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Original article (Orijinal araştırma)

Mortality effect of wettable powder formulations containing entomopathogenic fungal spores against some stored-product pests[1]

Entomopatojen fungus sporları içeren ıslanabilir toz formülasyonların bazı depolanmış ürün zararlılarına karşı ölüm etkisi

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Abstract

In this study, the effectiveness of wettable powder formulations containing entomopathogenic fungal spores of *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) and *Metarhizium robertsii* Sorokin (Hypocreales: Clavicipitaceae) was examined against major stored-product pests in 2021 in Bioinsecticide Mass Production Laboratory of Plant Protection Department, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University. Twelve formulations were developed: six of which were based on *B. bassiana* isolate 5-4 and the other six on *M. robertsii* isolate S3. The formulations were applied on both wheat and concrete surfaces at predetermined dosages. Bioassays were conducted using *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) third-instar larvae, *Rhyzopertha dominica* (F.,1792) (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L.,1763 (Coleoptera: Curculionidae) adults, under controlled conditions at 30±2°C, 65±5% relative humidity, and in darkness. When applied to wheat, all formulations exhibited high mortality rates against *R. dominica* and *P. interpunctella*, while showing limited efficacy against *S. oryzae*. Conversely, applications on concrete surfaces demonstrated higher efficacy to all pests, particularly against *S. oryzae*. Amongst the formulations, those containing *B. bassiana* outperformed *M. robertsii*-based formulations in terms of efficacy, both on concrete surfaces and wheat. The findings suggest that the developed formulations have significant potential as an effective alternative for pest management in empty storage facilities. Notably, surface applications provided superior results compared to applications directly on stored products.

Keywords: Bioinsecticide, entomopathogenic fungi, mortality, stored product pests

Öz

Bu çalışmada, entomopatojen fungus *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) ve *Metarhizium robertsii* Sorokin (Hypocreales: Clavicipitaceae) sporlarını içeren ıslanabilir toz formülasyonların depolanmış ürün zararlılarına karşı etkinliği 2021 yılında Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Biyoinsektisit Kitlesel Araştırma Laboratuvarında incelenmiştir. *B. bassiana*'nın 5-4 nolu izolatından 6 adet ve *M. robertsii*'nin S3 nolu izolatından 6 adet olmak üzere toplam 12 formülasyon hazırlanmış ve belirlenen dozlarda hem buğdaya hem de beton yüzeye uygulanmıştır. Biyolojik testlerde, *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) 3. dönem larvaları ile *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) ve *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) erginleri kullanılmıştır. Testler, 30±2°C sıcaklık ve %65±5 nispi nemde karanlık ortam şartlarında gerçekleştirilmiştir. Tüm formülasyonlar ürüne uygulandığında, *R. dominica* ve *P. interpunctella* üzerinde yüksek ölüm etkisi gösterirken, *S. oryzae*'ye karşı daha yüksek etki göstermiştir. Beton yüzeye uygulandığında ise tüm zararlılara, özellikle *S. oryzae*' üzerinde etkisi düşük kalmıştır. Formülasyonlar arasında, *B. bassiana* içerenlerin üç zararlı türüne karşı hem yüzeyde hem de üründe, *M. robertsii* içerenlere kıyasla daha etkili olduğu tespit edilmiştir. Bu sonuçlar, hazırlanan ıslanabilir toz formülasyonların, boş depolarda zararlılara karşı mücadelede etkili ve önemli bir alternatif olabileceğini göstermektedir. Özellikle beton yüzeye uygulamanın, ürüne uygulamaya kıyasla daha başarılı sonuçlar sunduğu belirlenmiştir.

Anahtar sözcükler: Biyoinsektisit, entomopatojen fungus, ölüm etkisi, depolanmış ürün zararlıları

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INTRODUCTION

Cereals are subjected to both direct and indirect damage by a variety of pest species during the post-harvest storage process. These pests contribute to both qualitative and quantitative losses in stored grains. The damage caused by feeding includes reductions in grain weight and nutritional value, seed germination loss, accumulation of excreta, presence of webbing, and insect body fragments, all of which result in a decline in market value (Abdel-Raheem et al., 2015). These losses can rise to as much as 9% in developed countries and 20% or higher in developing countries (Barra et al., 2013). Therefore, effective management of stored product pests is of critical importance.

Synthetic chemicals have been widely and intensively used in pest management of stored products. However, the use of chemicals in the control of stored-product pests has led to concerns regarding resistance development, residue problems, toxic effects on non-target organisms and environmental contamination (Khan & Khan, 2023). As a result, alternative pest control strategies have been sought. Among these alternatives, entomopathogenic fungi stand out as a promising solution. Notably, *Beauveria* Vuill. (Hypocreales: Cordycipitaceae) and *Metarhizium* Sorokin (Hypocreales: Clavicipitaceae) have been shown, through numerous studies, to be significantly effective against major pests of stored products. Studies have reported the effectiveness of *Beauveria bassiana* Vuillemin (Hypocreales: Cordycipitaceae) in controlling pests such as *Rhyzopertha dominica* (F.,1792) (Coleoptera: Bostrichidae) (the lesser grain borer), *Sitophilus oryzae* (L., 1763) (Coleoptera: Dryophthoridae) (the rice weevil), and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) (the Indian meal moth) (Moino et al., 1998; Rice & Cogburn, 1999; Padin et al., 2002; Vassilakos et al., 2006; Lord, 2007; Batta, 2008; Mahdneshin et al., 2009; Sabbour et al., 2012; Kavallieratos et al., 2014; Wakil & Schmitt, 2015; Er et al., 2016, 2018; Barış & Er, 2021). Similarly, *Metarhizium* spp. have demonstrated high efficacy against the same pest species, with supportive evidence from numerous studies (Dal Bello et al., 2001; Batta, 2005; Kavallieratos et al., 2006; Athanassiou et al., 2008; Mahdneshin et al., 2009; Sewify et al., 2014). *Metarhizium robertsii* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) among *Metarhizium* species has the ability to infect over 200 insect species across various orders, including Lepidoptera, Coleoptera, Hemiptera, Diptera, Dermaptera, and Orthoptera (Brunner-Mendoza et al., 2019).

