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Biocontrol of Red Flour Beetle, Tribolium castaneum (Herbst) in Stored Wheat Using Entomopathogenic Fungi

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Abstract

This study was conducted to evaluate the efficacy of four entomopathogenic fungi against the red flour beetle, Tribolium castaneum (Herbst) as an alternative to chemical pesticides. The adult stage inside stored wheat was subjected to different concentrations of Beauveria bassiana (Balsamo), Metarhizium anisopliae (Metsch), Verticillium lecanii (Zimmerman), and Paecilomyces ilacinus (Thoms) in either Petri-plates at 7, 15 and 22 days or bag storage at 20, 40 and 60 days exposure times. The first experiment indicated that V. lecanii achieved maximum mortality (58.89 and 57.5%) via interaction with the highest dose of 0.748 × 107 and maximum exposure time of 22 days respectively. The adults required the lowest concentration of V. lecanii to achieve an average LC50 (6233697 mg) within various exposure times. Regarding the latter experiment (liquid assay), the least survivorship (41.25) was observed by P. ilacinus followed by V. lecanii (56.25) during 60 days of exposure time. On the other hand, the powder assay revealed that the highest mortality (57.08 and 48.75) of adults inside bags was recorded respectively via the interaction terms between V. lecanii with both the highest concentration (1000 mg) and exposure times (60 days) and the LC50 value of the aforementioned bioagent was (240.02) for 60 days treatment followed by B. bassiana. The study suggests that the use of biopesticides can provide significant insight in the management of pests while minimize the weight loss of stored wheat and thus, can be used as an effective alternative or integrated with chemical pesticides in pest management strategies.

Keywords

Biocontrol, Tribolium castaneum, Entomopathogenic fungi, Pest management, Stored wheat

ORIGINAL ARTICLE

Biocontrol of Red Flour Beetle, *Tribolium castaneum* (Herbst) in Stored Wheat Using Entomopathogenic Fungi

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Abstract

This study was conducted to evaluate the efficacy of four entomopathogenic fungi against the red flour beetle, *Tribolium castaneum* (Herbst) as an alternative to chemical pesticides. The adult stage inside stored wheat was subjected to different concentrations of *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch), *Verticillium lecanii* (Zimmerman), and *Paecilomyces ilacinus* (Thoms) in either Petri-plates at 7, 15 and 22 days or bag storage at 20, 40 and 60 days exposure times. The first experiment indicated that *V. lecanii* achieved maximum mortality (58.89 and 57.5%) via interaction with the highest dose of 0.748×10^7 and maximum exposure time of 22 days respectively. The adults required the lowest concentration of *V. lecanii* to achieve an average LC_{50} (6,233,697 mg) within various exposure times. Regarding the latter experiment (liquid assay), the least survivorship (41.25) was observed by *P. ilacinus* followed by *V. lecanii* (56.25) during 60 days of exposure time. On the other hand, the powder assay revealed that the highest mortality (57.08 and 48.75) of adults inside bags was recorded respectively via the interaction terms between *V. lecanii* with both the highest concentration (1000 mg) and exposure times 60 days and the LC_{50} value of the aforementioned bioagent was (240.02) for 60 days treatment followed by *B. bassiana*. The study suggests that the use of biopesticides can provide significant insight in the management of pests while minimize the weight loss of stored wheat and thus, can be used as an effective alternative or integrated with chemical pesticides in pest management strategies.

Keywords: Biocontrol, *Tribolium castaneum*, Entomopathogenic fungi, Pest management, Stored wheat

1. Introduction

Diverse studies have attempted to tackle the basic issue of how to sustainably feed the world's expanding population in the coming years. A number of these publications have focused on enhancing food production to meet present and future demands, and the major economic staple crop (i.e. wheat) has been prioritized as a solution [1,2]. Wheat is significant not just because it is an effective source of carbohydrates, protein, and fibre, but also because it is possible to grind the seeds into flour, the main component of bread and other baked products [3,4].

The practice of storing wheat in grain storage bags after harvesting is a common procedure that is widely adopted in many developing countries., particularly in tropical and semi-tropical regions which makes it vulnerable to a wide range of pests, including insects. These insects have the potential to inflict significant amounts of damage to the products, which can result in a reduction in quantity due to feeding damage, as well as a fall in quality due to the contamination of the product with cast skin and frass [5]. To give an illustration of such damages, the red flour beetle *Tribolium castaneum* (Herbst) is a cosmopolitan insect pest of cereal products in which the larvae stage feed vigorously on food products

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such as flour and wheat, lowering its nutritive value and creating a condition that encourages the growth of fungal pathogens [6,7].

Many control strategies for post-harvest insects have been applied during the past decades, nonetheless, heavy reliance on the use of chemical fumigants (i.e. phosphine) as a grain protectant in a variety of habitats resulted in the appearance of resistant strains of *T. castaneum* towards these pesticides [8,61]. Besides, the toxic effect of insecticides on non-target organisms, mammals, as well as the environment, has motivated the search for alternatives such as biological control, including entomopathogenic fungi (EPF) for the management of stored product insects [9,10].

The EPF was found to be a very efficient tool for managing insect pests of stored grains; that's due to their target-specific and eco-friendly nature [9,11]. These insect pathogens have a unique mode of infection which can first penetrate the integument, grow in the hemocoel of infected insects, and sporulate on the outer surfaces of cadavers, thus producing epizootics that overcome the population of insects quickly [12,13]. This will occur with the aid of several hydrolytic enzymes in insect cuticles (i.e. chitinases, proteases, and lipases) that endorse the germination and growth of parasitic fungi across the surface of insect hosts [14,15].

Several fungi, which belong to Zygomycota and Ascomycota, are known to be entomopathogenic to several economic insect pests including stored pests. *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch), *Verticillium lecanii* (Zimmerman), and *Paecilomyces ilacinus* (Thoms.) are the most effective fungal agents, which have relatively wide host ranges and are amenable to mass production, used in microbial control of insects [16,17].

