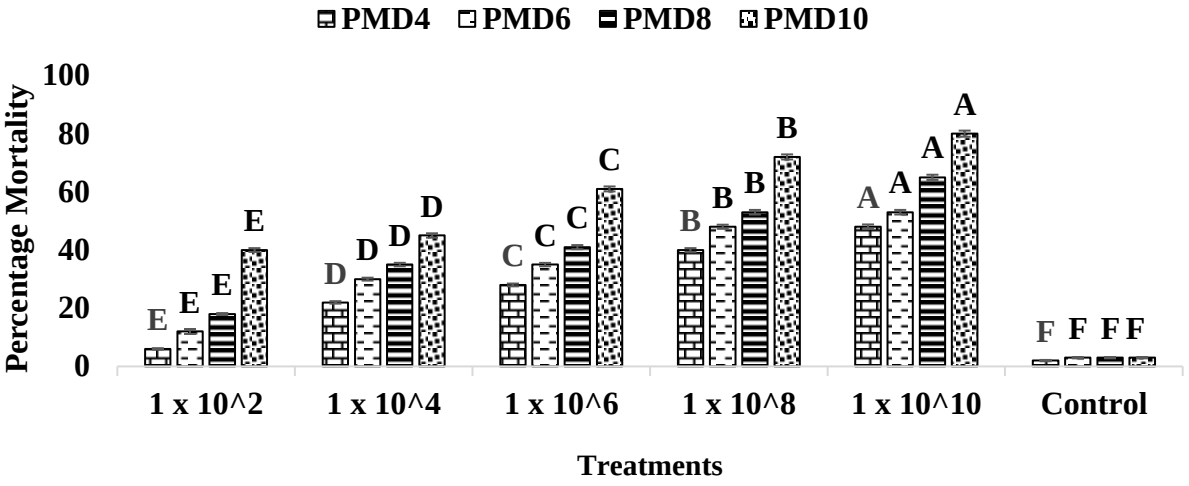

Figure 2: Percentage Mortality of *T. castaneum* Exposed to the Entomopathogenic Fungus *B. bassiana* at Various Time Intervals (4, 6, 8, and 10 days)

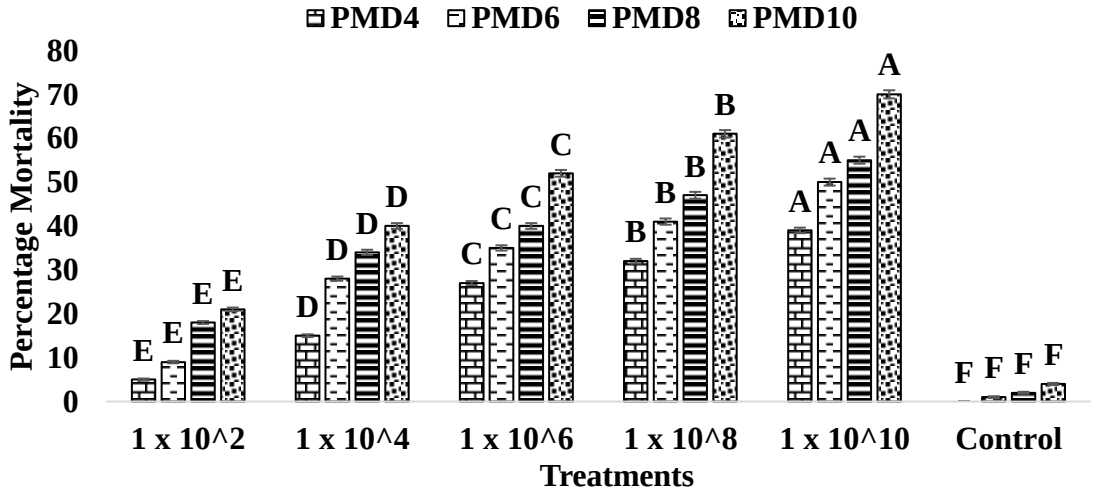

Figure 3: Percent mortality of *Tribolium castaneum* against entomopathogenic *fungi T. harzianum at* different intervals after exposure of 4,6,8 and 10 days