In literature, the use of entomopathogenic fungi as a dust application on stored-product is commonly tested for the control of stored-product pests. While numerous studies have focused on the application of chemicals on structural surfaces, there are very few studies regarding the use of entomopathogenic fungi with the same approach (Athanassiou et al., 2017; Wakil et al. 2023). Since entomopathogenic fungal spores can be acquired by insects from treated surfaces, much like insects feeding on fungus-treated foliage, applying fungal spore suspensions onto surfaces seems to be a feasible approach in treatments of empty storage and processing facilities of stored commodities. It has been suggested that formulations of entomopathogenic fungi, which can be applied in water, may be effective in reducing the use of chemicals for pest control in empty warehouses. The potential of entomopathogenic fungal isolates, which has been demonstrated in numerous studies against stored product pests, raises interest regarding their performance once formulated.

Unlike formulations that are used in powder form, there is no research on the development of formulations that will be mixed in water prior spraying for the control of pests in stored products. This study was conducted to demonstrate the mortality effects of *B. bassiana* and *M. robertsii* on three insects, *R. dominica, S. oryzae* and *P. interpunctella* by application on both commodity and concrete surface, and to explore probable enhancement of the effects by formulating the spores of these entomopathogenic fungi as wettable powders.

Materials and Methods

Test Insects and their cultures

In the Entomology Laboratory of the Department of Plant Protection at Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, insect species used in bioassay tests were reared under controlled laboratory conditions. Rearing of *R. dominica* and *S. oryzae* was conducted using wheat as substrate. For the preparation of the rearing medium of *P. interpunctella*,, a mixture consisting of 2 kg of wheat bran, 350 g of corn flour, 350 ml of glycerin, and one teaspoon of yeast was utilized (Henteş, 2020). Cultures of *S. oryzae* were maintained at 26±2°C and 65±5% relative humidity (r.h.) in complete darkness. The cultures of *P. interpunctella* and *R. dominica* were kept at 30±2°C and 65±5% r.h. For the tests, one-week-old *R. dominica* and *S. oryzae* adults, third-instar larvae of *P. interpunctella*, were selected to ensure uniformity in developmental stage and age.

Entomopathogen fungi culture

Beauveria bassiana and *M. robertsii* used in this study were obtained from the collection of the Department of Plant Protection at Faculty of Agriculture, Kahramanmaraş Sütçü İmam University. The *B. bassiana* isolate 5-4 (single spore culture of isolate 151138) was originally obtained from a field-infected *R. dominica* adult (Er et al., 2016), while *the M. robertsii* isolate S3 (single spore culture of isolate F17-2-1) was derived using the *Galleria mellonella* trap method. Spore production was conducted following the protocol described by Barış & Er (2021). Rice (100 g) was soaked overnight, drained, and then mixed with 1.5 g of $CaSO_4$ and $CaCO_3$ each. The mixture was autoclaved and allowed to cool. Under sterile conditions, the cooled rice was inoculated with 10 ml of a spore suspension containing $2x10^7$ spores/ml. The inoculated rice was sealed in bags and incubated at 25±2°C with a 12/12 hour light/dark photoperiod for 14 days. Post-incubation, the bags were opened, and the rice was dried at 25±2°C. Dried spores were separated using a 38 µm sieve and collected in glass bottles for formulation studies.

Germination test

Prior to the bioassay tests, the germination rate of the fungal spores was assessed to ensure their viability. A diluted spore suspension was prepared and inoculated onto potato dextrose agar (PDA). The plates were then incubated at 25±2°C for 24 hours. Spore germination was evaluated under a light microscope. Germinated spores were defined as those exhibiting germination tubes at least equal in length to the diameter of the spore. The analysis revealed a germination rate exceeding 98%, indicating high viability of the spores and their suitability for use in bioassays.

Test surface

A concrete surface was used to test the insecticidal effectiveness of wettable powder (WP) formulations against *P. interpunctella, R. dominica,* and *S. oryzae.* Plastic Petri dishes with a surface area of 100.0 cm² were used for the bioassays and each one had a bottom covered with ordinary concrete (Teknoflex, Türkiye) commercially available for multiple purposes. Concrete and tap water were combined in a 5:1 ratio to create a thick, long-lasting paste. Approximately 20 ml of this concrete mixture was applied to each Petri dish, and the mixture was allowed to set and dry for a period of two weeks.

WP formulations

All solid materials used in the wettable powder formulation, including fillers and additives, were dried at 70±2°C, while fungal spores were dried at 25±2°C. All materials were then sieved through a 38 µm mesh. When preparing the formulations, blank formulations were first prepared for each different formulation. Then, fungal spores were added to the prepared blank formulations and mixed homogeneously. Details of the formulation composition per 1 kg of product are provided in Tables 1 and 2. The formulation includes carboxymethylcellulose and a non-ionic, organosilicon-based surfactant.