The dedication of EPF for biocontrol agents against insect pests has been well known and documented in many previous works; however, insufficient studies are available on the use of these EPFs as control agents that can provide the elements required against stored product insects to be an efficient practical use inside silos and warehouses [18,19,56]. Hence, the study explores the efficacy of various biopesticides via dual assay forms (dry powder and liquid) against *T. castaneum* inside wheat grains, offering insights into which forms are most effective for each fungus and promoting sustainable agriculture. Furthermore, estimate the weight loss of the cereals over a long time as practical application during storage.

This work was undertaken to highlight the potential impact of entomopathogenic fungi on stored product insects to provide useful advice to manage stored product insects, more precisely the works aimed to explore the potential role of various commercial formulations of *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch), *Verticillium lecanii* (Zimmerman) and *Paecilomyces ilacinus* (Thoms.), as effective biocontrol agents against the most destructive stored pest *T. castaneum* in stored wheat to reduce grain loss during post-harvest practices. Thus, the resistance and susceptibility of red flour beetle to various concentrations of treatments were tested based on LC_{50} values and the insecticidal effects were further estimated by measuring the fresh weight and weight loss of wheat products after two months of storage.

Thus, this study emphasises the potential of entomopathogenic fungi to be used either alone or in integrated pest management to reduce reliance on harmful chemical fumigants in silos and warehouses as it looks at how these EPF affect the pest and wheat weight loss when in storage which is essential because it directly links to food security. It also provides actual data on the efficiency of EPF not only as liquid via spraying but also as powder assay via mixing with grains, and there is a scarcity of research employing this practise that could influence future pest management research and policy in stored products.

2. Material and methods

2.1. Insect rearing and experimental conditions

The laboratory study was carried out in the College of Agricultural Engineering Sciences at Salahaddin University-Erbil. Pure cultures of red flour beetle, *Tribolium castaneum* adults maintained in the plant protection department were mass-produced in large plastic containers (600 ml) covered with a piece of fine mesh cloth and contained an artificial diet of 95% wheat flour mixed with 5% yeast powder [20]. All cultures were maintained in an incubator at a temperature of 30 ± 2 °C and relative humidity of 60 ± 2. Furthermore, the identity of *T. castaneum* was previously confirmed via the use of species-specific primers [21].

2.2. Experiment 1: petri-plates bioassay

2.2.1. Preparation of fungal formulation

The commercially produced formulation of EPF (Rajan Labs, India) containing 1.87×10^8 conidia per gram powder was used in this study. These EPF formulations were *Beauveria bassiana* (B.b), *Metarhizium anisopliae* (M.a), *Verticillium lecanii* (V.l), and *Paecilomyces ilacinus* (P.i). To generate a stock

solution (0.748×10^7 spore/ml) of each EPF, 1 g of the commercial powder was mixed with 25 ml of distilled water containing 0.1% Tween-80, Sigma–Aldrich, Germany. Following that, the stock solution (0.748×10^7 spore/ml) along with diluted concentrations of 0.748×10^6 and 0.748×10^5 spore/ml has been used [22]. The control treatment (0 spore/ml) was created when adults of red flour beetle were subjected to filter papers treated with distilled water only. The germination of the commercial product was assessed by counting a total of 200 conidia within the dish under a microscope at 400 × magnification and prior to the assay, >93% germination was ensured [23].

2.2.2. Bioassay of insects

A total sample size of 480 adults of *T. castaneum* aged 3–7 days old was used in the experiment. Three replicates, each with ten adults, were placed on filter papers inside Petri plates and were subjected to the spray application of various concentrations of the fungal spores of EPF and to avoid any possible cross contamination, separate sterilized hand amber glass Boston sprayers were dedicated for each bioagent. Control treatment (0 spore/ml) was created when adults of red flour beetle on filter papers were sprayed with distilled water only. Adult mortality was recorded after 7, 15, and 22 days of exposure, and was considered dead when probed with a sharp object or fine paintbrush without any response.

2.3. Experiment 2: bag storage assay

2.3.1. Spore suspension assay

Clean bread wheat grains, *Triticum aestivum* var. Hawler 4 was utilized in the experiment [24]. To eliminate grains from possible previous and undetected insect stages i.e. the eggs, grains were stored at −15 °C for 7 days following the methods used by [58]. Following Petri-plates bioassay efficacy, the spore suspension of 0.748×10^7 spore/ml of *B. bassiana*, *M. anisopliae, V. lecanii,* and *P. ilacinus* was applied to 250 g of wheat lots placed inside paper bags. Then each treatment of sprayed wheat grains was introduced to 20 adults aged 3–7 days old and the bags were sealed later. Four replicates of each treatment were utilized, and adult mortality was recorded after 20, 40, and 60 days of exposure. Moreover, the insecticidal effect of various EPF on red flour beetle was further tested and the percentage weight loss was calculated by counting and weighing damaged and undamaged wheat in a representative sample consisting of 1000 grains. Accordingly, the damaged and undamaged kernels inside each bag, treated with various bioagents after 60 days of exposure, were compared with those not treated with EPF (Untreated control) according to the following equation:[57]

$$\text{Wheat weight loss}\,(\%) = \frac{(\text{UNd}) - \text{DN}_4}{\text{U}(\text{Nd} + \text{N}_4)} \times 100$$

where: U = Weight of undamaged grains, N_4 = Number of undamaged grains, D = Weight of damaged grains, Nd = Number of damaged grains.

