

EFFICACY OF *Cordyceps fumosorosea* AND Silico-Sec® AGAINST STORED PRODUCT PESTS: IMPACT ON MORTALITY, FECUNDITY, MYCOSIS, AND SPORULATION

M. Yasin^{1*}, W. Wakil^{2,3}, M. A. Qayyum⁴, M. Ashraf⁵, A. S. Aldawood⁶, M. Hussain⁷, H. M. B. Yousuf^{1,8} and A. Mahfooz¹

¹Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

²Department of Entomology, University of Agriculture, Faisalabad 38040, Pakistan

³Senckenberg German Entomological Institute, 15374 Müncheberg, Germany

⁴Institute of Plant Protection, Muhammad Nawaz Shareef (MNS) University of Agriculture, Multan 60000, Pakistan

⁵Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad 38850, Pakistan

⁶Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, Riyadh, P.O.Box 2460, Riyadh, 11451 Saudi Arabia

⁷Guangdong Pest Control Engineering and Technology Centre, Guangdong Province 510000, China

⁸Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011, USA.

*Corresponding author's e-mail: yasin_1876@yahoo.com

ABSTRACT

Stored grain insect pests threaten global food security by reducing the quality and quantity of grain, highlighting the urgent need for sustainable and eco-friendly alternatives to chemical insecticides. In current laboratory tests, entomopathogenic fungi, *Cordyceps fumosorosea*, and a diatomaceous earth (DE) formulation, Silico-Sec®, were tested against the adult stages of *Liposcelis paeta*, *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, and *Tribolium castaneum* using a completely randomized design (CRD) with three replications. Diatomaceous earth was applied at a concentration of 200 ppm, while *C. fumosorosea* was introduced at 1×10^6 and 1×10^8 conidia kg^{-1} of wheat, either individually or in integrated manners. After 21 days of exposure, the highest mortality in all insect species was observed in T₁ (*L. paeta*: 69.53%, *C. ferrugineus*: 56.76%, *R. dominica*: 51.58%, and *T. castaneum*: 43.90%), followed by T₃ and T₂ in individual treatments. In combined treatments, T₅ resulted in the highest mortality (*L. paeta*: 100.0%, *C. ferrugineus*: 95.12%, *R. dominica*: 92.47%, and *T. castaneum*: 83.65%). The lowest LT₅₀ and LT₉₀ values were recorded for *L. paeta*, indicating their higher susceptibility, while the *T. castaneum* exhibited the highest resistance towards all the treatments. Maximum progeny emergence was recorded in T₂ (*L. paeta*: 44.33%; *C. ferrugineus*: 49.44%; *R. dominica*: 54.88%; and *T. castaneum*: 61.44%) compared to the control treatment. On the other hand, the highest mycosis (T₂: 87.21%; T₃: 77.32%; T₄: 55.20%; and T₅: 41.10%) and sporogenesis (T₂: 185.22; T₃: 167.67; T₄: 152.22; and T₅: 140.67 spores) were recorded in *L. paeta*. The results indicated that *C. fumosorosea* and Silico-Sec® can be used as effective grain protectants and provide significant control when used alone or in combination against major storage insect pests. Their exceptional mode of action qualifies them as an efficient and viable solution, reflecting their importance as critical aspects in IPM interventions in the preservation of stored grains.

Keywords: *Cordyceps fumosorosea*, Silico-Sec®, Progeny emergence, Mycosis, Sporogenesis

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INTRODUCTION

Cereal grains are a vital source of nutrition for humans and animals. Postharvest storage exposes these grains to diverse insect pests, such as psocids and mites, leading to annual losses of about 10-20% (Hassuba *et al.*, 2024; Yousuf *et al.*, 2025b). Moreover, it causes loss of nutrients, decreased germination, and reduced grain weight, making it unfit to be consumed by humans, and in most cases leads to market rejection (Deshwal *et al.*,

2020; Yasin *et al.*, 2024). Among the stored grains and grain products, there are various insect pests, such as the grain psocids, *Liposcelis paeta* Pearman (Psocoptera: Liposcelididae); rusty grain beetle, *Cryptolestes ferrugineus* Stephens (Coleoptera: Laemophloeidae); lesser grain borer, *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae); and red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Curculionidae), which causes substantial qualitative and quantitative losses when grains are stored for an extended duration

(Riasat *et al.*, 2011; Saeed *et al.*, 2020; Đukić *et al.*, 2016, 2020).

Both larvae and adults of *R. dominica* are very detrimental to grains, as the larvae feed on the endosperm of the kernels, thereby promoting secondary pest infestations by other pests like *T. castaneum* and *C. ferrugineus* (Hill, 2003; Wakil *et al.*, 2021). Wheat germ is preferred by the larvae of *C. ferrugineus*, which further augments the damage (Rees, 2004). However, *T. castaneum* larvae cannot feed on whole grains and cause qualitative losses in flour through direct feeding, discoloration, and the production of benzoquinones (BQs), which render the flour viscous and elastic (Yousuf *et al.*, 2025a). These BQs create a disgusting taste, making the flour unfit for human consumption. Over the past ten years, *L. paeta* has increasingly posed a significant threat to stored products in various regions worldwide (Wakil and Schmitt, 2015), especially to starch-rich commodities such as grains and flours (Nayak *et al.*, 2014). Estimated losses caused by these insects ranged between 5-15% in stored oilseeds and cereals (Padin *et al.*, 2002).