Table 1. Ingredients of wettable powder formulations containing *Beauveria bassiana*

Formulation names	Fungal spores	Filler	Adjuvant	Other substances
BbWP1	25% *B. bassiana* (3×10^{13})	68% Diatomaceous Earth (Detech)	5% Silicon dioxide	2% Surfactants
BbWP2	50% *B. bassiana* (6×10^{13})	43% Diatomaceous Earth (Detech)	5% Silicon dioxide	2% Surfactants
BbWP3	75% *B. bassiana* (9×10^{13})	18% Diatomaceous Earth (Detech)	5% Silicon dioxide	2% Surfactants
BbWP4	25% *B. bassiana* (3×10^{13})	68% Kaolin	5% Silicon dioxide	2% Surfactants
BbWP5	50% *B. bassiana* (6×10^{13})	43% Kaolin	5% Silicon dioxide	2% Surfactants
BbWP6	75% *B. bassiana* (9×10^{13})	18% Kaolin	5% Silicon dioxide	2% Surfactants

Table 2. Ingredients of wettable powder formulations containing *Metarhizium robertsii*

Formulation names	Fungal spores	Filler	Adjuvant	Other substances
MrWP1	50% *M. robertsii* (2×10^{13})	45.33% Diatomaceous Earth (Detech)	3.33% Silicon dioxide	1.34% Surfactants
MrWP2	75% *M. robertsii* (3×10^{13})	21.5% Diatomaceous Earth (Detech	2.5% Silicon dioxide	1% Surfactants
MrWP3	90% *M. robertsii* (3.6×10^{13})	7.2% Diatomaceous Earth (Detech)	2% Silicon dioxide	0.8% Surfactants
MrWP4	50% *M. robertsii* (2×10^{13})	45.33% Kaolin	3.33% Silicon dioxide	1.34% Surfactants
MrWP5	75% *M. robertsii* (3×10^{13})	21.5% Kaolin	2.5% Silicon dioxide	1% Surfactants
MrWP6	90% *M. robertsii* (3.6×10^{13})	7.2% Kaolin	2% Silicon dioxide	0.8% Surfactants

Bioassays

Grain treatments

Formulations containing *B. bassiana* were applied at the concentration of 1 g/kg wheat. *M. robertsii* formulations were applied at concentrations that will deposit equivalent number of spores of the formulations containing *B. bassiana*; MrWP1 and MrWP4 at 1.5 g/kg wheat, MrWP2 and MrWP5 at 2.0 g/kg wheat, MrWP3 and MrWP6 at 2.5 g/kg wheat. In addition to these concentrations (1X), all the formulations were also tested doubling the dosage (2X). Additionally, pure fungal spore suspensions ($3x10^{10}$, $6x10^{10}$, $9x10^{10}$, $12x10^{10}$, and $18x10^{10}$ spores/kg) were tested to demonstrate the effect of formulating spores. Furthermore, all three insects were exposed to blank formulations with the highest filler contents (WP1 and WP4) as well. Each treatment was prepared with 10 mL of water and uniformly applied to 1 kg of wheat using a compressor-equipped HSENG Airbrush AS18. Control wheat was treated with 1% Tween 80 solution. *S. oryzae* and *R. dominica* bioassays were conducted in 50 mL Falcon tubes containing 40 g of treated wheat per replicate (five replicates). *P. interpunctella* assays were conducted using 1-liter glass jars with 300 g of treated wheat per replicate (three replicates). Twenty insects were added to each replicate. Experimental units were maintained at 30±2°C, 65±5% relative humidity (regulated using saturated $NaNO_2$ solutions), and in darkness. Mortality was recorded on days 3, 5, and 7 for *P. interpunctella* larvae, and on days 7 and 14 for *S. oryzae* and *R. dominica* adults.

Surface treatments

Formulations containing *B. bassiana* were applied at the concentration of 1 g/m² of surface area. The concentrations of *M. robertsii* formulations were determined using the same approach as that used for grain treatments. The concentrations were 1.5 g/m² for MrWP1 and MrWP4, 2.0 g/m² for MrWP2 and MrWP5, 2.5 g/m² for MrWP3 and MrWP6. Fungal spore suspensions ($3x10^{10}$, $6x10^{10}$, and $9x10^{10}$ spores/m²) were also tested. Blank formulations with the highest filler content (WP1 and WP4) were applied as fungus-free formulation. Each treatment was applied using 250 µL of water and a compressor-equipped HSENG Airbrush AS18 for uniform coverage. Control surfaces were treated with 1% Tween 80 solution. Twenty insects and three wheat grains were placed on each treated surface (five replicates per treatment). Bioassays were conducted at 30±2°C, 65±5% RH (maintained using saturated $NaNO_2$ solutions), and in darkness. Mortality was recorded on days 3, 5, 7, 9, 11, and 13 post-exposures.

Statistically analysis

One-way ANOVA was used to analyze the mortality rates of the wettable powder formulations against pests on both the product and the surface. Tukey's multiple comparison test ($p \leq 0.05$) was used to determine significant differences between means. For grain treatments (bioassay), an independent t-test ($p \leq 0.05$) was used to compare mortality rates between the two application doses (1X and 2X). Mortality data were arcsine-transformed prior to statistical analysis.

Results

Grain treatments

Insect mortalities after application of unformulated *B. bassiana* 5-4 and *M. robertsii* spores are illustrated in Figure 1. Both fungi showed quite high mortalities on *R. dominica* and *P. interpunctella* depending on spore concentration and time. However, *S. oryzae* adult mortalities were rather low exceedingly only 20% after 14 days at the highest concentrations. These data set a baseline to recognize the effects of formulations on mortalities.