The efficiency of each treatment following their insecticidal activity was determined by using this equation:[59]

$$\text{Efficiency}\,(\%) = \frac{\text{No.of dead insects in treatment} - \text{untreated control}}{\text{Untreated control}} \times 100$$

2.3.2. Powder-formulated assay

The powder product of each bioagent was applied at four concentrations (0, 250, 500, and 1000 mg/250 g wheat grain). The experiment was set out with four replications and 20 adults were introduced to each treatment beetle [25]. Before applying the treatments and eliminating any possible previous grain infestations, the same procedure of spore suspension assay was also applied here. A sample size of 1280 individuals of *T. castaneum* was used for the experiment. We examined the protectant ability of all four EPFs by mixing them with wheat grains inside paper bags [26]. Insect mortality was recorded for various concentrations after 20, 40, and 60 days of exposure. Besides, both percentages of weight loss and treatment efficiency were calculated as pointed out in the above section.

2.4. Statistical analysis

The significance of variations in mortality of red flour beetles treated with entomopathogenic fungi was determined using an analysis of variance. The data was examined using multi-factor analysis of variance (ANOVA) in Statgraphics Centurion XV, followed by Fischer's least significant difference (LSD)

test to establish statistical differences between mean mortality rates at $P \leq 0.05$. To achieve normal distribution, the data were Arcsine converted to minimize variability.

The lethal concentration fifty (LC_{50}) values and associated statistics were estimated by exposing the mortality data to the maximum likelihood program of probit analysis using SPSS software version 20, after which the data was corrected according to Abbott's formula [55]. Using mortality and LC_{50} values and time as explanatory variables, we examined the efficacy of several bio-insecticides.

3. Results and discussion

3.1. Experiment 1: petri-plates bioassay

3.1.1. Mortality of Trbolium castaneum

The laboratory test via utilizing different EPF significantly affected the mortality of *Tribolium castaneum* adults ($F_{(3,1140)} = 52.27.$, $P < 0.001$, see Fig. 1a). Red flour beetle treated with *Verticillium lecanii* achieved the highest mortality (31.11%) which differs significantly from all other treatments. The results exhibited no significant difference between both *Paecilomyces ilacinus* and *Metarhizium anisopliae* (26.11, 24.17 %) subsequently. In addition, the mortality of red flour beetles, as in Fig. 1b, was varied in accordance to the bioagents concentrations ($F_{(3,1140)} = 157.56$, $P < 0.001$) with recording maximum and minimum mortality of 43.61 and 2.22 %, via the use of concentration one and zero, respectively. The exposure duration had a significant influence on adult mortality ($F_{(2,1140)} = 301.61.$, $P < 0.001$). The highest mortality recorded after 22 days was 41.25% while the lowest recorded after 7 days was 0.63 % (explained in Fig. 1c).

The adults of the *T. castaneum* beetle have a thick coating of cuticle and the efficiency of the entomopathogenic fungal may be more likely at exposure to higher concentrations of conidia for a longer time. This is in agreement with previous studies that stated that beetle mortality is positively correlated with the dose of conidial suspension and the duration of exposure [27,28].

Moreover, the findings are also consistent with the research conducted by Hassuba et al. [29] who investigated the effect of various concentrations of *M. anisopliae* against stored product beetle inside wheat grains and the result revealed that the highest concentration (2.0 × 109 spores/kg) of EPF achieved the highest mortality rate and minimized weight loss up to 0.7% with providing significant protection to wheat grains during storages.

The results indicated that treatment types also had a considerable impact on adult mortality via interaction with both doses ($F_{(9,1140)} = 5.85.$, $P < 0.001$) and exposure times ($F_{(6,1140)} = 14.34.$, $P < 0.001$). Hence, maximum average mortality for both doses and exposure times recorded by *V. lecanii* were 58.89 and

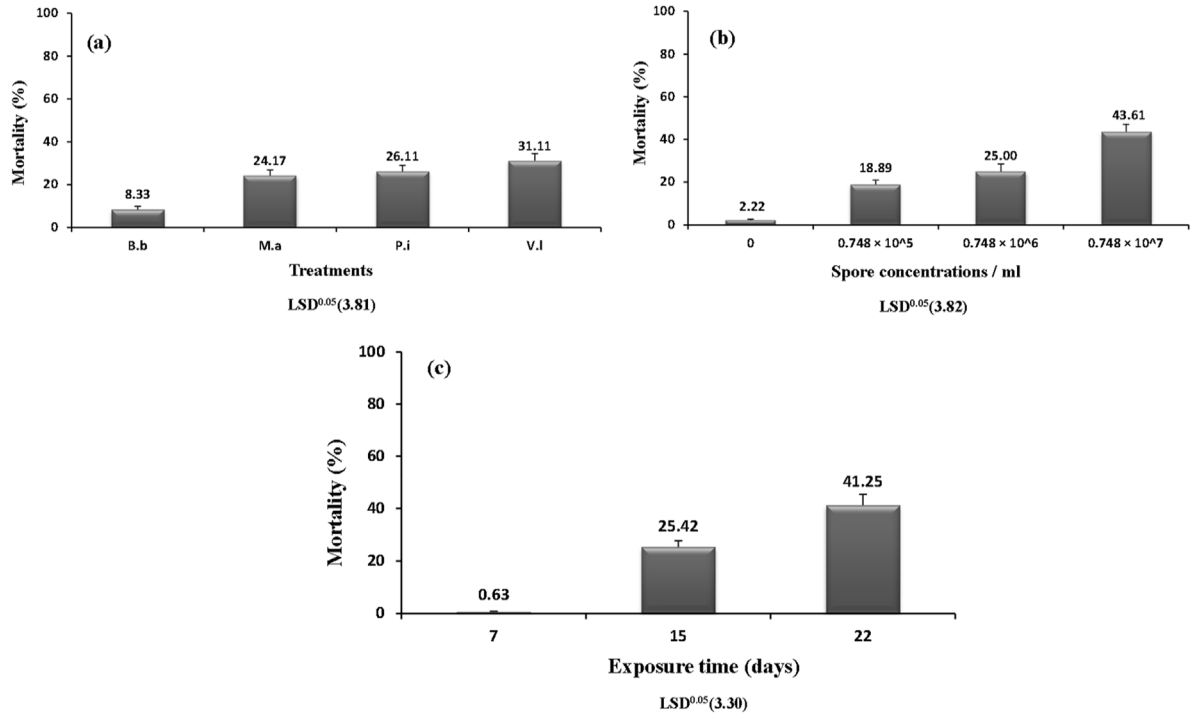

Fig. 1. Mortality of T. castaneum adults in response to (a) treatment types (b) spore concentrations (c) exposure time.