Conventional grain protectants, particularly chemical insecticides and fumigants, are the mainstay of the farming community to combat these insect pests, and majority of the insects have developed resistance to one more insecticides. Moreover, chemical insecticides pose serious threats to human and animal health and the environment (Ashraf *et al.*, 2017; Yousuf *et al.*, 2024).

Entomopathogenic fungi (EPFs) are known to be an effective alternative in the management of a broad spectrum of economical insect pests (Batta, 2018; Saeed *et al.*, 2020; Wakil *et al.*, 2021). Their distinct mechanism of action reduces the risk of physiological resistance development, positioning them as a promising solution for sustainable pest control. Generally, fungal spores adhere to the insects' bodies, followed by germination and penetration of the host cuticle, and ultimately kill the host (Akbar *et al.*, 2004; Ali *et al.*, 2022; Iqbal *et al.*, 2022). Furthermore, they exist in nature; hence, are safer for the environment and less hazardous to the non-target organisms (Rumbos and Athanassiou, 2017).

The use of inert dusts, especially diatomaceous earths (DEs), has increasingly become important in stored-product protection due to their long-lasting effectiveness and ability to control storage pests (Athanassiou *et al.*, 2014; Losic and Korunić, 2018; Korunić *et al.*, 2020). However, their use often necessitates high dosages, which can impact certain physical properties of commodities (Korunić, 1998). To enhance effectiveness, the combined application of EPFs and DEs has been explored within integrated pest management (IPM) programs (Ak, 2019), with multiple studies highlighting the synergistic effects of various DE formulations with EPFs (Akbar *et al.*, 2004; Vassilakos *et al.*, 2006; Ak, 2019; Rizwan *et al.*, 2019). This synergy

occurs as DEs compromise the insect cuticle, facilitating EPF penetration and accelerating mortality. In this context, the current study was designed to evaluate the effectiveness of *C. fumosorosea*, both individually and in combination with DE, against four major storage pests: *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum*. Additionally, the study examined progeny emergence, mycosis, and sporulation on insect cadavers.

MATERIALS AND METHODS

Test insects: Specimens of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* were collected from grain markets, grain storage, and processing facilities of Faisalabad, Punjab, Pakistan (31.4294° N, 73.0750° E).

Insect rearing: Insect populations were subsequently maintained for four years in the Microbial Control Laboratory, University of Agriculture Faisalabad (UAF), Pakistan. The adults and nymphs of *L. paeta* were reared on a diet composed of 2% rice krispies, 97% cracked wheat, and 1% brewer's yeast under controlled conditions at 30±1°C with 70±5% relative humidity (R.H.) in an incubator (Sanyo, Japan) (Opit and Throne, 2008). *C. ferrugineus* was maintained on wheat mixed with 5% wheat germ and 5% cracked wheat at above mentioned laboratory conditions. *R. dominica* adults were reared on whole wheat grains at the same controlled conditions mentioned earlier. Meanwhile, *T. castaneum* adults were raised on wheat flour supplemented with 5% brewer's yeast at 28±2°C and 65±5% R.H. (Kavallieratos *et al.*, 2022).

Wheat grains: For the bioassay, clean, insect-free wheat (*Triticum aestivum* L., var. Miraj-2008) kernels with minimal dockage and no prior pesticide exposure were used. The grains were sterilized by heating at 50°C for four hours. Before the experiment, initial moisture content was determined to be 11% using a calibrated moisture meter (Dickey-John, Multigrain CAC II; Dickey-John Co., USA). The grains were then incubated at 25±1°C and 75±5% RH for seven days to allow moisture equilibration, reaching a final moisture content of 13.8 ± 0.4%.

DE formulation: The DE formulation Silico-Sec® (Biofa GmbH, Münsingen, Germany), a freshwater-derived DE composed of 92% SiO₂, 3% Al₂O₃, 1% Fe₂O₃, and 1% Na₂O, with an average particle size ranging from 8 to 10 µm was used. Prior to the experiments, the DE sample was kept under standard laboratory conditions for about three weeks to equilibrate to the laboratory conditions.

Fungal formulations: To prepare the conidial suspension for mortality assessments, *C. fumosorosea* was isolated from the cadavers of mole crickets, *Gryllotalpa orientalis* Burmeister (Orthoptera:

Gryllotalpidae). The specimen underwent initial disinfection using a 5% sodium hypochlorite solution, followed by 2-3 rinses with distilled water before being transferred onto Potato Dextrose Agar (PDA) medium (BD, France) for culturing. Conidia from 2-3 week old cultures were scraped with a sterilized scalpel and suspended in 10 ml of distilled water containing 3 mm glass beads and 0.01% Triton X-100. The suspension was filtered through muslin cloth and homogenized using a vortex mixer for 5 minutes. Serial dilutions were made with an improved Neubauer hemocytometer (Marienfeld, Germany) to obtain final concentrations of 1.7×10^6 and 1.7×10^8 conidia kg^{-1} of wheat.