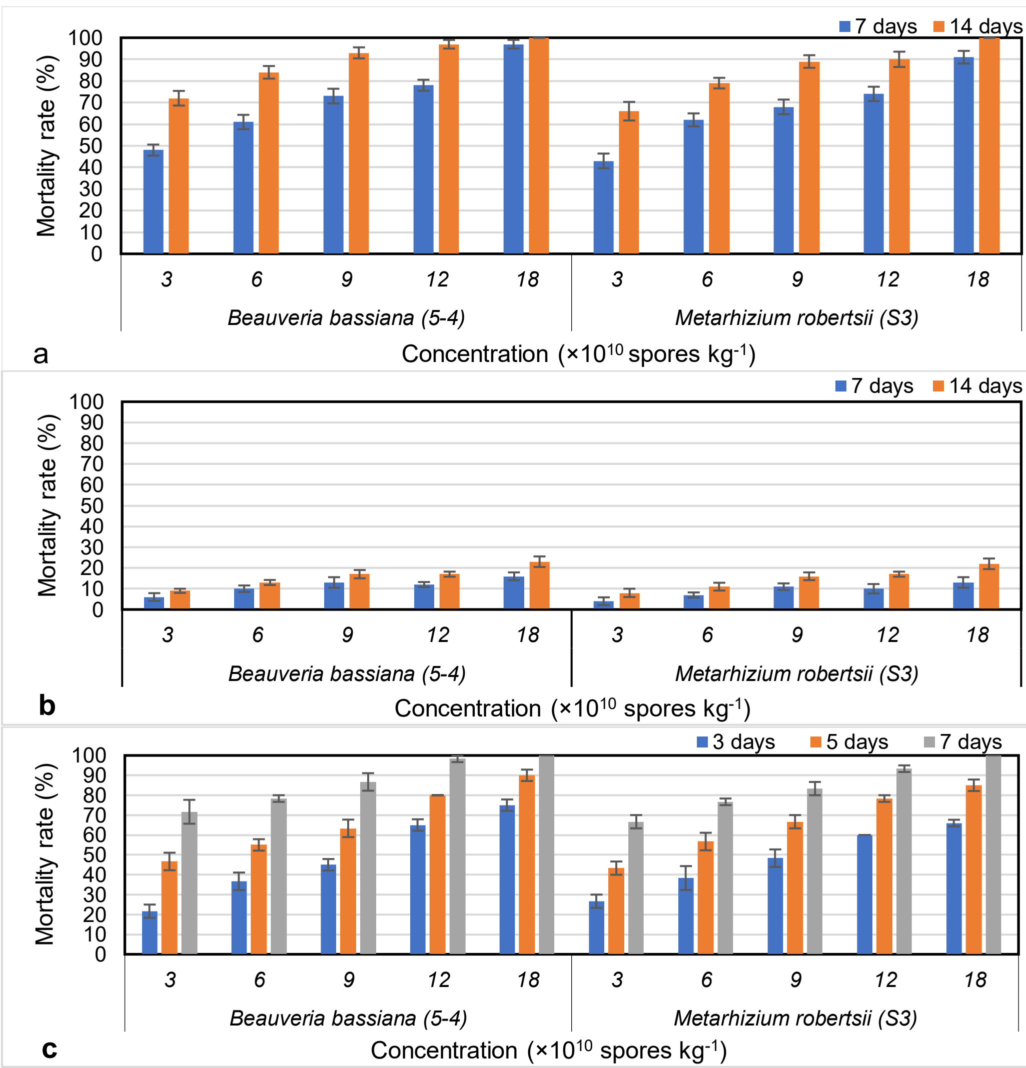

Figure 1. Mean (%)±SE mortality of a) *Rhyzopertha dominica,* b) *Sitophilus oryzae* and c) *Plodia interpunctella* on wheat treated with *Beauveria bassiana* and *Metarhizium robertsii* spores at five different concentrations.

Rhyzopertha dominica adult mortalities after applying formulations are presented in Table 3. Statistically significant differences in mortality were observed 7 and 14 days after application of wettable powder formulations at the standard dose (1X). Formulations BbWP2, BbWP3, BbWP5, BbWP6, MrWP2, MrWP3, MrWP5, and MrWP6 exhibited higher mortality than other formulations on both days. BbWP3 showed the highest mortality (87%) at 1X on day 7, while both BbWP2 and BbWP3 reached 100% mortality by day 14. All formulations achieved 100% mortality at the double dose (2X) on both days. Significant differences in mortality between the 1X and 2X doses were observed for all formulations on day 7 and for BbWP1, BbWP4, MrWP1, MrWP2, and MrWP4 on day 14. The blank formulations (WP1 and WP4) caused no mortality by day 14. Incorporation into wettable powder formulations significantly enhanced the insecticidal activity of both fungal isolates (5-4 and S3) across all tested applications. This demonstrates the improved efficacy of formulated entomopathogenic fungi against *R. dominica* under laboratory conditions.

Table 3. Mean (%)±SE mortality of *Rhyzopertha dominica* adults after 7 and 14 days of exposure to wheat treated with two different doses (1X and 2X) of wettable powder formulations

Exposure	7th day			14th days		
Treatment	1X	2X		1X	2X	
Control	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0	-
BbWP1	59.0±4.30 bc	100.0±0.0	***	83.0±3.74 bc	100.0±0.0	**
BbWP2	81.0±2.91 a	100.0±0.0	***	100.0±0.0 a	100.0±0.0	-
BbWP3	87.0±2.00 a	100.0±0.0	***	100.0±0.0 a	100.0±0.0	-
BbWP4	57.0±4.10 c	100.0±0.0	***	76.0±4.0 c	100.0±0.0	***
BbWP5	77.0±2.55 a	100.0±0.0	***	97.0±2.0 a	100.0±0.0	-
BbWP6	81.0±4.00 a	100.0±0.0	**	99.0±1.0 a	100.0±0.0	-
MrWP1	49.0±2.45 c	100.0±0.0	***	72.0±2.55 c	100.0±0.0	***
MrWP2	76.0±3.32 ab	100.0±0.0	***	93.0±2.55 ab	100.0±0.0	*
MrWP3	81.0±3.67 a	100.0±0.0	***	97.0±2.0 a	100.0±0.0	-
MrWP4	56.0±1.87 c	100.0±0.0	***	77.0±2.0 c	100.0±0.0	***
MrWP5	77.0±3.90 a	100.0±0.0	***	96.0±1.87 a	100.0±0.0	-
MrWP6	84.0±2.91 a	100.0±0.0	**	97.0±2.0 a	100.0±0.0	-
F and *p* values	$F_{11,59}$=14.403 p<0.0001	-		$F_{11,59}$=16.442 p<0.0001	-	

- Within each column, different letters indicate significant differences according to Tukey's multiple comparison test (p<0.05).
* p<0.05, ** p<0.001, and ***p<0.0001 indicate differences according to the significance level and T-test.

