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Original Research

Insecticidal and Antimicrobial Effect of Entomopathogenic Fungi in Stored Wheat

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Abstract

In developing countries, the loss of food grain is about 10–20% of their total yield due to poor storage infrastructure. During storage under such unproductive storage conditions, approximately 600 species of insects deteriorate the stored grains, and 100 of those species cause financial damage to the grains. In addition to directly causing weight loss through feeding damage, insects also drastically reduce nutrients, lower the proportion of seeds that germinate, lower the grade, and decrease the market value of the crop as a result of waste accumulation, webbing, and insect dead bodies. The use of antiquated and conventional chemical fumigants to control stored grain insect pests has been a prevalent practice for a long time, which results in environmental contamination, residual toxicity, and resurgence. Due to high pathogenicity and minimal human toxicity, entomopathogenic fungi may be a viable option over traditional techniques for managing stored grain insect pests and can be treated as a novel solution for protecting the stored grains. Under consideration of these facts, the goal of the current study was to examine how three distinct entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii*) affected wheat grain insects (*Sitophilus oryzae*, *Rizopertha dominica,* and *Tribolium castaneum*) that were kept in storage.

Keywords: entomopathogenic fungi, stored grain, insects pests, toxicity, germination

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Introduction

Each year, enormous amounts of globally stored human food resources are spoiled due to the infestation of several insect pests, such as *S. oryzae*, *R. dominica*, and *T. castaneum* insects, in wheat during storage. In addition to primary agricultural output, for human food security, agricultural commodities and food items must be distributed and stored in an appropriate manner after harvest. To find alternatives to conventional chemical fumigants, a range of plant-based essential oils were assessed for their ability to inhibit insect pests from stored goods and for their fumigant toxicity, contact toxicity, ovicidal impact, and mortality [1-4].

Approximately 600 kinds of insects are linked to stored grain, and 100 of those species cause grain losses financially. In addition to directly causing weight loss through feeding damage, insects also drastically reduce nutrients, lower the proportion of seeds that germinate, lower grade, and decrease the value of the products they produce owing to waste buildup, webbing, and insect cadavers [5].

The rice weevil, or *Sitophilus oryzae*, is a member of the order Coleoptera's family Curculionidae. The damage caused by *Sitophilus oryzae* adults and grubs to grains of wheat, rice, maize, etc. The grubs are squishy, legless, white, and have a head that is yellowish brown. They are curled and stay inside the grain. Weevil adults are small, reddish brown to chocolate in color, with four yellow spots on their elytra and nose. Adults drilled circular holes in the damaged grain, deforming the kernels into powder, and, in cases of severe infestation, "dry heating" the grain.

The use of antiquated, conventional chemical fumigants to control stored grain insect pests is a typical management strategy. However, this approach might lead to contamination of the environment, residual toxicity, resurgence, and resistance. The great pathogenicity of entomopathogenic fungi makes them a viable option for searching for novel ways to manage stored grain insect pests. Common microorganisms with little toxicity to mammals and safety for the environment are known as entomopathogenic fungi and are found all over nature. When kept in storage, they can spread quickly and release continuously.

Among biocontrol pathogens, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Lecanicillium lecanii* are the most significant due to their extensive host range of insects. One type of pathogen that creates sickness symptoms and infects insects fatally is called an entomopathogenic fungus (EPF). While infection hyphae directly pierce the host's exoskeleton or cuticle to infect insects through touch, fungal conidia adhere to the cuticle of the host and germinate [6-9]. *Beauveria bassiana, Metarhizium anisopliae,* and *Lecanicillium lecanii* are a few naturally occurring entomopathogenic funguses that are among the more than 700 species of EPFs and have a wide host range [10, 11]. Because they are prevalent in nature, they are safe alternatives to non-target creatures, good for the environment, and have little toxicity to mammals [12, 13].