57.5%, followed by *P. ilacinus* (47.78 and 48.33 %) at 0.748×10^7 and 22 days exposure period respectively (see Fig. 2a & b). In addition, *V. lecanii* recorded the highest mortality (1.67, 34.17 & 5 7.5%) while *B. bassiana* recorded the lowest mortality (0, 10 & 15%) at 7, 15 & 22 days respectively (illustrated in Fig. 2b).

The interaction term of bioagent doses with exposure times was also significant ($F_{(6,1140)} = 37.3.$, $P < 0.001$). Accordingly, the least adult survivorship (75% mortality, explained in Fig. 2c) was obtained using the highest concentration (0.748×10^7) and 22 days of the exposure period.

The LC_{50} values for various biopesticide types and doses on *T. castaneum* adult stage were evaluated following different exposure times. The results shown in Table 1 indicated that the red flour beetle was more susceptible to *V. lecanii* with an average of 6,233,697 mgL^{-1} and thus required less concentration to induce 50 % mortality at various exposure periods. On the other hand, the adults were more tolerant to *B. bassiana* with an average LC_{50} of 15,668,738 mgL^{-1} within the same period.

Table 1. The LC_{50} values (conidia/ml) for the adults of T. castaneum for measuring mortality using the bioagents according to different exposure times.

EPF	Time (days)	LC $_{50}$ (conidia/ml)	Average
B.b	7	29,763,089.49	
	15	9,417,096.69	
	22	7,826,026.42	15,668,738
M.a	7	29,467,316.15	
	15	6,381,242.73	
	22	3,101,402.24	12,983,320.4
P.i	7	18,539,857.83	
	15	5,612,963.05	
	22	2,190,159.14	8,780,993
V.l	7	13,932,981.60	
	15	4,006,744.26	
	22	761,364.44	6,233,697

3.2. Experiment 2: bag storage assay

3.2.1. Spore suspension assay

The results illustrated in Fig. 1a revealed that the mortality of red flour beetle inside paper bags was significantly influenced by the type of bioagents applied ($F_{(4,59)} = 29.57.$, $P < 0.001$). Thus, the highest mortality was achieved via the use of *P. ilacinus* (27.08 %) while the least mortality recorded by the untreated control was 2.50%.

The mortality of red flour beetles showed significant variation in accordance to the time were subjected to cereal seeds treated with the bioagents ($F_{(2,59)} = 83.37.$, $P < 0.001$, see Fig. 3b). Likewise, the interaction term between treatment types and exposure times was also significant ($F_{(8,59)} = 11.32.$, $P < 0.001$). As a result, the maximum mortality of the insect (58.75 & 43.75, Fig. 3c) was recorded by both biopesticides (*P.i* & *V.l*) respectively after 60 days of exposure.

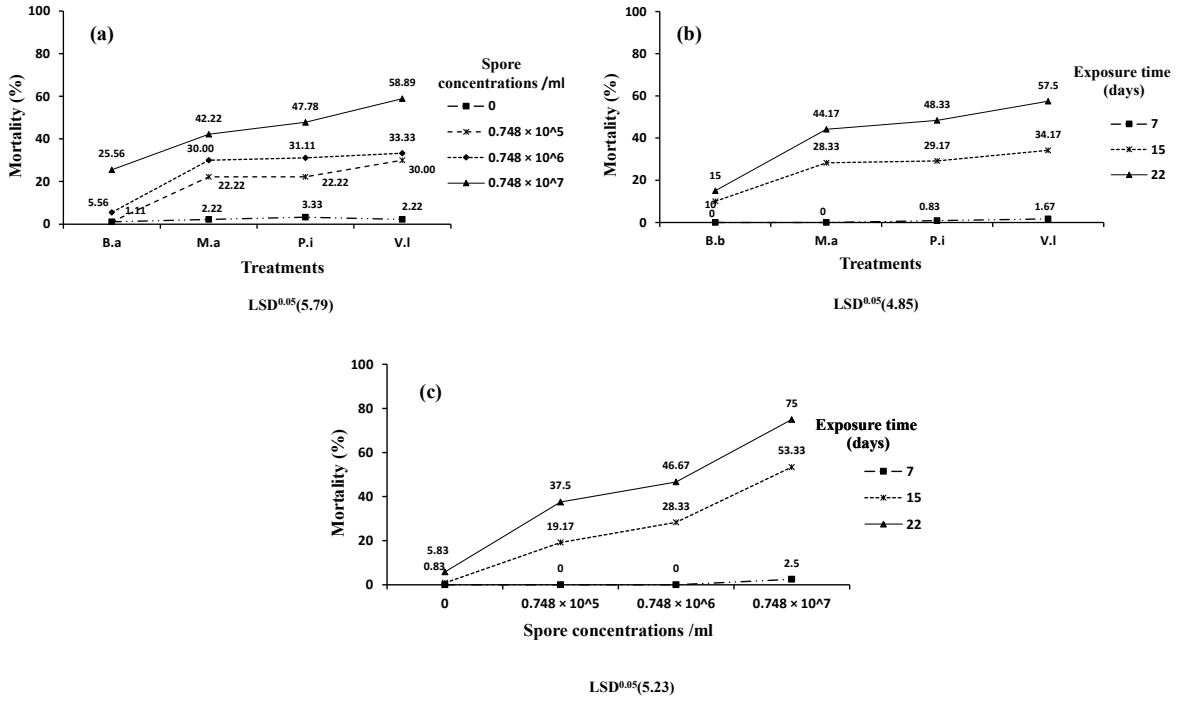

Fig. 2. Mortality of T. castaneum adults in response to interaction term between (a) treatment types and spore concentrations (b) treatment types and exposure times (c) spore concentrations and exposure times T. castaneum mortality based on LC50 values.