The mortality effects of formulations on *S. oryzae* adults are given in Table 4. Statistically significant differences were observed in the mortality effects at 1X and 2X doses on day 14. However, by day 7, a significant difference occurred at the 2X dose, while no significant difference was detected at the 1X dose. For all the formulations, the mortalities significantly increased by doubling the application dosage. Among the formulations, BbWP1 at the 2X dose exhibited the highest mortality rate against *S. oryzae* on day 14, reaching 65%. The blank formulations, WP1 and WP4, showed mortality rates of 24±1.87% and 18±1.22%, respectively, against *S. oryzae* on day 14. Comparing the mortalities caused by unformulated and formulated spores (Figure 1), formulating both isolates enhanced their efficacy against *S. oryzae* adults.

According to *P. interpunctella* larval mortalities (Table 5), statistically significant differences were observed between the formulations at the 1X dose on days 3 and 5. On both days, mortalities increased with the increasing number of spores in formulations. At the 1X dose, MrWP6 showed the highest mortality (90%) on day 3, reaching 100% by day 5. At the 2X dose, all formulations achieved 100% mortality by day 3. A significant difference in mortality between the 1X and 2X doses was observed across all formulations on day 3. The blank formulations (WP1 and WP4) resulted in 15±2.89% and 10±0.0% mortality, respectively, against *P. interpunctella* on day 7. The incorporation of entomopathogenic fungal isolates (5-4 and S3) into the wettable powder formulations significantly increased mortality in *P. interpunctella*.

Table 4. Mean (%)±SE mortality of *Sitophilus oryzae* adults after 7 and 14 days of exposure to wheat treated with two different doses (1X and 2X) of wettable powder formulations

Exposure	7 days			14 days		
Treatment	1X	2X		1X	2X	
Control	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0	-
BbWP1	21.0±2.91	47.0±2.55 a	***	43.0±2.55 a	65.0±2.74 a	***
BbWP2	23.0±2.00	41.0±2.91 ab	**	39.0±1.87 ab	62.0±3.00 ab	**
BbWP3	21.0±2.91	41.0±4.85 ab	*	39.0±2.91 ab	60.0±4.18 ab	**
BbWP4	18.0±3.00	30.0±2.24 b	*	35.0±3.16 ab	49.0±1.87 ab	**
BbWP5	15.0±1.58	33.0±3.00 ab	**	29.0±1.87 ab	51.0±2.91 ab	***
BbWP6	13.0±2.55	30.0±4.47 b	*	25.0±3.53 b	48.0±4.64 ab	**
MrWP1	21.0±1.87	44.0±5.79 a	**	37.0±2.55 ab	62.0±5.78 ab	**
MrWP2	19.0±2.91	43.0±5.39 a	**	34.0±4.58 ab	57.0±6.44 ab	*
MrWP3	19.0±2.91	40.0±5.39 ab	**	33.0±3.39 ab	58.0±4.64 ab	**
MrWP4	22.0±1.22	34.0±3.32 ab	*	36.0±1.87 ab	49.0±2.91 ab	*
MrWP5	17.0±3.39	30.0±3.54 b	*	31.0±2.92 ab	47.0±3.39 ab	*
MrWP6	18.0±3.00	31.0±3.67 b	*	31.0±4.30 ab	45.0±3.87 b	*
F and *p* values	$F_{11,59}$=1.40 *p*=0.204	$F_{11,59}$=2.499 *p*<0.05		$F_{11,59}$=2.628 *p*<0.05	$F_{11,59}$=2.906 *p*<0.05	

- Within each column, different letters indicate significant differences according to Tukey's multiple comparison test ($p<0.05$).
* $p<0.05$, ** $p<0.001$, and *** $p<0.0001$ indicate differences according to the significance level and T-test.

Table 5. Mean (%)±SE mortality of *Plodia interpunctella* 3rd instar after 3, 5, and 7 days of exposure to wheat treated with two different doses (1X and 2X) of wettable powder formulations

Exposure	3rd day			5th day			7th day	
Treatment	1X	2X		1X	2X		1X	2X
Control	0.0±0.0	0.0±0.0	-	0.0±0.0	0.0±0.0		0.0±0.0	0.0±0.0
BbWP1	65.0±2.89 d	100.0±0.0	***	83.33±1.67 c	100.0±0.0	***	100.0±0.0	100.0±0.0
BbWP2	71.0±3.33 bcd	100.0±0.0	***	90.0±0.00 abc	100.0±0.0	**	100.0±0.0	100.0±0.0
BbWP3	85.0±0.00 abc	100.0±0.0	***	98.33±1.66 ab	100.0±0.0	*	100.0±0.0	100.0±0.0
BbWP4	66.67±3.33 cd	100.0±0.0	***	83.33±3.33 bc	100.0±0.0	**	100.0±0.0	100.0±0.0
BbWP5	76.67±4.41 abc	100.0±0.0	**	93.33±4.41 abc	100.0±0.0		100.0±0.0	100.0±0.0
BbWP6	83.33±3.33 abc	100.0±0.0	**	95.0±2.89 abc	100.0±0.0		100.0±0.0	100.0±0.0
MrWP1	70.0±5.00 cd	100.0±0.0	***	90.0±2.89 abc	100.0±0.0	*	100.0±0.0	100.0±0.0
MrWP2	78.33±4.41 abc	100.0±0.0	**	95.0±2.89 abc	100.0±0.0		100.0±0.0	100.0±0.0
MrWP3	88.33±1.67 ab	100.0±0.0	***	98.33±1.67 ab	100.0±0.0		100.0±0.0	100.0±0.0
MrWP4	70.0±2.89 cd	100.0±0.0	***	86.67±1.67 bc	100.0±0.0	***	100.0±0.0	100.0±0.0
MrWP5	80.0±5.00 abc	100.0±0.0	*	96.66±1.67 abc	100.0±0.0		100.0±0.0	100.0±0.0
MrWP6	90.0±2.89 a	100.0±0.0	*	100.0±0.0 a	100.0±0.0		100.0±0.0	100.0±0.0
F and *p* values	$F_{11,35}$=6.038 *p*<0.0001			$F_{11,35}$=4.399 *p*<0.001				

Within each column, different letters indicate significant differences according to Tukey's multiple comparison test ($p<0.05$).
* $p<0.05$, ** $p<0.001$, and *** $p<0.0001$ indicate differences according to the significance level and T-test.