The naturally occurring soil-borne fungus *Beauvaria bassiana* and *Metarhizium anisopolae* infects pests like grasshoppers, beetles, and moths, absorbing their energy and using it as a supply of nitrogen and carbon [14]. Several studies have shown that these entomopathogenic fungi can independently control the amount of grain insects that are stored [15-18].

Therefore, the current study was conducted to evaluate the effectiveness of three different entomopathogenic fungi, namely *B. bassiana, M. anisopolae*, and *L. lecanii*, against the aforementioned insects and pests in wheat during storage. This was done in light of the facts that the economic harm caused by these storage insects to the stored grains and entomopathogenic fungi's ability to combat these pests. Additionally, the effect of entomopathogenic fungi on the quality of wheat seed germination was examined at specific intervals throughout storage and contrasted with the control sample that was left untreated.

Experimental

Culture of Test Insects

The Entomological Laboratory at Veer Kunwar Singh College of Agriculture, Dumraon, Buxar, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India, was used as a site for testing the culture of the insects. Inoculation temperature and RH (relative humidity) for the pure cultures of *S. oryzae, R. dominica*, and *T. castaneum* were 27±1°C and 70±5%, respectively. For raising, a 1000 ml plastic jar was used. To promote air exchange within the jar, a copper wire net with a mesh size of 30 was placed over a hole that measured 1.8 cm in diameter and was cut out of the plastic jar lid. *T. castaneum* was raised on wheat flour (95%), yeast powder (5%), and wheat kernels (variety HD-2967). *S. oryzae* and *R. dominica* were raised on wheat kernels. Plastic jars containing the proper feed were filled, and 100 adults of mixed sex were released into each jar before being stored in the incubator. First-generation adults (0–7 days old) were used for all the tests.

Preparation of Grain

Every experiment was conducted using fresh wheat seed variety HD-2967. The grains were dried for a whole day at 60 °C in a hot air oven before being used. After that, the grain's moisture content was tested, and the required amount of water was added to raise it to 13.5 percent. To guarantee that the moisture was distributed evenly, the grain samples were spread out on laboratory slabs, and the necessary amount of water was sprayed over them with a hand sprayer. After thoroughly mixing the grain, it was kept for a week in a polythene bag to achieve the proper equilibrium moisture content.

To carry out additional tests, the 100g of wheat was subsequently put into polycarbonate vials with a 200 ml capacity.

Acquisition of Entomopathogenic Fungi

The nominated entomopathogenic fungi were purchased for the purpose of the experiments. A commercially prepared product containing CFU 6×10^9 of every entomopathogenic fungus was utilized.

In the BOD incubator, a pure culture of test insects was created at a temperature of 27±1°C and at a relative humidity (RH) of 70±5%. For raising, one kilogram capacity plastic jars were utilized. To help with aeration in the jar, a 30 mesh copper wire net was placed over a 1.8 cm diameter hole that had been cut out of the lid. While *T. castaneum* was raised on wheat flour, adults of *S. oryzae* and *R. dominica* were raised on the grains of the wheat variety HD-2967. The grain was disinfested in a hot air oven for 12 hours at 60°C prior to use. Following infestations, the grain's moisture content was assessed and increased to 13.5% through the addition of water using the Pixton method [19]. *S. oryzae*, *R. dominica*, and *T. castaneum* were used in the studies to verify the effectiveness of entomopathogenic fungi in controlled settings with a relative humidity of 70% and a temperature of 27±1°C. Each plastic vial contained fifty grams of wheat grains of the HD-2967 variety with a moisture content of 13.5%. Each vial contained twenty adult *S. oryzae* (0–7 days old). Each vial was filled with the necessary amount of the prepared product, following which the insects were released for 24 hours. The vial screw caps were then firmly shut. After that, the insects were given a month to feed and procreate. Counting began with the appearance of the first generation of insects and continued until the final insect emerged or the grain went bad.