Grain treatment: The experiment consisted of 6 treatments: **T₁** (DE: 200 ppm), **T₂** (*C. fumosorosea*: 1×10^6 conidia kg^{-1}), **T₃** (*C. fumosorosea*: 1×10^8 conidia kg^{-1}), **T₄** (DE: 200 ppm + *C. fumosorosea*: 1×10^6 conidia of wheat), and **T₅** (DE: 200 ppm + *C. fumosorosea*: 1×10^8 conidia kg^{-1}), along with a control (**T₆**). Six plastic jars (D: 16 cm and H: 24 cm) were used to prepare 1 kg wheat lots for each treatment, with 5% cracked wheat admixed in it to promote the growth of secondary insect pests (psocids, rusty grain beetle, and red flour beetle). Silico-Sec[®] was used as dust, while *C. fumosorosea* was introduced as a fungal spore. The designated doses were added to the wheat and thoroughly blended for 5 minutes to ensure uniform dispersion throughout the grain mass. A separate, untreated batch was maintained as a control.

Bioassays: Experiments were conducted using less than two-week-old adults of all insect species. For the bioassay, 80 g of wheat were procured from both treated and untreated jars and weighed using an electronic balance (Shimadzu, ELB 300, Japan). The grains were then transferred into small cylindrical plastic vials (D: 5 cm and H: 8 cm) with an opening of 1.50 cm, covered with a muslin cloth of 60 mesh size to allow adequate aeration while preventing insect escape. Sixty adults from each rearing box were placed in vials and incubated at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ R.H., and a photoperiod of 12:12 (L:D). After 7, 14, and 21 days of exposure, the mortality data were recorded. Cadavers from treatments with *C. fumosorosea*, alone or combined with Silico-Sec[®], were stored at 4°C for mycosis and sporulation assessment. After the final count, adults of all species were removed from the experimental jars, and the jars were then returned to the incubator to track progeny emergence, after 30 days for *L. paeta* and 60 days for beetles. Each treatment had three replications, and the entire experiment was repeated three times to minimize pseudo-replication. Each time, fresh wheat and new vials were used.

Mycosis and sporulation: Before initiating the investigation, insect cadavers were surface sterilized by

immersing them in a 0.5% solution of NaClO for 2-3 minutes, followed by 2-3 washes with sterilized distilled water. Then, all the sterilized cadavers were cultured on PDA medium and incubated under the same conditions as described above. After seven days of incubation, mycosed adults were identified by observing fungal growth on the cadavers under a stereomicroscope. The mycosed individuals were then suspended in 20 ml of sterilized distilled water containing 3% Tween-80, with separate suspensions prepared for each replication. The solution was thoroughly stirred for 2-3 minutes to ensure complete detachment of spores from the insect bodies (Tefera and Pringle, 2003). A 1 ml aliquot of the suspension was taken from the suspension of each species, and the spore count was made using a Neubauer hemocytometer under a compound microscope.

Statistical analysis: Since mortality in the control groups did not exceed 5%, the corrected mortalities were calculated using Abbott's formula (Abbot, 1925). Mortality data for each insect species and exposure interval were analyzed using two-way ANOVA, with treatment type and exposure time as independent variables. F1 progeny emergence, mycosis, and sporulation were analyzed similarly. Statistical analyses were performed in Minitab 17, with mean comparisons using Tukey-Kramer (HSD) test at a 1% significance level (Sokal and Rohlf, 1995). To calculate LT_{50} and LT_{90} , the mortality data were subjected to Probit analysis (LeOra, 2003), and estimation of 95% fiducial limits was made for each exposure interval (Wakil *et al.*, 2018).

RESULTS

Mortality of storage insects: Mortality of the four stored product insect species was significantly affected by both the main effects and their interactions, except for *L. paeta*, where the interaction effect was not significant (Table 1).

Significant differences ($p \leq 0.01$) in mortality rates were observed among all species at various exposure durations. After seven days, recorded mortality values were as follows: *L. paeta*: $F_{4,44} = 111$, $p \leq 0.01$; *C. ferrugineus*: $F_{4,44} = 98.2$, $p \leq 0.01$; *R. dominica*: $F_{4,44} = 179$, $p \leq 0.01$; and *T. castaneum*: $F_{4,44} = 173$, $p \leq 0.01$ (Figure 1). At 14 days, significant differences persisted: *L. paeta*: $F_{4,44} = 213$, $p \leq 0.01$; *C. ferrugineus*: $F_{4,44} = 191$, $p \leq 0.01$; *R. dominica*: $F_{4,44} = 341$, $p \leq 0.01$; and *T. castaneum*: $F_{4,44} = 322$, $p \leq 0.01$ (Figure 2). The trend continued through 21 days, with the following mortality values: *L. paeta*: $F_{4,44} = 152$, $p \leq 0.01$; *C. ferrugineus*: $F_{4,44} = 196$, $p \leq 0.01$; *R. dominica*: $F_{4,44} = 374$, $p \leq 0.01$; and *T. castaneum*: $F_{4,44} = 451$, $p \leq 0.01$ (Figure 3).

Table 1. ANOVA parameters for the main effects and their interactions on the mortality rates of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* adults

SOV	df	<i>T. castaneum</i>		<i>R. dominica</i>		<i>C. ferrugineus</i>		<i>L. paeta</i>	
		F	P	F	P	F	P	F	P
Treatment	4	963.73	≤ 0.01	830.94	≤ 0.01	545.69	≤ 0.01	486.50	≤ 0.01
Exposure interval	2	856.65	≤ 0.01	641.48	≤ 0.01	718.10	≤ 0.01	719.34	≤ 0.01
Treatment*Exposure Interval	8	48.97	≤ 0.01	14.69	≤ 0.01	10.14	≤ 0.01	2.31	0.02