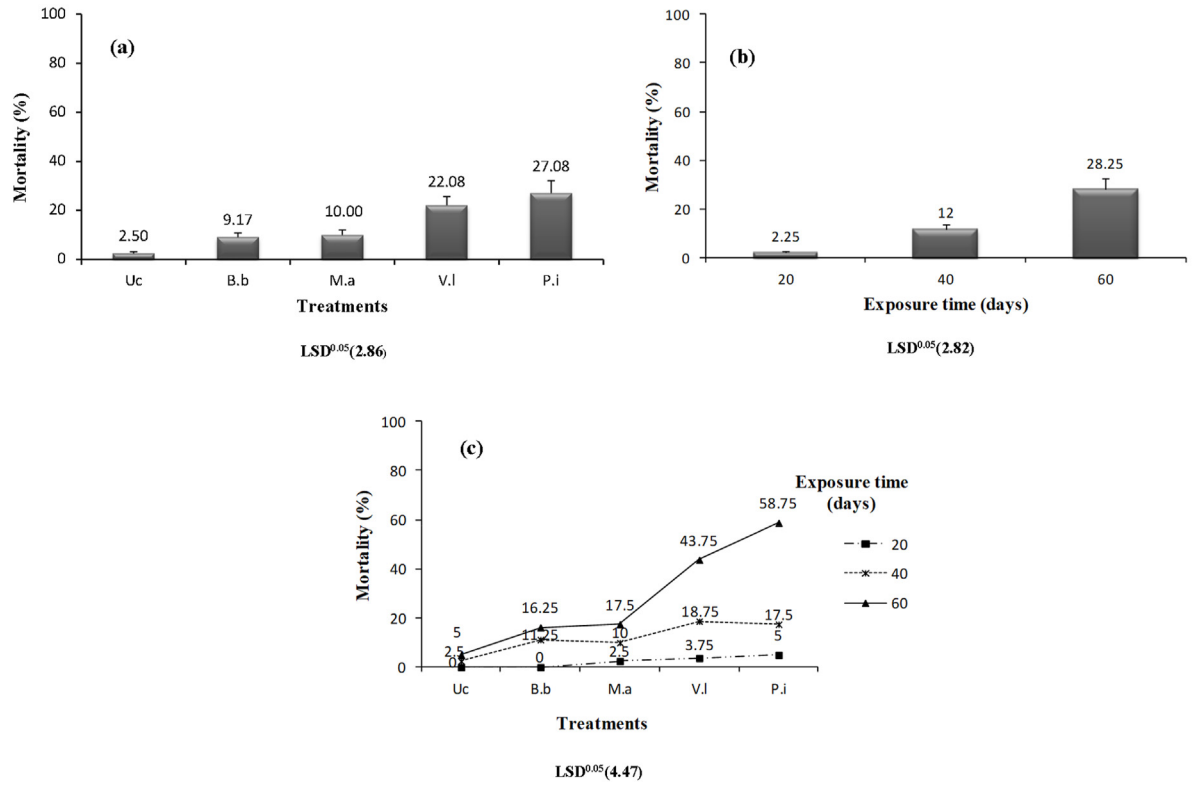

Fig. 3. Percentage mortality of T. castaneum adults inside bags following (a) treatment types (b) exposure times (c) interaction term between treatment types and exposure times.

Thus, the results suggests that the highest mortality rates (58.75% for *P. ilacinus* and 43.75% for *V. lecanii*) observed after 60 days followed by *M. anisopliae* 17.5% and *B. bassiana* 16.25% which indicates that the choice of bioagent is crucial factors in effectively controlling *T. castaneum*. On the other hand, the spraying method resulted in the highest mortality rate of 100% in *T. castaneum* adults attributed to *M. anisopliae*, followed by *B. bassiana* at 86.67% and *V. lecanii* at 60% at the concentration 1×10^8 spores/ml [56].

3.2.2. Powder-formulated assay

In response to powder-formulated assay analysis, the present data revealed that the mortality rate was significantly influenced by the three main factors (treatment type, doses applied, and duration time) in the experiment ($F_{(3,191)} = 60.16.$, $P < 0.001$, Fig. 4a; $F_{(3,191)} = 324.74$, $P < 0.001$, Fig. 4b; $F_{(2,191)} = 356.73$, $P < 0.001$, explained in Fig. 4c), respectively.

The combination of treatment type and doses exhibited a significant influence on red flour beetle mortality ($F_{(9,191)} = 8.9.$, $P < 0.001$), the maximum mortality (57.08, 53.33 %) was observed via the use of *V. l* and *B. b* respectively at 1000 mg which didn't differ significantly from each other. The control treatment recorded the lowest adult mortality, as in Fig. 5a, when wheat grains were not treated with any entomopathogenic doses. Besides, insect survivorship was significantly influenced by both the treatment type and the time adults were subjected to the biopesticide powders ($F_{(6,191)} = 7.7.$, $P < 0.001$). The highest and the lowest mortalities (48.75 & 28.75, shown in Fig. 5b) were recorded by *V.l* & *M.a* respectively after 60 days of exposure.

Likewise, *T. castaneum* mortality on treated wheat grains increased significantly with an increase of both doses and the time of exposure ($F_{(6,191)} = 37.54.$, $P < 0.001$). As a result, the maximum mortality (68.75, illustrated in Fig. 5c) was recorded by utilizing the highest concentration (1000 mg) and longest duration time (60 days).