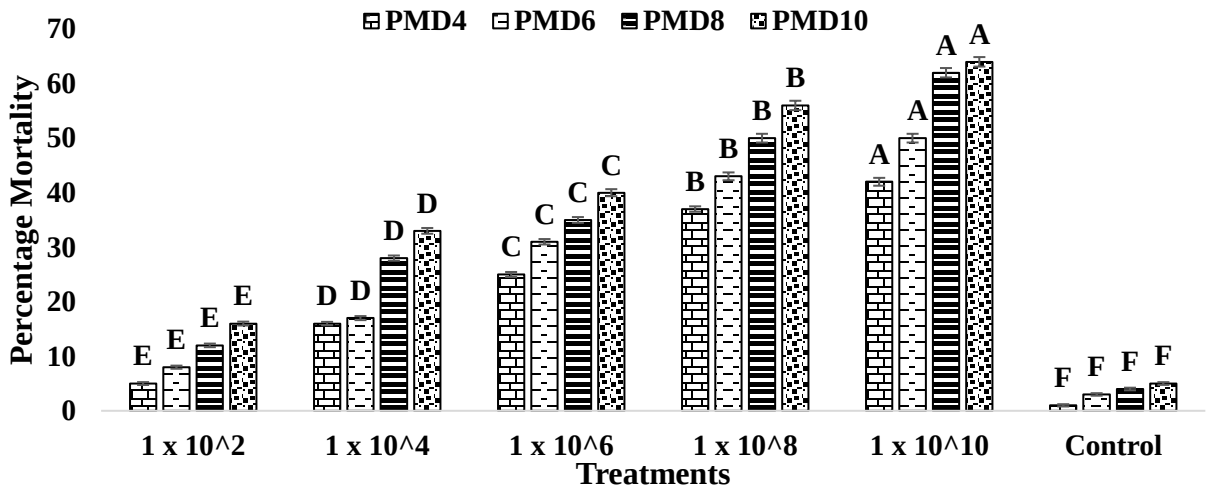

Figure 4: Percent mortality of *Tribolium castaneum* against entomopathogenic fungi *I. cateniannulata* at different intervals after exposure of 4, 6, 8 and 10 days

LC_{50} and LT_{50} values of entomopathogenic Fungi against *Tribolium castaneum* exposed to different post-exposure interval

The lethal concentration (cfu/ml) of M. attenuatum was determined, and the LC50 values were identified as: 1.69955x 10^{10}, 6.1x 10^{9}, 8.1x 10^{8}, 3.6x 10^{7}, and 1.9x 10^{5} after intervals of 4, 6, 8, and 10 days, respectively (refer to Table 1). The lethal time (days) of *M. attenuatum* was also recorded, showing LT50 values of 13.5, 13.4, 9.2, 5.6, and 3.2 days for concentrations 102, 104, 106, 108, and 1010 (see Table 2). The lethal concentration (cfu/ml) of *B. bassiana* was documented, indicating LC50 values of 4.53x 10^{15}, 1.31x 10^{11}, 1.31x 10^{11}, 2.5x 10^{8}, and 1.9x 10^{5} after 4, 6, 8, and 10 days, respectively (refer to Table 3). The lethal time (days) of *B. bassiana* was noted, with LT50 values of 1.9x 10^{4}, 15.7x 10^{3}, 10x 10^{3}, 11x 10^{3}, and 7.2x 10^{3} days for concentrations 102, 104, 106, 108, and 1010 (see Table 4). Similarly, the lethal concentration (cfu/ml) of *T. harzianum* was identified, with LC50 values of 2.2 x 10^{12}, 9.6x 10^{10}, 3.2x 10^{9}, 4.3x 10^{8}, and 2.2x 10^{6} after 4, 6, 8, and 10 days, respectively (refer to Table 5). The lethal time (days) of *T. harzianum* was recorded, showing LT50 values of 17.1, 11.86, 9.6, 7.8, and 5.9 days for concentrations 102, 104, 106, 108, and 1010 (see Table 6). Lastly, the lethal concentration (cfu/ml) of *I. cateniannulata* was determined, with LC50 values of 2.86x 10^{11}, 2.6 x 10^{10}, 2.8x 10^{10}, 1.7x 10^{9}, and 4.08x 10^{8} after intervals of 4, 6, 8, and 10 days, respectively (refer to Table 7). The lethal time (days) of *I. cateniannulata* was also noted, indicating LT50 values of 20.79, 15.83, 20.29, 7.90, and 5.50 days for concentrations 102, 104, 106, 108, and 1010 (see Table 8).