Surface treatments

Insect mortalities after exposure to concrete surface sprayed with unformulated *B. bassiana* 5-4 and *M. robertsii* spores are illustrated in Figure 2. Similar to grain treatment results (Figure 1), both fungi were quite effective against *R. dominica* and *P. interpunctella* depending on spore concentration and time. *Sitophilus oryzae* adult mortalities were still lower but not as low as those in grain treatments. Except for one formulation, mortalities were above 40% reaching up to 64%. The data set a baseline to understand the effects of formulations on mortalities.

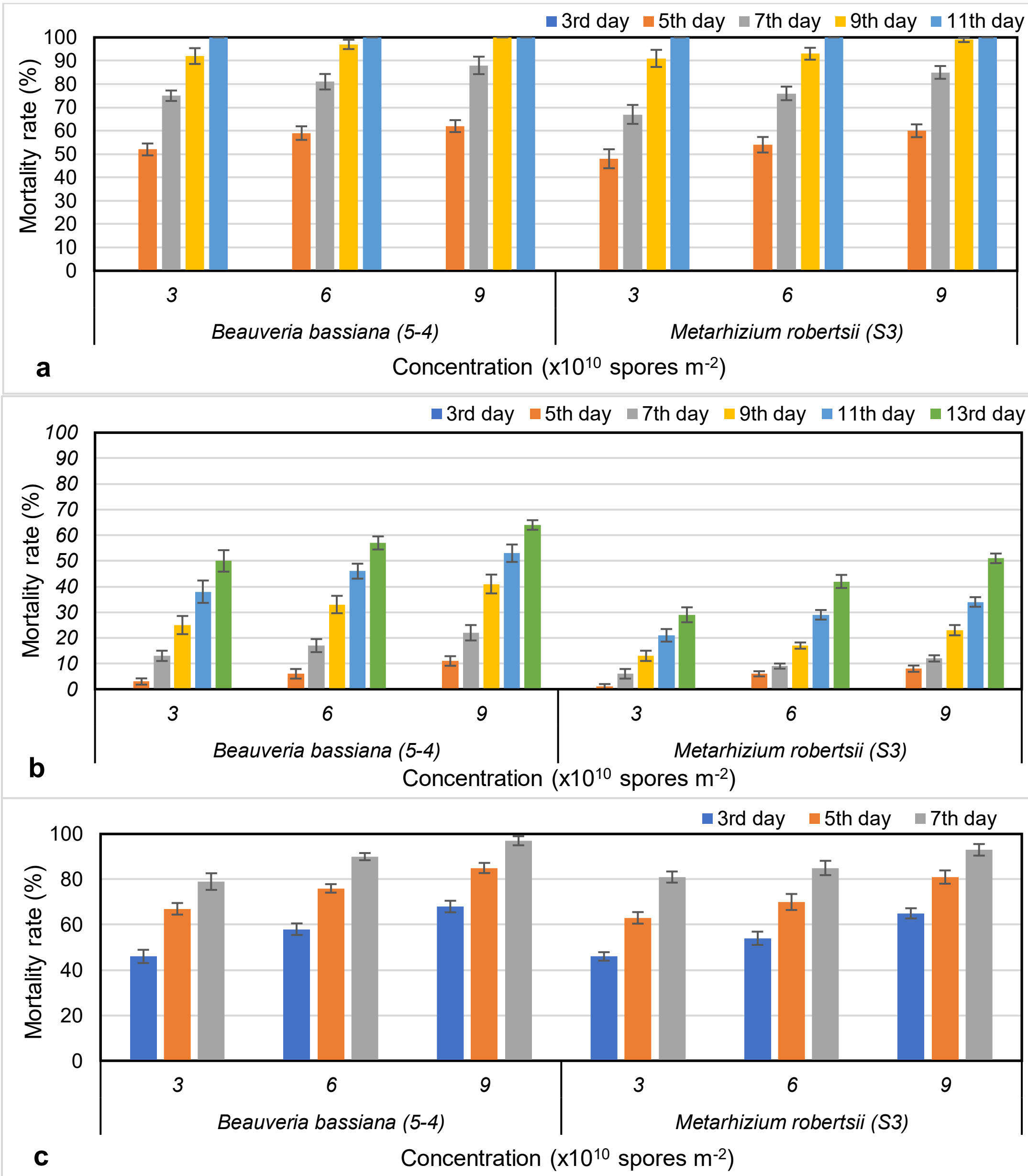

Figure 2. Mean (%)±SE mortality of (a) *Rhyzopertha dominica*, (b) *Sitophilus oryzae* and (c) *Plodia interpunctella* on concrete surfaces treated with *Beauveria bassiana* and *Metarhizium robertsii* spores at three different concentrations.

Table 6 shows the *R. dominica* adult mortalities after exposure to treated surface. None of the wettable powder formulations exhibited lethal effects on *R. dominica* after 3 days of exposure. Statistically significant differences in mortality were observed between the formulations on days 5, 7, and 9. BbWP3 showed the highest mortality, with 75% on day 5 and 94% on day 7. Most of the formulations caused quite high mortalities reaching 100% by day 9. On day 11, all the formulations achieved 100% mortality. The blank formulations had no measurable effect on *R. dominica* adults.