To investigate the impact of various entomopathogenic fungi on wheat germination, samples from several tests were gathered. The test for germination was carried out by using Chalam et al.'s methodology [20]. The calculation of the vigor index (seedling vigor) followed Abdul-Baki and Anderson's recommendations [21]. The method outlined by Fahad et al. [22] was used to compute the vigor index, germination percentage, and viability significance.

Statistical Analysis

The experiments were carried out by adopting a completely randomized design (CRD), and STPR 3 software was used to analyze the experimental data.

Results

The inhibitory impact of all the selected entomopathogenic fungi was assessed at doses of 1, 3, 5, and 7 g/Kg of seed against four stored grain pests. The first-generation progeny's observations in the preliminary and confirmatory tests were compared with the untreated control samples.

Inhibitory Effect of Entomopathogenic Fungi Against Sitophilus Oryzae in Stored Wheat

Figs. 1 and 2, respectively, show the average percent inhibition of different entomopathogenic fungi against *Sitophilus oryzae* as determined by preliminary and confirmatory tests in treated wheat after six and twelve months of storage. The inhibition percentage in the preliminary test is determined to be between 28.7% and 87%. At a dosage of 3 grams per kilogram of seed, *L. lecanii* demonstrated a maximum inhibition of 87.0%. The lowest percentage of inhibition, however, is 28.7% for *B. bassiana* at 1.0 g/Kg dose of seed. For *M. anisopliae*, at 3.0 g and 7.0 g/kg doses, respectively, the percentage inhibition varies from 47.2% to 65.8%. The percentile range for inhibition in a confirmatory test is 42% to 100%. M. anisopoliae, B. bassiana, and L. lecanii all reached the maximum 100% inhibition at higher dosages, i.e., 5.0 g and 7.0 g per kg. In comparison to the controlled sample, 42.0% inhibition is caused by *B. bassiana* at a dose of 1 g/kg.

Rhyzopertha Dominica is Inhibited by Entomopathagenic Fungi in Stored Wheat

After six and twelve months of storage, respectively, treated wheat is subjected to preliminary and confirmatory tests to determine the percent inhibition of several entomopathogenic fungi against *Rhyzopertha dominica*. The results are displayed in Figs. 3 and 4. In a preliminary test, the use of *B. bassiana* and *M. anisopliae* at doses of 5 g/Kg and 7 g/Kg of seed, respectively, demonstrated 100% inhibition when compared to the untreated control. As compared to the untreated control, the confirmatory test showed complete 100% suppression for *M. anisopoliae* at 3.0, 5.0, and 7.0 g/kg dosages and at 1.0, 3.0, and 5.0 g/Kg dosages of *B. bassiana*.

Entomopathogenic Fungi's Inhibitory Effect on Tribolium Castaneum in Stored Wheat

Figs. 5 and 6 depict the outcome of applying varying concentrations of entomopathogenic fungi against *Tribolium castaneum*, as measured by percent inhibition through preliminary and confirmatory tests conducted on treated wheat after six and twelve months of storage, respectively. In the initial test, doses of *M. anisopoliae* (5.0 and 7.0 g), *B. bassiana* (3.0, 5.0, and 7.0 g), and *L. lecanii* (5.0 and 7.0 g/Kg of seed) showed 100% inhibition in comparison to that of untreated control, which showed no inhibition at all. Comparing the confirmatory test results to the untreated control, 100% inhibition was observed at 3.0 and 7.0 g of *M. anisopoliae* and 5.0 and 7.0 g of *B. bassiana* per kilogram of wheat seed.