Khater et al [30] discovered that increasing the fungal concentration of both *M. anisopliae* and *B. bassiana* isolates against *T. catsaneum* resulted in an increase in the total amount of conidia that were attached to the insect. Furthermore, they discovered a high reduction percentage in F1 progeny following inoculation with both fungi, which was evaluated after fifty days of treatment. The current investigation also displayed high rate of mortality among the beetle after being exposed to higher doses of the

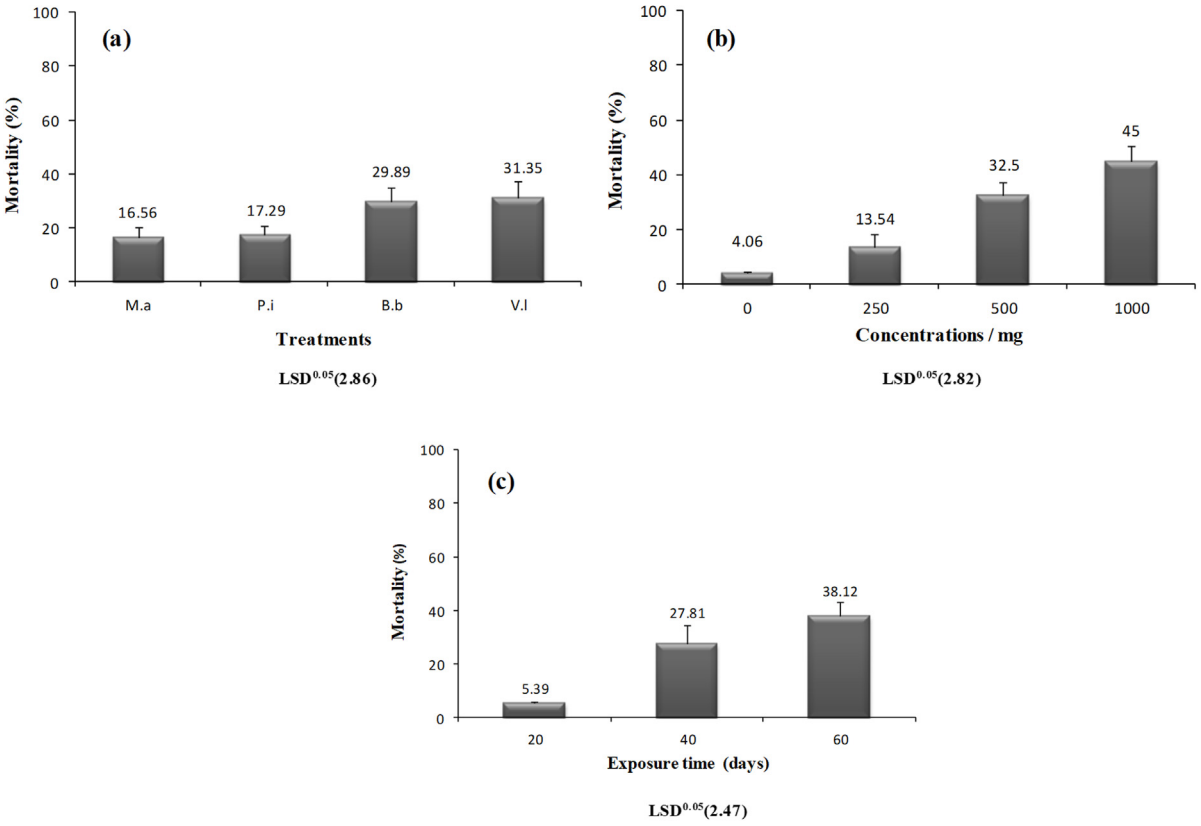

Fig. 4. Percentage mortality of T. castaneum adults inside bags with (a) treatment types (b) powder concentrations (c) exposure times.