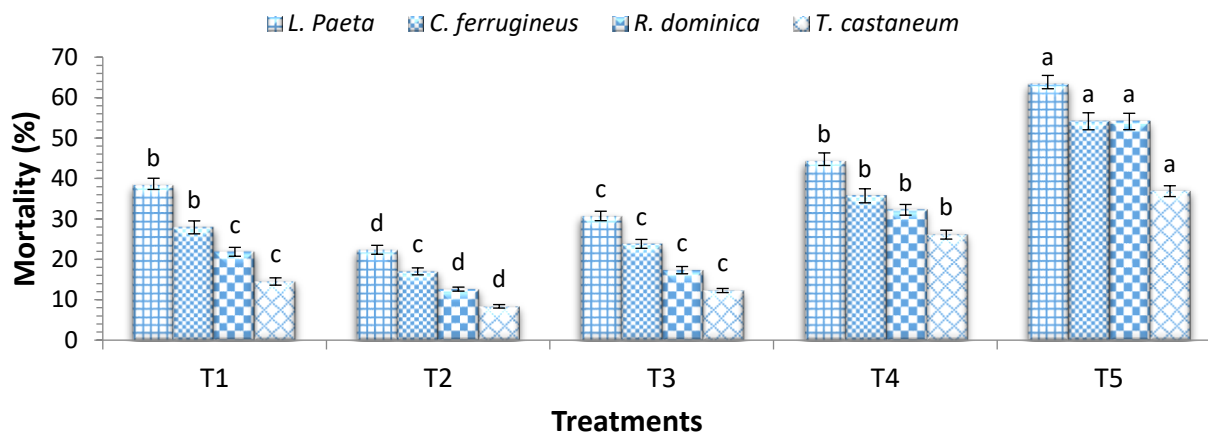


Fig. 1. Mean percent mortality (±SE) of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with Silico-Sec® and *Cordyceps fumosorosea*, either individually or in combination, after 7 days of application. Mortality values (%) followed by the same letters indicate no significant difference based on the Tukey-Kramer (HSD) test at a 1% significance level

After 21 days, the highest mortality rates were observed in the combined treatment T₅ (*L. paeta*: 100%; *C. ferrugineus*: 95.12%; *R. dominica*: 92.47%; *T. castaneum*: 83.65%), followed by T₄ (*L. paeta*: 76.36%; *C. ferrugineus*: 70.67%; *R. dominica*: 67.56%; *T. castaneum*: 58.89%), T₁ (*L. paeta*: 69.53%; *C. ferrugineus*: 56.76%; *R. dominica*: 51.58%; *T. castaneum*: 43.90%), T₃ (*L. paeta*: 61.98%; *C. ferrugineus*: 51.08%; *R. dominica*: 40.14%; *T.*

castaneum: 26.27%), and T₂ (*L. paeta*: 52.71%; *C. ferrugineus*: 40.95%; *R. dominica*: 29.42%; *T. castaneum*: 20.43%).

The treatment effectiveness was further reflected in the LT₅₀ values, which ranged from 8.89 to 19.47 days for *L. paeta*, 5.73 to 24.78 days for *C. ferrugineus*, 5.46 to 33.92 days for *R. dominica*, and 10.68 to 40.77 days for *T. castaneum* (Table 2).

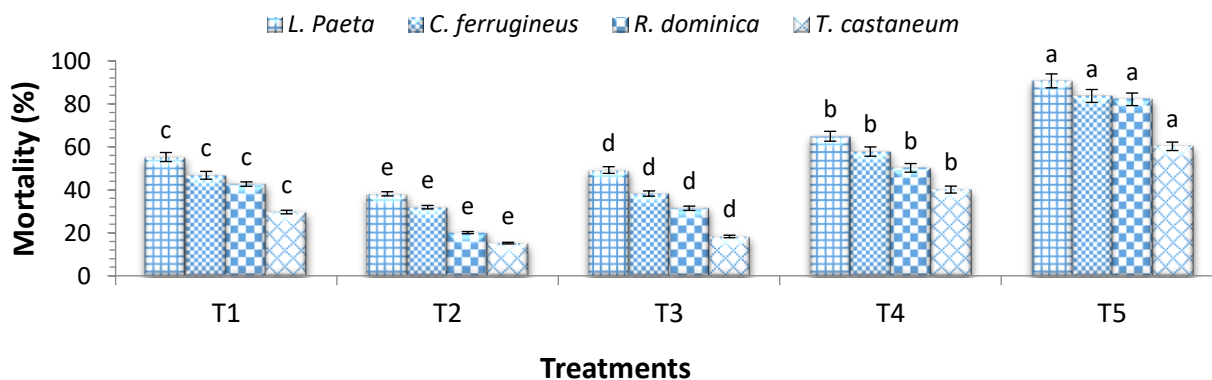


Fig. 2. Mean percent mortality (±SE) of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with Silico-Sec® and *Cordyceps fumosorosea*, either individually or in combination, after 14 days of application. Mortality values (%) followed by the same letters indicate no significant difference based on the Tukey-Kramer (HSD) test at a 1% significance level