Table 1: LC_{50} values of *M. attenuatum* against *T. castaneum* after different exposures intervals

Days	LC_{50} (cfu/ml)	FL Limit	Slope ± S.E.	χ^2	D.F.	P
2nd day	1.6 x 10^{10}	2.2 x 10^{9}-3.7 x 10^{11}	0.10 ± 0.02	3.45882	3	0.326
4th day	6.15 x 10^{9}	8.4 x 10^{8}-1.2 x 10^{11}	0.09 ± 0.02	5.57793	3	0.134
6th day	8.1 x 10^{8}	1.09 x 10^{8}-1.46 x 10^{10}	0.08 ± 0.01	5.15135	3	0.161
8th day	3.6 x 10^{8}	6.5 x 10^{7}-2.61 x 10^{9}	0.08 ± 0.01	2.11043	3	0.550
10th day	1.9 x 10^{6}	1.03 x 10^{4} -1.4 x 10^{6}	0.11 ± 0.02	3.45882	3	0.326

Table 2: LT_{50} of *M. attenuatum* against *T. castaneum* after different exposures interval

Concentrations	LT_{50} (Days)	FL Limit	Slope ± S.E.	χ^2	D.F.	P
1 x 10^{2} cfu/ml	13.5644	11.2531- 19.1665	1.78 ± 0.31	8.38890	3	0.039
1 x 10^{4} cfu/ml	13.4653	10.0657- 25.3769	0.78 ± 0.17	0.827475	3	0.843
1 x 10^{6} cfu/ml	9.23845	7.41581- 13.6683	0.71 ± 0.14	7.78120	3	0.051
1 x 10^{8} cfu/ml	5.68994	4.67038- 6.84168	0.72 ± 0.13	4.77167	3	0.189
1 x 10^{10} cfu/ml	3.41628	2.17810- 4.34673	0.56 ± 0.12	9.46553	3	0.024

Table 3: LC_{50} *B. bassiana* against *T. castaneum* after different exposure intervals

Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
2nd day	4.53 x 10^{15}	2.6 x 10^{13} -2.6 x 10^{20}	0.07 ± 0.01	6.876	3	0.07
4th day	1.31 x 10^{11}	4.3 x 10^{9} - 7.5 x 10^{13}	0.05 ± 0.01	2.81	3	0.42
6th day	1.31 x 10^{11}	4.3 x 10^{9} - 7.5 x 10^{13}	0.04 ± 0.01	1.70	3	0.64

8th day	2.5 x 10^8	2.4 x 10^7 - 3.5 x 10^9	0.05 ± 0.009	0.755	3	0.86

Table 4 LT_{50} *B. bassiana against T. castaneum* after different exposure intervals

Conc.	LT_{50} (days)	FD Limit	Slope ± S.E.	χ 2	D.F.	P
1 x 10^2	1.9x10^4	34.7x10^4- 703.2x10^3	1.04 ±0.26	3.73	2	0.15
1 x 10^4	15.7x10^3	28.3x10^4-180.1x10^3	1.05± 0.22	1.74	2	0.42
1 x 10^6	16.0x10^3	28.0x10^3- 172.2x10^3	1.09± 0.23	0.35	2	0.83
1 x 10^8	11.1x10^3	22.4x10^4-72.0x10^3	1.10 ±0.198	0.99	2	0.60
1 x 10^{10}	7.2x10^3	21.4x10^4 -89.8x10	0.78 ±0.156	0.66	2	0.71

Table 5 Calculation of LC50 of *T. harzianum* against *T.castaneum* after different exposure intervals

Days	LC_{50} (cfu/ml)	FL Limit	Slope ± S.E.	χ^2	D.F.	P
2nd day	2.23×10^{12}	1.18x 10^{11}-6.03× 10^{14}	0.14 ±0.025	3.30	3	**0.347**
4th day	9.60×10^{10}	6.9x10^{10}-8.96×10^{12}	0.09 ± 0.015	5.28	**3**	**0.152**
6th day	3.2x 10^9	3.5x10^9-9.98×10^{10}	0.079± 0.012	6.91	**3**	**0.075**
8th day	4.38 x 10^9	4.28x10^8-1.46×10^{10}	0.063 ± 0.011	2.79	**3**	**0.424**
10th day	2.67x 10^6	4.2x10^6-1.4 x10^8	0.077±0.0105	3.80	**3**	**0.283**

Table 3 Calculation of LT_{50} values of *T. harzianum* against *T.castaneum* after different exposure intervals