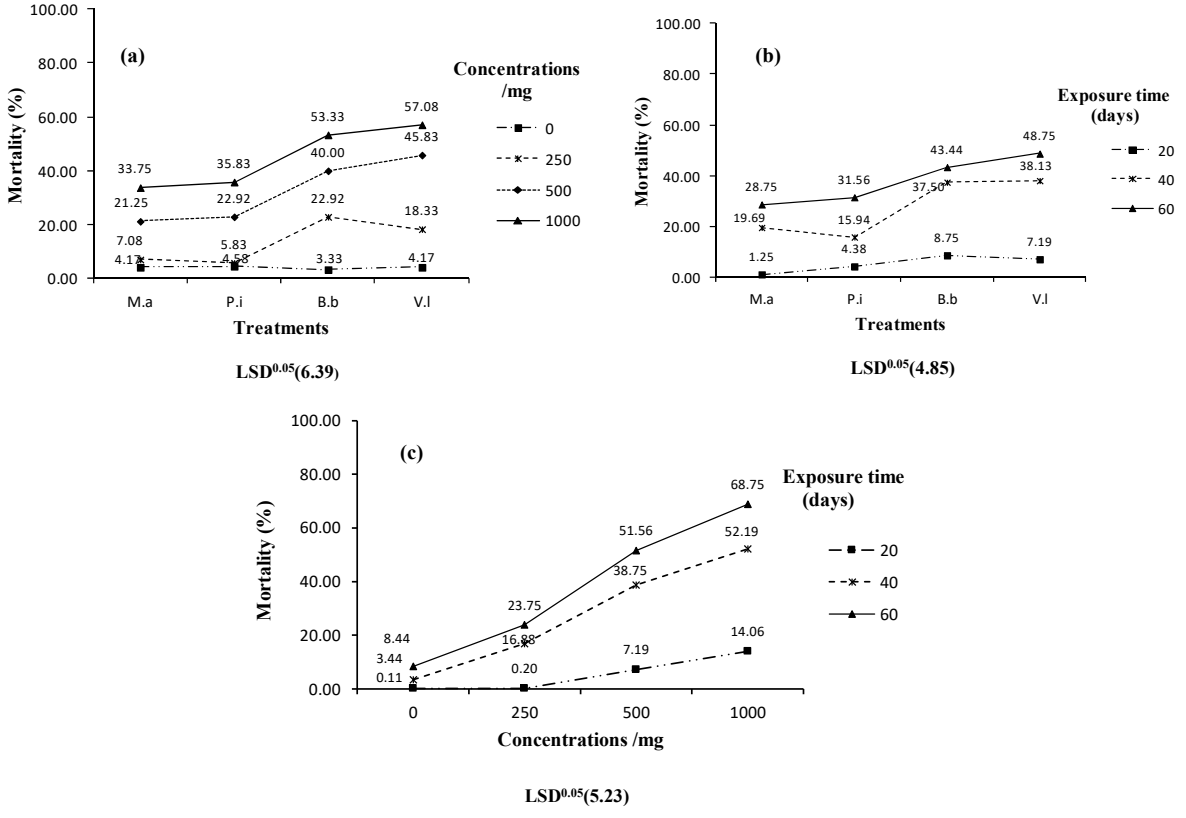

Fig. 5. Percentage mortality of T. castaneum adults inside bags concerning interaction terms between (a) treatment types and powder concentrations (b) treatment types and exposure times (c) powder concentrations and exposure times.

EPF for a period of 60 day as the dry conidial concentrations of various utilized EPF in the experiment exhibited different virulence against the insect pest in contingent upon the exposure interval and dose rate. According to [10,31,32], the total potential of a fungus may only be expressed after long incubation periods.

3.3. T. castaneum based on LC50 values

The LC_{50} values of different entomopathogenic fungus types and doses revealed a constant decrease in the value whenever the adult beetle was exposed for a longer period to the agents. For instance, the LC_{50} values of the red flour beetle subjected to wheat grains treated with *V. lecanii* for 20, 40, and 60 days were 1627.85, 522.87, and 240.02 mgL^{-1}, respectively. Whereas the beetle was more tolerant to *M. anisopliae* agent which displayed the least insecticidal activity, as explained in Table 2, in comparison with the remainder of biopesticides within the same period and thus required more doses to induce 50 percent of mortality.

Our findings suggested that the mortality of *T. castaneum* was concentration and time-dependent and can be justified by low LC_{50} with exposure of the beetle to the EPF for a longer time. The findings of the current study conform with Akmal et al. [33] who tested *B. bassiana*. in a laboratory evaluation and found that the LC_{50} values of *T. castaneum* were 4.36×10^8 and 3.31×10^8 spores/ml on the 6th and 7th day post-infection. The results are also in agreement with Bhatti et al. [34] as found that LC_{50} of *T. castaneum* decreased with increasing time of exposure to *Beauveria bassiana, Isaria cateniannulata, Trichoderma harzianum, and Metarhizium attenuatum* under controlled laboratory conditions.

Table 2. The LC_{50} values for the adults of T. castaneum for measuring mortality using the bioagents according to different exposure times.

Entomopathogenic fungi agents	Time (days)	LC_{50}[a]	Average
	20	2141.32	
M.a	40	1079.49	
	60	856.61	1359.14
	20	1718.88	
P.i	40	1187.68	
	60	800.31	1235.62
	20	1381.03	
B.b	40	654.86	
	60	547.76	861.22
	20	1627.85	
V.l	40	522.87	
	60	240.02	796.91

[a] LC_{50} values needed (mg) of commercial powder for each 250 g of wheat grains.

3.4. Bioassay by treatment efficiency

Analysis of variance showed significant differences in the performance of the bioinsecticides against the red flour beetle pest. Hence, wheat grains infested with *T. castaneum* without the entomopathogenic fungi were significantly more damaged by beetles than grains treated with the bioagents.

Table 3 shows that the percentage weight loss of the wheat varied with not only the type of EPF but also the type of bioagent's structure applied to the wheat whether in the form of powder or liquid. Hence, *P. ilacinus* was the most efficient agent (98.33%) followed by *V. lecanii* (78.33%) which recorded 16.36 and 12.01 weight loss in the treated grains, respectively, in comparison with the untreated control that exhibited maximum weight loss of 18.43 %.

Regarding the powder assay, *V. lecanii* was the most effective agent (89.49%) that achieved minimum weight loss (11.58 %) followed by *B. bassiana* (12.44%) and was significantly smaller than the loss in the untreated kernels (17.22%).

According to the results, *T. castaneum* exhibited susceptibility towards some bioagents whereas there was more resistance towards other types. The variations in percentage mortality and lethal concentration 50 of insects might refer to the stage, type, and physiology of the target insect species [21,35]. In addition, the potential of the EPF often varies among

Table 3. Potential efficiency of various treatments and their impact on wheat weight loss after two months of storage.

No.	Treatment symbol	% Efficiency		% Weight loss	
		Liquid assay	Powder assay	Liquid assay	Powder assay
1	V.l	78.33	89.49	12.01	11.58
2	B.b	26.67	85.38	13.06	12.44
3	M.a	30	40.94	14.71	13.54
4	P.i	98.33	42.99	16.36	15.27
5	Uc	–	–	18.43	17.22
	$LSD^{0.05}$			0.63	2.25

fungal species and might be dependent on the mode of action of the pathogen [13,36].