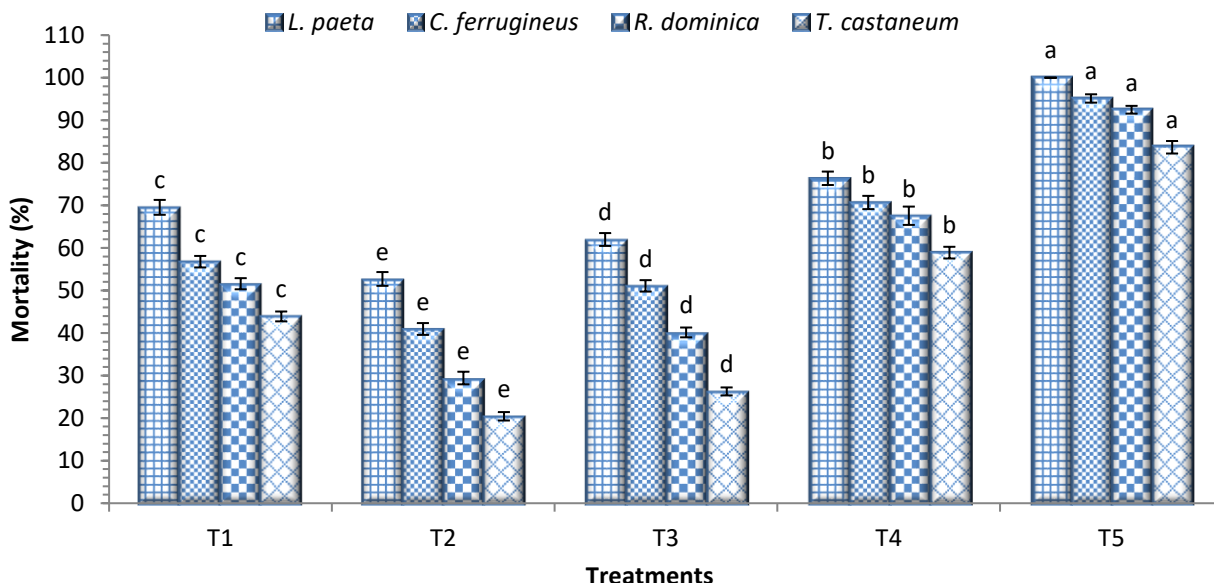


Fig. 3. Mean percent mortality (±SE) of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with Silico-Sec® and *Cordyceps fumosorosea*, either individually or in combination, after 21 days of application. Mortality values (%) followed by the same letters indicate no significant difference based on the Tukey-Kramer (HSD) test at a 1% significance level

Table 2. LT₅₀ and LT₉₀ values for *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with *Cordyceps fumosorosea* alone and in combination with diatomaceous earth. The LT₅₀ and LT₉₀ values are presented with their respective confidence intervals (CIs)

Insect spp.	Treatments	LT ₅₀ (conidia ml ⁻¹) (CI)	LT ₉₀ (conidia ml ⁻¹) (CI)	Slope	Intercept	χ ² (df =1)	P
<i>L. paeta</i>	T ₁	11.59 (10.30-12.70)	34.07 (30.64- 39.08)	0.05	-7.86	1.07	0.78
	T ₂	19.47 (18.15-21.17)	41.62 (37.09-48.28)	0.04	-12.70	1.04	0.83
	T ₃	14.69 (13.59-15.84)	37.21 (33.33-42.89)	0.05	-9.83	2.07	0.15
	T ₄	8.89 (7.423-10.06)	28.93 (26.44-32.41)	0.06	-6.73	2.35	0.12
	T ₅	4.86 (3.85-5.68)	13.37 (12.70-14.917)	0.15	-6.46	3.58	0.06
<i>C. ferrugineus</i>	T ₁	16.59 (15.35-18.06)	41.57 (36.62- 49.15)	0.05	-9.96	3.15	0.07
	T ₂	24.78 (22.45-28.36)	51.61 (44.33- 63.44)	0.04	-12.91	1.40	0.23
	T ₃	19.49 (17.99- 21.52)	45.16 (39.44-54.08)	0.04	-11.16	1.79	0.18
	T ₄	11.47 (10.52-12.32)	28.08 (26.08-30.73)	0.07	-10.35	1.12	0.97
	T ₅	5.73 (4.59- 6.66)	17.28 (16.39-18.36)	0.11	-6.79	1.34	0.55
<i>R. dominica</i>	T ₁	18.95 (17.64-20.66)	42.18 (37.39- 49.35)	0.08	-11.89	2.96	0.13
	T ₂	33.92 (29.15- 42.70)	66.30 (54.07- 89.34)	0.05	-9.66	1.01	0.81
	T ₃	25.20 (22.72- 29.11)	53.16 (45.33- 66.16)	0.04	-12.63	2.99	0.08
	T ₄	13.64 (12.66-14.61)	33.30 (30.35-37.40)	0.06	-10.43	1.29	0.67
	T ₅	5.46 (4.11- 6.54)	18.76 (17.69-20.08)	0.09	-5.80	1.48	0.24
<i>T. castaneum</i>	T ₁	22.74 (21.17-24.87)	42.90 (38.41- 49.39)	0.06	-11.15	1.36	0.24
	T ₂	40.77 (34.01- 54.29)	73.30 (58.39-103.54)	0.03	-12.61	1.24	0.22
	T ₃	36.94 (31.21- 48.08)	70.81 (56.82- 98.50)	0.03	-10.85	1.01	0.45
	T ₄	17.45 (16.41- 18.69)	37.31 (33.80- 42.25)	0.06	-9.81	2.22	0.13
	T ₅	10.68 (9.77-11.49)	25.75 (24.11- 27.85)	0.08	-7.57	1.31	0.21

A significant reduction in progeny emergence was also observed across treatments, with *L. paeta*: $F_{5,53} = 475, p \leq 0.01$; *C. ferrugineus*: $F_{5,53} = 368, p \leq 0.01$; *R. dominica*: $F_{5,53} = 268, p \leq 0.01$; *T. castaneum*: $F_{5,53} = 211, p \leq 0.01$, and control: $F_{5,53} = 268, p \leq 0.01$. Progeny production was significantly higher ($p \leq 0.01$) in T₆ than in T₂, T₃, T₁, T₄, and T₅ for all insect species (Figure 4). The highest number of offspring emerged in *L. paeta* (153.56), followed by *C. ferrugineus* (140.33), *R. dominica* (130.44), and *T. castaneum* (122.11).