Days	LT50	FL Limit	Slope ± S.E.	χ 2	D.F.	P
1 x 10^2	17.18	13.11- 31.69	1.83 ± 0.38	0.707	3	0.871
1 x 10^4	11.86	9.94- 15.99	1.32 ± 0.213	1.22	3	0.747
1 x 10^6	9.66	8.23- 12.36	1.07 ± 0.173	1.51	3	0.679
1 x 10^8	7.840	6.78- 9.46	0.986 ± 0.154	0.99	3	0.802
1 x 10^{10}	5.31	5.3-9.1	0.88 ±0.142	0.49	3	0.719

Table 7 Calculation of LC_{50} values of *I. cateniannulata* against *T.castaneum* after different exposure intervals

Days	LC_{50} (cfu/ml)	FL Limit	Slope ± S.E.	χ^2	D.F.	P
2nd day	2.86 x 10^{11}	3.88 x 10^{13}–7.19 x 10^{18}	0.130 ± 0.02	13.88	3	**0.003**
4th day	2.64 x 10^{10}	5.71 x 10^{13}– 2.14 x 10^{19}	0.101 ± 0.015	4.71	**3**	**0.194**
6th day	2.8 x 10^{10}	8.75 x 10^{12} –2.09 x 10^{17}	0.10± 0.01	3.69	**3**	**0.296**
8th day	1.7 x 10^9	1.75 x 10^{12}–9.92 x 10^{15}	0.095 ± 0.012	2.50	**3**	**0.475**
10th day	4.08 x 10^8	1.53 x 10^{12}- 1.71 x 10^{16}	0.085 ± 0.011	2.94	**3**	**0.400**

Table 8 Calculation of LT_{50} values of *I. cateniannulata* against *T.castaneum* after different exposure intervals

Concentrations	LT_{50}	FL Limit	Slope ± S.E.	χ^2	D.F.	P
1 x 10^2	20.79	14.51- 55.69	1.78± 0.44	1.80	3	0.614
1 x 10^4	15.83	11.99 - 28.25	1.16± 0.23	1.62	**3**	0.653
1 x 10^6	20.29	11.81- 165.83	0.49± 0.15	0.34	**3**	0.952
1 x 10^8	7.90	6.31- 11.27	0.61± 0.13	0.11	**3**	0.990
1 x 10^{10}	5.50	4.44- 6.66	0.68± 0.12	0.57	**3**	0.902

(**χ^2** = Chi square value, P = Probability value, LT= Lethal Time, D.F = Degree of freedom, FL = Fudicial Limit; SE = Standard error

Discussion

The red flour beetle, *T. castaneum* (Coleoptera: Tenebrionidae), is recognized as a significant pest in stored wheat grains, displaying a cosmopolitan distribution and adaptability to various environments. Typically found in stored products, these beetles thrive in temperatures between 28-30 degrees Celsius. Their infestation can lead to the development of foul-smelling and sticky flour, attributed to the secretion of excreta and shed skin into the grains (Bosly and Kawanna, 2014). Consumption of contaminated flour containing beetle feces and skin poses potential health risks (Gorham, 1979). Over time, the invasion of additional pests, combined with a rise in the beetle population, leads to significant deterioration in the quantity and quality of stored wheat grains across various varieties (Ali et al., 2009); (Ali et al., 2012).

Acknowledging the significance of microbial control, researchers are actively investigating diverse microorganisms, particularly fungi. Our investigation used four fungal strains (B. bassiana, I. catteniannulata, M. annutatum, T. harzianum) against T. castaneum adults at consistent concentrations (102, 104, 106, 108, 1010). The mortality of adult beetles was observed throughout 2, 4, 6, 8, and 10 days. Our findings indicate that *B. bassiana* emerged as the most effective fungus against *T. castaneum*, causing a 79 percent mortality rate. In contrast, *T. harzianum* exhibited the least effectiveness, with a mortality rate of approximately 64 percent. *I. cateniannulata* and *M. annutatum* showed mortality rates of 70 and 75 percent, respectively. The study illustrated that fungal growth within *T. castaneum* body parts commenced after 2-3 days, reaching maximum impact within 10-20 days.