Adaptations and mechanisms involved are varied for instance building resting spores during extreme conditions, the lifestyle of EPF whether saprophyte or parasite, penetration into the vital organ of the insect body, type of fungal structures, whether hyphae or blastospores, the ability to establish new infestations, and the capability to exert mycotoxins are possible mechanisms of manipulations of the fungal bioagents [36]. For instance, the entomopathogenic fungus *V. lecanii* may penetrate the host cuticle soon after germination or during substantial surface colonization of the host cuticle [37]. While, the conidia of *B. bassiana* penetrate the host and the mycelia then spread within the host hemocoel which eventually led to the host's death [62,60]. In addition, *M. anisopliae*, which marks the start of the invasion of insects, often produces an appressorium that usually marks the start of the invasion of the insect via the hyphae to penetrate the cuticle soon after germination and might cause rapid death to the host [38,39].

Alongside the physical invasion of the fungal pathogen into the host, several enzymes (i.e. glucosidase, acid trehalase, protease, and mycotoxins) might contribute to the death of the insect [40–42]. The secondary infections which the renewing inocula also fasten the mortality of insect pests when the further spread of pathogens occurs after the death of the insect and the production of innumerable amounts of conidia from internal or external sporulation [43,44]. As a result, such characteristics of the fungal entomopathogen can enhance their insecticidal effects as the stored insect pests can pick up gradually the lethal doses of the entomopathogen from sporulating cadavers as time passes by.

Another possible activity of EPF to kill the coleopteran adults is through the ingestion of the fungi during feeding on the wheat grains contaminated with EPF, the mortality of *Sitophilus granarius* was revealed when the growth of ingested conidia appeared in the insect gut causing the death of infected insects [45]. The presence of high humidity is considered an essential parameter in the use of EPF against pests. The study indicated that *P. ilacinus* was more efficient when used as liquid and the conidiospore is in favor of moisture. A similar result was obtained by [46] who stated that *P. ilacinus* was effective when the insect pests were subjected to wet filter papers inside Petri plates.

On the other hand, the current result indicated that *B. bassiana* was more effective when applied as a dry powder than liquid formulation [47]. Consequently, the above-mentioned bio agent was capable of achieving high levels of mortality via the use of dry conidia to manage stored product insects. Similar to our finding, several other previous studies revealed that the longevity of conidia of *B. bassiana* is generally more stable under dry conditions and thus leads to an increase in their effectiveness as potential bioagents to manage stored product insects [48–50]. This is in agreement with Wakil et al. [28] as they used entomopathogenic fungi of genera, *Beauveria* and *Metarhizium* with concentrations of (1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia/kg wheat) as dry powder and found that *B. bassiana* caused higher mortality to *T. castaneum* compared to *M. anisopliae*.

Consistent with the aforementioned, research has shown that dried conidia of entomopathogenic fungus can effectively control insect pests in stored grain. The results of this investigation demonstrated that after four months of typical storage conditions, dry conidia of *B. bassiana* could decrease weight loss in wheat grains caused by stored product beetle [25]. Furthermore, prior research has shown that entomopathogenic fungi, particularly *B. bassiana*, are more effective in storage facilities when grains are subjected to low moisture levels [51,52].

The current study found that using *B. bassiana* and *M. anisopliae* as powder was more efficient than spraying method against *T. castaneum*. This supports a previous study conducted by Wakil et al [26] who concluded that dusting was more efficient than spraying when using these fungal agents alone or in combination with diatomaceous earth. Thus, in the framework of Integrated Pest Management, substituting biopesticides for conventional chemical pesticides can help to lower the dosage of chemical insecticides, so preventing any negative environmental consequences [53].

Hence, this research is in line with the increasing prevalence of microbial biopesticide control, which involves the investigation of a variety of fungal strains used to determine their efficacy against *T. castaneum* when applied either via spraying or dipping techniques [33,34]. Nevertheless, the potential for contamination is higher when we are dealing with stored wheat flour and grains, particularly when spraying and dipping methods were employed to control insect pests that infest stored products, particularly in places with elevated temperatures and humidity, such as side storage and warehouses.

As a result, it is worth mentioning to highlight the fact that the majority of the bioagents employed in the current study were far more effective than the liquid assay when applied as a powder, even though the powder assay is rarely used in prior investigations compared to the liquid test. Additionally, the powder assay leads to a reduction in the weight loss of the

wheat grain, which is the primary objective of commercial grain storekeepers and farmers.

Furthermore, our study revealed that *V. lecanii* was more efficient than both *B. bassiana and M. anisopliae* as powder and liquid assay with a chieving 89.49 and 78.33% respectively, despite the fact that it has not been utilised as frequently as these two latter species for the management of stored insect and as a result this fungus can be used as a potential biopesticide for managing this devastating pest inside stored products. The outcomes also line up with research carried out by Broumandnia and Rajabpour [54] who found that all *Lecanicillium lecanii* isolates were virulent to *T. castaneum* and caused high mortality with the *L. lecanii* isolate PAL7, with a concentration of 10^7 conidia/mL, exhibited strong virulence against the stored product pest.

4. Conclusions

The present results demonstrated that *V. lecanii and B. bassiana* can be used as a dry powder with success against *T. castaneum* injurious which can consider an ideal practice for insect pest management inside stored cereals. Furthermore, the liquid formulation of *P. ilacinus* and *V. lecanii* proved their efficiency as a suitable fungal insecticide against the pest. However, in either case, it is recommended that the insect pest be exposed to the biopesticide for a relatively long period to achieve efficient control of the pest in stored grains. The EPF can be employed independently or in conjunction with chemical fumigants to decrease the dependence on conventional pesticides by implementing integrated pest management techniques. This can improve the quality and quantity of wheat products during storage. Hence, this would probably benefit commercial grain storekeepers and millers would lessen the environmental impact resulting from the use of chemical pesticides, and improve food security for farmers.

Ethical statement

All the authors declare that they have no competing interests and no objections to publish this manuscript.

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Author contribution

Sahand K.Khidr and Qasim A. Marzani designed the study. Sahand K. Khidr performed the data analysis and all three authors performed the laboratory experiments and approved the final manuscript.

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