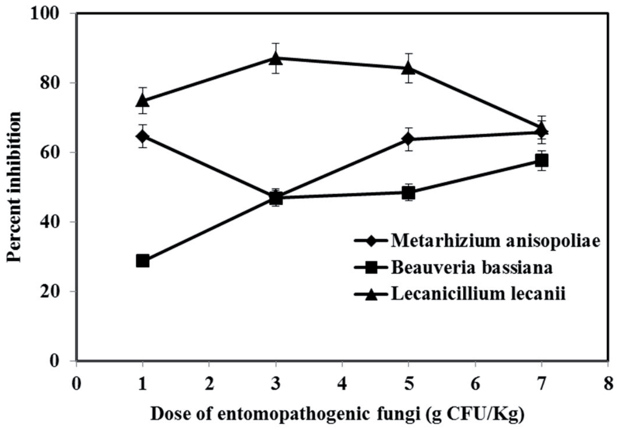

Fig. 1. Effect of various doses of entomopathogenic fungi on percent inhibition of Sitophilus oryzae after six months of wheat storage.

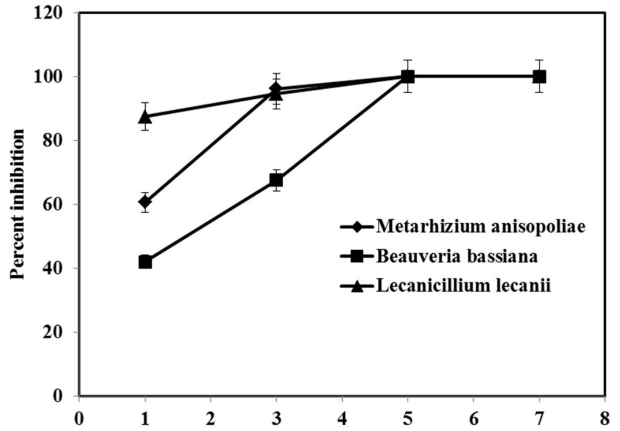

Fig. 2. Effect of various doses of entomopathogenic fungi on percent inhibition of Sitophilus oryzae after twelve months of wheat storage.

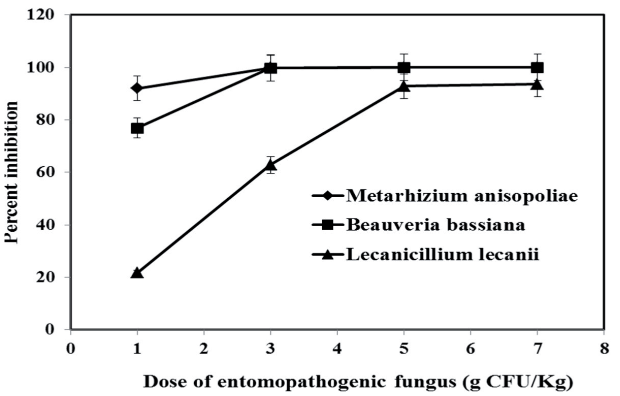

Fig. 3. Effect of various doses of entomopathogenic fungi on percent inhibition of Rhyzopertha dominica after six months of wheat storage.

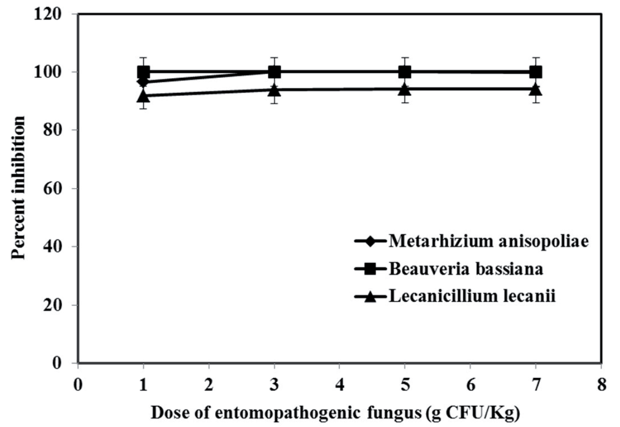

Fig. 4. Effect of various doses of entomopathogenic fungi on percent inhibition of Rhyzopertha dominica after twelve months of wheat storage.