The study also emphasized the susceptibility of stored wheat flour and grains to contamination, particularly under elevated temperature and humidity conditions. Flour beetles, in conjunction with specific fungal species, contribute to this contamination, potentially deteriorating the quality and quantity of stored products. Severe infestations by insect pests can lead to significant harm to stored grains on a global scale. The red flour beetle's ability to adapt to various environmental changes underscores its role as a significant pest affecting cereal grains and their derivatives. Earlier research has identified four primary pests in stored wheat, with T. castaneum ranking second after Sitophilus oryzae. The moisture accumulated through beetle activity creates an ideal environment for fungal growth and the accumulation of mycotoxins (Mukhtar et al., 2021).

Our study aligns with the growing trend of microbial control, with various fungal strains being explored for their efficacy against *T. castaneum*. Al-Ani's study (Al-Ani et al., 2018) on the mortality rate of *T. castaneum* using *B. bassiana* and F. proliferatum supports our findings, emphasizing the economic damage caused by *Tribolium* as a serious pest of wheat flour. Combining entomopathogenic fungi with essential oils and other materials has been noted to enhance efficacy results (Jamali et al., 2021). Furthermore, the effectiveness of Trichoderma harzianum as an insecticidal control agent has been supported by other studies, emphasizing the need for novel alternatives to control pests, particularly those affecting stored grains (Gad et al., 2020).

Trichoderma and *I. cateniannulata* have been investigated for their efficacy in managing stored grain pests, while *B. bassiana* and *M. annutatum* have been widely adopted due to their consistent and high mortality rates, reaching 80-85 percent. However, it is crucial to acknowledge that certain studies express reservations regarding fungi, highlighting potential harm and spoilage to stored products, especially in situations of elevated moisture and mishandling. Increased moisture levels can trigger hot spots in grains, making it challenging to control attacks despite preventive measures, leading to substantial grain spoilage (Fleurat-Lessard, 2017).

Conclusion

In conclusion, it is crucial to continue investigating various bioagents and microbes that are specific to targets and offer effective control against insects and pests in stored grains. Subsequent experiments should extend these findings to warehouses and storage facilities to validate their practical applicability.

References

Al-Ani, L. K. T., Yonus, M. I., Mahdii, B. A., Omer, M. A., Taher, J. K., Albaayit, S. F. A., and Al-Khoja, S. B. (2018). First record of use Fusarium proliferatum fungi in direct treatment to control the adult of wheat flour Tribolium confusum, as well as, use the entomopathogenic fungi Beauveria bassiana. *Ecology, Environment and Conservation* **24**, 29-34. 10.22004/ag.econ.288998

Alexandratos, N., and Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. 10.22004/ag.econ.288998

Ali, A., Ahmad, F., Biondi, A., Wang, Y., and Desneux, N. (2012). Potential for using Datura alba leaf extracts against two major stored grain pests, the khapra beetle Trogoderma granarium and the rice weevil Sitophillus oryzae. *Journal of Pest Science* **85**, 359-366. 10.1007/s10340-012-0426-1

Ali, A., Sarwar, M., Khanzada, S., and Abro, G. H. (2009). Reaction of certain wheat varieties to the action of red flour beetle, Tribolium castaneum (Herbst)(Coleoptera) under insectary conditions. *Pakistan Journal of Zoology* **41**.

Bosly, H. A., and Kawanna, M. A. (2014). Fungi species and red flour beetle in stored wheat flour under Jazan region conditions. *Toxicology and Industrial Health* **30**, 304-310. 10.1177/0748233712457449

Devi, M. B., and Devi, N. V. (2015). Biology of rust-red flour beetle, Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). *In* "Biological Forum", Vol. 7, pp. 12-15. Satya Prakashan.

Fleurat-Lessard, F. (2017). Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins–An update. *Journal of Stored Products Research* **71**, 22-40. 10.1016/j.jspr.2016.10.002

Gad, H. A., Al-Anany, M. S. M., mohamed Sameer, W., and Al-Anany, F. S. M. (2020). Control of Acanthoscelides obtectus with Trichoderma harzianum applied alone or in combination with diatomaceous earth on a stored common bean. *Plant Protection Science* **56**, 107-115. 10.17221/104/2019-PPS

Gorham, J. R. (1979). The significance for human health of insects in food. *Annual Review of Entomology* **24**, 209-224. 10.1146/annurev.en.24.010179.001233

Greeley, M. (1986). FOOD, TECHNOLOGY AND EMPLOYMENT: THE FARM-LEVEL POST-HARVEST SYSTEM IN DEVELOPING COUNTRIES. *Journal of Agricultural Economics* **37**, 333-347. 10.1111/j.1477-9552.1986.tb01602.x