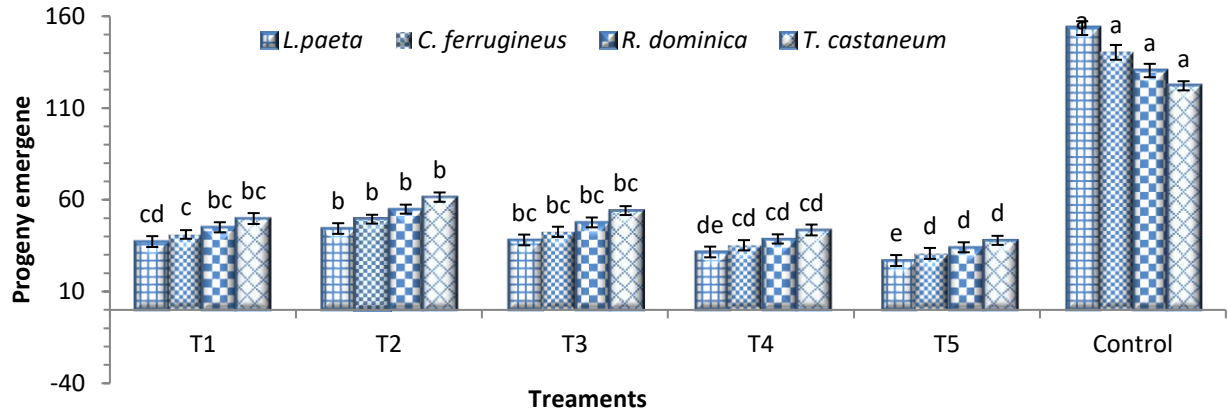


Fig 4. Mean progeny emergence (\pm SE) of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with Silico-Sec[®] and *Cordyceps fumosorosea*, either individually or in combination, after the removal of parental adults (30 days for *L. paeta* and 63 days for the other species). Data followed by the same letters indicate no significant difference based on the Tukey-Kramer (HSD) test at a 1% significance level

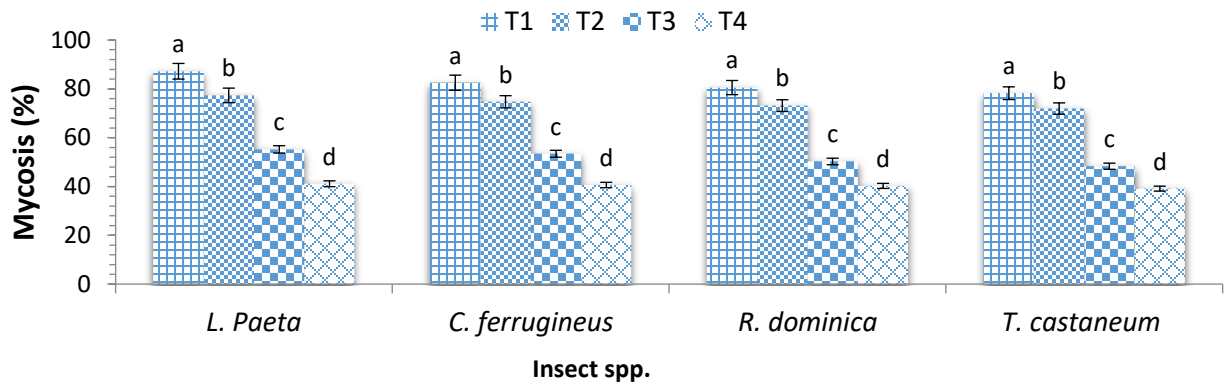


Fig. 5. Mean percentage mycosis (\pm SE) in cadavers of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with *Cordyceps fumosorosea*, either alone or in combination with Silico-Sec[®]. Mycosis percentages followed by the same letters indicate no significant difference based on the Tukey-Kramer (HSD) test at a 1% significance level