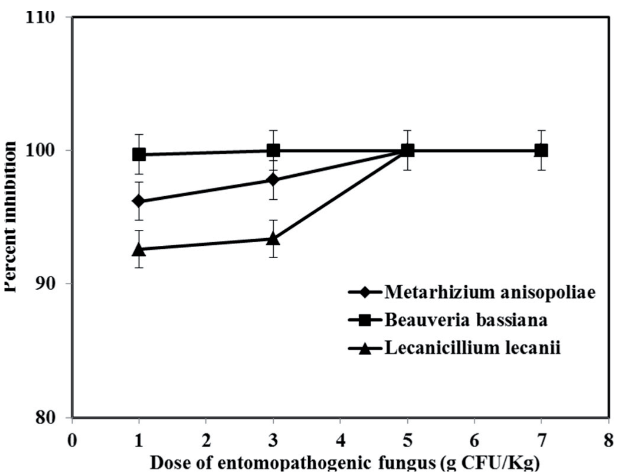

Fig. 5. Effect of various doses of entomopathogenic fungi on percent inhibition of Tribolium castaneum after six months of wheat storage.

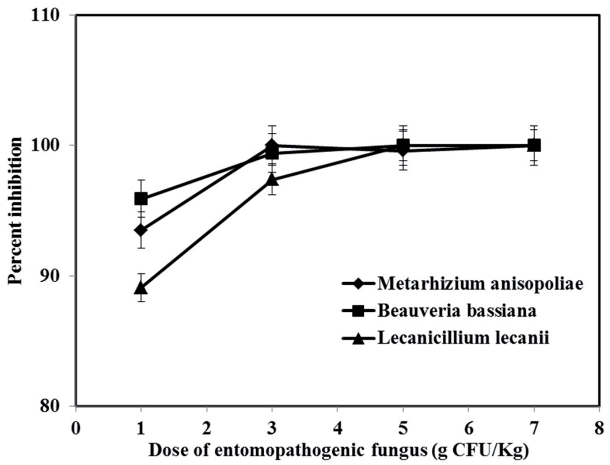

Fig. 6. Effect of various doses of entomopathogenic fungi on percent inhibition of Tribolium castaneum after twelve months of wheat storage.

Impact of Entomopathogenic Fungi Treatment on the Viability of Wheat Seed: Its Significance, Vigor Index, and Germination Percentage

Table 1 displays the results of various entomopathogenic fungal doses on germination percentage, vitality index, and the importance of wheat seed viability following a twelve months storage period. After twelve months of storage, it was discovered that wheat seed treated with all fungi had a higher proportion of germination (86.00%) than the untreated control. The wheat treated with *B. bassiana* exhibited the maximum germination percentage (97.64) at a dose of 7.0 g/Kg of seed. At a dose of 1 g/Kg of seed, wheat treated with *B. bassiana* had the lowest germination percentage (94.66). *M. anisopliae* had the lowest vigor index, whereas *B. bassiana* and *L. lecanii* showed the highest vigor index. Every treated fungal sample had viability values greater than 1, indicating a higher percentage of seed germination compared to the control group.

Discussion

The following headings provide a description of the discussion surrounding the experimental results:

Assessment of Entomopathogenic Fungi's Inhibition Effect Against Various Stored Grain Insect Pests

At dosages of 5 and 7 g/kg of grain, *Metarhizium anisopoliae*, *Beauveria bassiana*, and *Lecanicillium lecanii* all showed 100% suppression of *Sitophilus oryzae* in the examined samples. 100% inhibition of *Rhyzopertha dominica* was observed at each dosage of *M. anisopolae* and *B. bassiana*. However, *T. castaneum* and *C. chinensis* indicated complete reticence of all three tested fungi at high levels of dosages (5.0 g and 7.0 g/Kg).