Jahromi, A. T., Stakhovych, S., and Ewing, M. (2014). Managing B2B customer churn, retention and profitability. *Industrial Marketing Management* **43**, 1258-1268. 10.1016/j.indmarman.2014.06.016

Jamali, F., Sohrabi, F., and Kohanmoo, M. A. (2021). Entomopathogenic fungi and plant essential oils are not compatible in controlling Tribolium castaneum (Herbst). *Journal of Plant Diseases*

and Protection **128**, 799-808. 10.1007/s41348-021-00430-5

Kitinoja, L., Saran, S., Roy, S. K., and Kader, A. A. (2011). Postharvest technology for developing countries: challenges and opportunities in research, outreach and advocacy. *Journal of the Science of Food and Agriculture* **91**, 597-603. 10.1002/jsfa.4295

Molina, J., Sikora, M., Garud, N., Flowers, J. M., Rubinstein, S., Reynolds, A., Huang, P., Jackson, S., Schaal, B. A., and Bustamante, C. D. (2011). Molecular evidence for a single evolutionary origin of domesticated rice. *Proceedings of the National Academy of Sciences* **108**, 8351-8356. 10.1073/pnas.1104686108

Mukhtar, S., Mukhtar, S., Hani, U., and Iram, S. (2021). Scenario Of Aspergillus Indoor Contamination In Pakistan (2000-2020)–A Review. *Environmental Contaminants Reviews (ECR)* **4**, 24-28. 10.26480/ecr.01.2021.24.28

Nadeem, M., Iqbal, J., Khattak, M. K., and Shahzad, M. A. (2012). Management of Tribolium castaneum (Hbst.)(Coleoptera: Tenebrionidae) using neem (Azadirachta indica A. Juss) and tumha (Citrullus colocynthis (L.)). *Pakistan Journal of Zoology* **44**.

Nakakita, H., and Winks, R. (1981). Phosphine resistance in immature stages of a laboratory selected strain of Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). *Journal of Stored Products Research* **17**, 43-52. 10.1016/0022-474X(81)90016-3

Nardi, J., Nascimento, S., Göethel, G., Gauer, B., Sauer, E., Fão, N., Cestonaro, L., Peruzzi, C., Souza, J., and Garcia, S. C. (2018). Inflammatory and oxidative stress parameters as potential early biomarkers for silicosis. *Clinica Chimica Acta* **484**, 305-313. 10.1016/0022-474X(81)90016-3

Outlook, F. (2018). come Food Deficit Countries (LIFDCs). This year the FAO took a longer-term view of that trend and found that countries may indeed be "paying more for less food," even though global production and trading conditions have been quite.

Pantenius, C. (1988). Storage losses in traditional maize granaries in Togo. *International Journal of Tropical Insect Science* **9**, 725-735. 10.1017/S1742758400005610

Shiferaw, B., Smale, M., Braun, H.-J., Duveiller, E., Reynolds, M., and Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security* **5**, 291-317. 10.1007/s12571-013-0263-y

Srivastava, C., and Subramanian, S. (2016). Storage insect pests and their damage symptoms: an overview. *Indian Journal of Entomology* **78**, 53-58. 10.5958/0974-8172.2016.00025.0

Tadesse, W., Zegeye, H., Debele, T., Kassa, D., Shiferaw, W., Solomon, T., Negash, T., Geleta, N., Bishaw, Z., and Assefa, S. (2022). Wheat production and breeding in ethiopia: retrospect and prospects. *Crop Breeding, Genetics and Genomics* **4**. 10.20900/cbgg20220003

Upadhyay, R. K., and Ahmad, S. (2011). Management strategies for control of stored grain insect pests in farmer stores and public ware houses. *World Journal of Agricultural Sciences* **7**, 527-549.

Villaverde, M. L., Juárez, M. P., and Mijailovsky, S. (2007). Detection of Tribolium castaneum (Herbst) volatile defensive secretions by solid phase microextraction–capillary gas chromatography (SPME-CGC). *Journal of Stored Products Research* **43**, 540-545. 10.1016/j.jspr.2007.03.003

Declaration

Acknowledgements

The authors express their gratitude to the University of the Punjab, Lahore, Pakistan, for providing the study facilities.

Data Availability statement

All data generated or analyzed during the study have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this research.

Consent for publication

Not applicable

Funding

There were no sources providing support, for this research.

Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.