Table 6. Mean (%)±SE mortality of *Rhyzopertha dominica* adults after 3, 5, 7, 9, and 11 days of exposure to concrete surfaces treated with wettable powder formulations

Exposure / Treatment	3rd day	5th day		7th day		9th day		11th day
BbWP1	0.0±0.0	62.0±3.39	ab	83.0±3.00	ab	96.0±1.87	abc	100.0±0.0
BbWP2	0.0±0.0	70.0±2.74	a	89.0±2.91	ab	99.0±1.00	ab	100.0±0.0
BbWP3	0.0±0.0	75.0±2.24	a	94.0±1.87	a	100.0±0.0	a	100.0±0.0
BbWP4	0.0±0.0	60.0±2.74	ab	79.0±2.91	ab	93.0±2.55	bc	100.0±0.0
BbWP5	0.0±0.0	69.0±2.92	a	88.0±2.55	ab	100.0±0.0	a	100.0±0.0
BbWP6	0.0±0.0	74.0±3.67	a	93.0±2.55	a	100.0±0.0	a	100.0±0.0
MrWP1	0.0±0.0	50.0±2.74	b	75.0±2.74	b	91.0±2.91	bc	100.0±0.0
MrWP2	0.0±0.0	67.0±4.36	a	87.0±4.36	ab	100.0±0.0	a	100.0±0.0
MrWP3	0.0±0.0	73.0±2.00	a	90.0±2.74	ab	100.0±0.0	a	100.0±0.0
MrWP4	0.0±0.0	50.0±3.53	b	73.0±3.00	b	91.0±1.87	c	100.0±0.0
MrWP5	0.0±0.0	65.0±3.16	ab	87.0±2.55	ab	99.0±1.00	ab	100.0±0.0
MrWP6	0.0±0.0	71.0±3.67	a	88.0±4.64	ab	96.0±2.45	abc	100.0±0.0
F	-	$F_{11,59}$=7.102		$F_{11,59}$=3.219		$F_{11,59}$=6.196		-
p	-	<0.0001		<0.005		<0.0001		-

-Within each column, different letters indicate significant differences according to Tukey's multiple comparison test (p<0.05).

The wettable powder formulations showed varying degrees of effectiveness against *S. oryzae* (Table 7). While no mortality was observed on day 3, significant differences emerged from day 5 onwards. BbWP1 consistently performed better than the rest, achieving 100% mortality by day 13. BbWP2 also reached 100% mortality on day 13. The blank formulations, WP1 and WP4, demonstrated lower mortality rates of 32±2.55% and 12±1.22%, respectively, by day 13. These results highlight the superior efficacy of BbWP1 and BbWP2 against *S. oryzae* compared to the other formulations and the controls.

Table 7. Mean (%)±SE mortality of *Sitophilus oryzae* adults after 3, 5, 7, 9, 11, and 13 days of exposure to concrete surfaces treated with wettable powder formulations

Exposure / Treatment	3rd day	5th day		7th day		9th day		11th day		13rd day	
BbWP1	0.0±0.0	38.0±3.39	abc	61.0±4.00	a	79.0±3.67	a	92.0±3.00	a	100.0±0.0	a
BbWP2	0.0±0.0	36.0±3.32	ab	53.0±4.10	ab	67.0±3.74	ab	89.0±1.87	ab	100.0±0.0	a
BbWP3	0.0±0.0	33.0±2.55	abc	47.0±4.36	abc	58.0±3.39	bc	73.0 ±3.39	bcd	87.0±3.39	bc
BbWP4	0.0±0.0	17.0±3.74	cd	30.0±3.53	cd	41.0±4.85	c	52.0±4.06	de	67.0±3.00	d
BbWP5	0.0±0.0	16.0±4.45	cd	32.0±3.00	cd	46.0±2.45	c	57.0±3.39	de	72.0±3.74	cd
BbWP6	0.0±0.0	18.0±2.55	cd	34.0±4.00	bcd	49.0±4.30	c	62.0 ±4.35	cde	76.0±3.67	cd
MrWP1	0.0±0.0	23.0±4.36	abcd	34.0±3.67	bcd	48.0±2.55	bc	62.0 ±3.39	cde	74.0±2.45	cd
MrWP2	0.0±0.0	39.0±4.30	a	52.0±5.15	ab	67.0±4.36	ab	80.0 ±4.18	abc	94.0±2.92	ab
MrWP3	0.0±0.0	32.0±4.67	abc	44.0±4.00	abcd	56.0±4.30	bc	66.0±4.30	cde	77.0±5.61	cd
MrWP4	0.0±0.0	21.0±3.32	abcd	31.0±3.32	cd	45.0±4.74	c	56.0±5.10	de	69.0±4.30	cd
MrWP5	0.0±0.0	20.0±3.53	bcd	32.0±3.74	cd	46.0±5.79	c	59.0±5.57	de	73.0±3.74	cd
MrWP6	0.0±0.0	15.0±3.16	d	26.0±3.32	d	38.0±4.10	c	48.0±4.10	e	62.0±4.63	d
F	-	$F_{11,59}$=6.523		$F_{11,59}$=8.184		$F_{11,59}$=9.033		$F_{11,59}$=13.347		$F_{11,59}$=24.099	
p	-	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

-Within each column, different letters indicate significant differences according to Tukey's multiple comparison test (p<0.05).

Larval mortalities of *P. interpunctella* are given in Table 8 Formulations BbWP3 and BbWP6 showed the greatest efficacy against *P. interpunctella*. BbWP3 achieved 86% mortality after three days and 100% mortality after five. BbWP6 reached 99% mortality on day 5 and 100% on day 7. By day 7, most formulations (except MrWP1 and MrWP4) achieved over 90% mortality. The blank formulations had no effect. These results suggest that BbWP3 and BbWP6 are the most promising formulations for controlling *P. interpunctella* infestations.