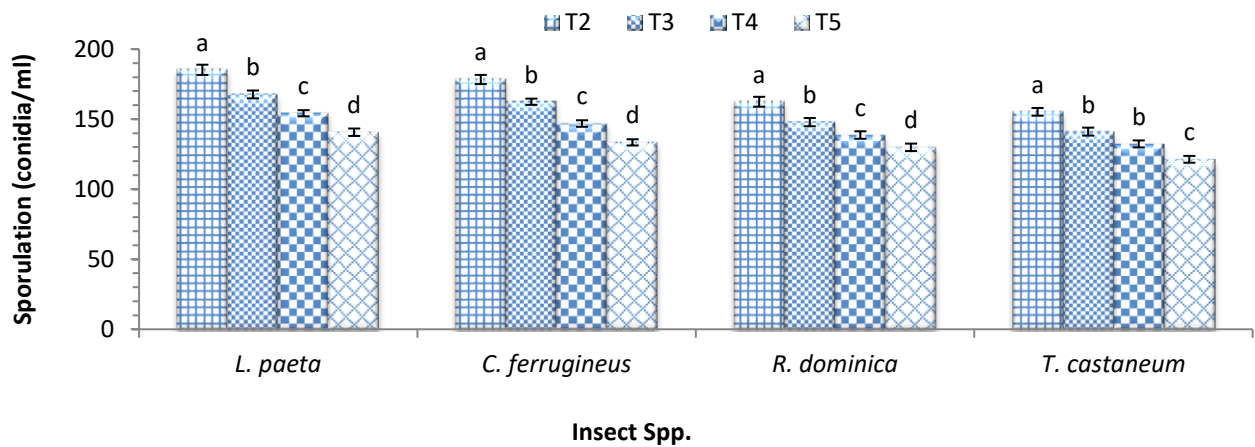


Fig. 6. Mean sporulation (\pm SE) in cadavers of *L. paeta*, *C. ferrugineus*, *R. dominica*, and *T. castaneum* on wheat treated with *Cordyceps fumosorosea*, either alone or in combination with Silico-Sec[®]. Treatments followed by the same letters indicate no significant difference according to the Tukey-Kramer (HSD) test at a 1% significance level

Mycosis and Sporulation: Significant variations in mycosis were observed across all insect species, with *L. paeta* ($F_{3,35} = 54$, $p \leq 0.01$), *C. ferrugineus* ($F_{3,35} = 45$, $p \leq 0.01$), *R. dominica* ($F_{3,35} = 268$, $p \leq 0.01$), and *T. castaneum* ($F_{3,35} = 61$, $p \leq 0.01$) displaying different levels of fungal infection (Figure 5). Among the treatments, the highest incidence of mycosis was observed in T₂, followed by T₃, T₄, and T₅, respectively. The maximum proportion of infected insects was recorded in *L. paeta* (87.21%), followed by *C. ferrugineus* (77.32%), *R. dominica* (55.20%), and *T. castaneum* (41.11%).

Similarly, sporulation varied significantly across all species, with *L. paeta* ($F_{3,35} = 37.7$, $p \leq 0.01$), *C. ferrugineus* ($F_{3,35} = 60.67$, $p \leq 0.01$), *R. dominica* ($F_{3,35} = 65.8$, $p \leq 0.01$), and *T. castaneum* ($F_{3,35} = 53.6$, $p \leq 0.01$) exhibiting notable differences (Figure 6). The highest sporulation was recorded in T₂, followed by T₃, T₄, and T₅. The greatest number of spores was recovered from *L. paeta* (185.22), followed by *C. ferrugineus* (167.67), *R. dominica* (154.22), and *T. castaneum* (140.67).

DISCUSSION

Despite decades of research on stored-product pest management, grain losses remain alarmingly high due to the limitations of conventional control strategies and the adaptive potential of insect pests. Addressing this challenge, the present study evaluated the combined efficacy of EPF and inert dust formulations as practical, integrated solutions for sustainable post-harvest pest management. The results demonstrated a synergistic interaction between Silico-Sec[®] and *C. fumosorosea*, resulting in significantly improved insecticidal efficacy against several economically important stored-product pests. The integration of these bioinsecticidal approaches enhances pest mortality through synergism, achieving greater efficacy as both agents have a novel mode of action that exhibits detrimental effects on the host. Such integration reflects a modern trend in sustainable pest management approaches, emphasizing the synergistic use of biopesticides and auxiliary stressors to strengthen pest suppression, delay resistance evolution, and ensure food safety by reducing chemical residues (Lord, 2001; Shah and Pell, 2003; Choudhary *et al.*, 2021). The individual and combined use of EPFs and DEs has been reported in several studies against storage insect pests (Ashraf *et al.*, 2017; Wakil *et al.*, 2021). Our results revealed that the combined use of Silico-Sec[®] in association with *C. fumosorosea* led to a considerable increase in the mortality of the adult population compared to using them separately. This increased effectiveness can be attributed to the capacity of DE to suppress the host's immune system, which is achieved through abrasion of the cuticle, lipid degradation, and loss of water, ultimately resulting in desiccation (Badii *et al.*, 2014). Moreover, the fluids

exuded help fungal spores adhere to the cuticle more effectively and multiply faster, thereby increasing mortality (Pourian and Alizadeh, 2021).

The enhanced virulence can be further promoted by the presence of nutrients, particularly hydrocarbons, such as alkanes and lipids, which DEs release or display during cuticle abrasion. These compounds are valuable carbon sources that sustain fungal metabolic processes, trigger virulence-related genes, and enhance effective host colonization (Dal Bello *et al.*, 2006). Moreover, the general physiological fitness of the fungal isolates, such as high conidial viability, high germination rates, and successful mycosis, also plays a significant role in the observed mortality increase (Rohrlich *et al.*, 2018). Our findings were corroborated several earlier studies that reported much more insect mortality when DE and EPF were combined than when applied separately (Riasat *et al.*, 2013; Shafiqhi *et al.*, 2014; Storm *et al.*, 2016; Wakil *et al.*, 2020; Hanif *et al.*, 2022; Hassuba *et al.*, 2024).

However, the effectiveness of the combined treatment was inconsistent across species, with *L. paeta* being the most susceptible, most probably because of their soft-bodied structure, thin cuticle, and high surface-area/volume ratio, which allows rapid desiccation and enables fungal infestation. On the other hand, *C. ferrugineus* and *R. dominica* were moderately susceptible to DE because of the small size and mobility or partially covered body parts, but *T. castaneum* was the most tolerant, probably because of a hardened cuticle and low cuticular permeability, which slows DE abrasiveness and fungal infection (Fields and Korunić, 2000; Athanassiou *et al.*, 2016). Similar results were documented by Wakil *et al.* (2021), who demonstrated that both individual and combined applications of DEBBM and *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) resulted in the highest mortality of *L. paeta* compared to other stored-product insect pests. Our results also confirmed other studies that soft-bodied insects exhibit high susceptibility to DEs, and the addition of EPFs further enhances this effect through synergistic interactions (Saeed *et al.*, 2020; Wakil *et al.*, 2021).

In progeny production, the combined application of DE and EPF significantly reduced progeny emergence compared to the untreated control. This decrease probably can be attributed to early adult death, which inhibits mating and oviposition, fungus-induced sterility, and extended mortality effects (Wakil *et al.*, 2020; Ozdemir, 2023). These findings are in line with those of Rizwan *et al.* (2019), who noticed that integrated applications were significantly more effective than single treatments in suppressing *T. castaneum* progeny development at low concentrations of conidial spores, but the relative control in reducing offspring development was relatively less effective. Moreover, the fungus-infested insect cadavers contribute to the secondary conidial spread, which leads to the horizontal

transmission and increases infection pressure over time (Fingu-Mabola *et al.*, 2021). This process helps achieve the persistence and residual activity of EPF over a long term in stored-product conditions, making DE-EPF integration a currently promising solution for the sustainable control of pests (Lord, 2001).

In the current study, mycosis and sporulation were found to be significantly higher when a lower concentration of *C. fumosorosea* was applied independently in all insect species tested. It is likely to be attributed to reduced intra-specific competition among conidia in lower concentrations, thereby increasing successful germination and the colonization of the host (Qayyum *et al.*, 2021; Tefera and Pringle, 2003). Additionally, reducing spore loads can potentially provide the optimum water and gaseous exchange on the insect cuticle, establishing more favorable micro-environmental conditions to support fungal growth (Wakil *et al.*, 2020; Pourian and Alizadeh, 2021). Conversely, lower mycosis and sporulation at increased conidial concentrations could be due to self-inhibition, whereby thick populations of spores suppress each other's development. This reduced sporulation could also be attributed to the desiccation effect of DE in combination treatments, which may influence fungal growth and mycelial development (Dal Bello *et al.*, 2006). Such inhibitory effects are not always life-limiting but may limit the recycling potential of EPFs in the long-term, which is an important source of biological sustainability. *L. paeta* was most successful in supporting mycosis and sporulation rates of the different species tested, possibly due to its permeable cuticle and lower body mass, which both promote internal colonization and external fungal development. Similar results were observed by Adane *et al.* (1996) with *Sitophilus zeamais* Motschulsky, as well as Vandenberg (1992) with *Megachile rotundata* (F.) in which sporulation varied radically with host species and conidial concentration.

Given these findings, the integration of DEs and EPF offers a promising and sustainable approach to stored-product pest management. This combination aligns with IPM principles by minimizing reliance on synthetic chemicals and addressing concerns over pesticide resistance, residues, and regulatory constraints (Zeni *et al.*, 2021; Wakil *et al.*, 2024). DEs cause physical desiccation, while EPF infects and kills insects biologically, offering complementary modes of action that enhance efficacy and reduce the risk of resistance (Vassilakos *et al.*, 2006). Their compatibility with standard grain storage practices and other non-chemical strategies, such as hermetic storage or modified atmospheres, further supports their practical integration into long-term, residue-free protection systems (Korunić, 1998; Lupu *et al.*, 2018; Wakil *et al.*, 2020). Since the experiments were performed in a controlled laboratory environment, the results may not be comparable in

commercial storage due to factors such as moisture in grains, temperature fluctuations, and aeration. Future research should emphasize field validation, efficacy dependence on fluctuation conditions, formulation optimization, and economic feasibility. Furthermore, it is vital to determine compatibility with other non-chemical approaches, including controlled atmospheres, hermetic storage, and botanical insecticides, to enhance integrated pest management approaches.

Eventually, this method is effective and sustainable in controlling stored-grain pests. This dual approach would be especially effective when used in conjunction with hermetic and modified-atmosphere storage systems to support pest control and enhance grain quality. For optimal results, treatments must be applied immediately after harvest, and moisture in the grain must not exceed 13% to encourage fungal growth. Although the startup expenses of EPFs can be higher, long-term benefits, such as reduced pesticide use, lower risk of resistance, and improved grain quality, are beneficial in terms of their cost-benefit analysis. Implementation of such an integrated approach can offer a convenient and environmentally friendly pest management control program across diverse storage environments.

Conclusion: The study indicated that the integrated application of *C. fumosorosea* and Silico-Sec[®] can significantly suppress populations of psocids and beetles. In stored products, this approach is promising for long-term storage protection. The distinct modes of action of these agents enhance their potential as effective biorational components in the Integrated Stored Grain Protection (ISGP) plan, supporting the combined use of insecticides for durable commodities. Further research is required to integrate low-risk biotic factors, such as commodity type and target species, along with abiotic factors like relative humidity, temperature, and physical modifications of storage facilities. This integration could facilitate the effective application of DEs and EPF at lower dosages within ISGP strategies while also mitigating resistance development to grain protectants.

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