Table 8. Mean (%)±SE mortality of *Plodia interpunctella* 3rd instar after 3, 5, and 7 days of exposure to concrete surfaces treated with wettable powder formulations

Exposure / Treatment	3rd day		5th day		7th day	
BbWP1	59.0±4.00	cde	77.0±4.06	bc	91.0±3.32	ab
BbWP2	60.0±2.74	cde	81.0±2.45	bc	97.0±1.22	ab
BbWP3	86.0±2.92	a	100.0±0.0	a	100.0±0.0	a
BbWP4	58.0±4.06	cde	81.0±2.92	bc	93.0±2.55	ab
BbWP5	56.0±4.30	de	79.0±4.58	bc	90.0±4.58	ab
BbWP6	82.0±3.39	ab	99.0±1.00	a	100.0±0.0	a
MrWP1	53.0±4.06	e	67.0±3.39	c	86.0±3.67	b
MrWP2	58.0±3.39	cde	74.0±2.92	bc	90.0±3.54	ab
MrWP3	74.0±2.45	abc	88.0±2.55	b	100.0±0.0	a
MrWP4	53.0±4.36	e	69.0±3.67	c	84.0±4.58	b
MrWP5	61.0±2.92	cde	76.0±3.32	bc	90.0±2.24	b
MrWP6	71.0±2.92	bcd	87.0±4.06	b	98.0±2.00	ab
F	$F_{11,59}$=10.833		$F_{11,59}$=21.281		$F_{11,59}$=5.113	
p	<0.0001		<0.0001		<0.0001	

-Within each column, different letters indicate significant differences according to Tukey's multiple comparison test (p<0.05).

DISCUSSION

This study assessed the efficacy of wettable powder formulations of entomopathogenic fungi against stored product pests *R. dominica*, *P. interpunctella*, and *S. oryzae*. The formulations were tested both on wheat grains and on a concrete surface.

The formulations exhibited significantly different mortality effects depending on the pest species and application method. *Rhyzopertha dominica* and *P. interpunctella* were notably more susceptible to the formulations than *S. oryzae*, regardless of the application method. This observation aligns with previous studies showing greater susceptibility of *R. dominica* and higher resistance of *S. oryzae* to entomopathogenic fungi (Moino et al., 1988; Rice & Cogburn, 1999; Dal Bello et al., 2001; Vassillakos et al., 2006; Kavallieratos et al., 2014). Formulations with a high spore content were more effective against *R. dominica* and *P. interpunctella*. Conversely, formulations with lower fungal content, but containing more fillers like diatomaceous earth and kaolin, were more effective against *S. oryzae*. The impact of these fillers on stored product pests has been documented in various studies. Abdelgaleil et al. (2021) specifically suggested the effect of kaolin against certain stored product pests. The efficacy of diatomaceous earth against stored product pests is well-established, as highlighted by numerous studies (Adane et al., 1996; Hidalgo et al., 1998; Storm et al., 2016; Lord, 2001; Dal Bello et al., 2001; Vassilakos et al., 2006; Kavallieratos et al., 2006; Sağlam et al., 2022). Mostly the effect of diatomaceous earth was found higher on *S. oryzae* adults and lower on *R. dominica* adults. These findings explain the results presented in this study. Although diatomaceous earth has an effect on *S. oryzae* mortality, blank formulations could only kill 32% of the adults in surface treatment, while fungal formulations reached up to 100%. For the other two tested insects, blank formulations caused much less mortalities, highlighting the efficacy of entomopathogenic fungi in the final formulations. Even though unformulated spores resulted in high mortalities for *R. dominica* and *P. interpunctella*, mortalities increased when spores were formulated. All the findings support that the tested formulations were suitable both for the fungal spores and for the targeted pests, as no adverse effects were noticed, and elevated efficacy was achieved. Overall, formulations containing *Beauveria bassiana* demonstrated greater efficacy against the tested stored product pests compared to formulations containing *M. robertsii*. The latter only showed better efficacy against *P. interpunctella* larvae when applied to wheat grains.

Effective concentration ranges for entomopathogenic fungal spores against stored product pests are typically between 500-1000 ppm equivalent to $1x10^{10}$-$1x10^{11}$ spores/kg grains according to the literature. The concentrations in our experiments were within this range.

The formulations were more effective against pests on concrete surfaces compared to grain applications. While many studies investigated applications of entomopathogenic fungi as powder on surfaces (Athanassiou et al., 2017; George et al., 2018), research on applying entomopathogenic fungi as suspensions in liquid is limited for stored-product pests. Existing surface application studies are primarily on chemical treatments. Wakil et al. (2023) demonstrated significant mortality effects against *T. castaneum* larvae and adults using *B. bassiana* and *M. anisopliae* by surface application. Further research on liquid surface applications of entomopathogenic fungi is needed. The findings presented here demonstrated that WP formulations of tested fungi can be considered for empty storage treatments against stored-product pests.

A commercially available diatomaceous earth-based insecticide, Protect-It, used for empty warehouses utilizes silicon dioxide and silica aerogel at concentrations of 70 mg/100 cm^2 (liquid) and 50 mg/100 cm^2 (powder). In the present study, significant pest mortality on concrete surfaces was achieved using formulations including considerably lower concentrations of diatomaceous earth.

Considering all the findings, the prepared wettable powder formulations are found to be appropriate for entomopathogenic fungi and may be more effective in empty storage areas. Specifically, the BbWP1 formulation demonstrated superior efficacy in general. Continued development and refinement of these formulations, incorporating new ingredients and technologies, can lead to further development for enhancing this pest control strategy.

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