The results of Rice et al. [16], who, after 21 days and treating the seed at high dosages of the *B. bassiana* isolates, demonstrated 80–100% mortality of *insects*, confirming the current outcomes. Compliant with our findings, Iqbal et al. [23] employed *B. bassiana* and *M. anisopliae* as biocontrol agents and observed a maximum 100% mortality rate against *Callosobruchus chinensis* at 1×10^8 spore/ml after 7 and 7.3 hours, respectively. Khan and Khan [24] also evaluated the effect of different entomopathogenic fungi that have the ability to control three major stored insect pests in a lab setting. They assessed the effects of four doses of each fungal isolate 1×10^4, 1×10^6, 1×10^8, and 1×10^{10} conidia/kg of wheat grains on adult mortality and progeny destruction in the next generation. The findings showed that the mortality of insects depended on both dose and time. Out of all the investigated insect species, *M. flavoviride* typically resulted in the highest mortality and progeny suppression. A. nidulans produced minimum mortality and progeny suppression, followed by *A. fumigatus* and *P. citrinum*. According to Pourian and Alizadeh [25], there was a notable decrease in the offspring production of treated *O. surinamensis* on wheat seeds and treated *C. maculatus* on cowpeas. According to their research, co-applications of the two

Table 1. Percentage of germination, vigor index, and viability of wheat seeds treated with different fungal dosages.

Names of Entomopathogenic fungi	Fungal dose (g/kg) CFU(6×10^9)	Germination Percentage	Vigour index	Significance of viability
M. anisopoliae	1.0	96.66	17271.33	1.1
	3.0	95.33	16554.00	1.1
	5.0	96.66	17813.33	1.1
	7.0	97.33	17838.00	1.1
B. bassiana	1.0	94.66	17866.67	1.1
	3.0	96.66	18041.33	1.1
	5.0	97.56	18043.75	1.1
	7.0	97.64	17271.88	1.1
L. lecanii	1.0	95.35	17838.00	1.1
	3.0	96.57	17866.67	1.1
	5.0	96.61	18041.33	1.1
	7.0	96.84	18043.75	1.1
Untreated Control		86.00	10882.70	1.1
S. Em±		1.37	496.9	
CD (p=0.05)		3.84	1457.76	

compounds were possible because of the low-lethal dose of Diatomeous Earth's synergistic enhancement of *B. bassiana* virulence in the varied mixes.

Effect on Percent Inhibition of Wheat Seed Treated with Entomopathogenic Fungi

The fatality rates of *S. oryzae* after 120 hours were found to be 90, 93.3, and 86.9% for *M. anisopliae*, *B. bassiana,* and *L. lecanii*, respectively, at high doses (7.0 g/Kg) of wheat seed. After 120 hours of treatment, the mortality rates for *R. dominica* against *M. anisopliae*, *B. bassiana*, and *L. lecanii* at high application rates (7.0 g/Kg of seed) were 90, 93.3, and 93.3%. Following 120 hours of treatment, the *Tribolium castaneum* showed 86.7%, 96.7%, and 96.7% of deaths at 7 g/Kg of grain containing *M. anisopliae, B. bassiana*, and *L. lecanii*. After seven days of treatment, Yanar et al. [26] demonstrated 94% mortality of *Sitophilus* against the *B.* isolates at a concentration of 1×10^9 conidia/ml, confirming the current findings. The aforementioned outcomes are consistent with the research conducted by Javid et al. [27], which revealed 90% mortality at a concentration of 1×10^8 spores/ml of *M. anisopliae* over a 72-hour treatment period.

According to Ak's study [28], *M. anisopoliae* (85.68%) demonstrated the highest effect when five different entomopathogenic fungi were subjected to *S. oryzae* and *B. bassiana at* 20°C and 25°C, respectively. *M. anisopliae* outperformed *B. bassiana* in the Abdel et al. [5] study against *S. oryzae* and *R. dominica*. For *B. bassiana*, the LC50 value was 1.2 ×10 conidia/g, while for *R. dominica* and *S. oryzae*, it was 1.6 x 10 conidia/g. It was discovered that *M. anisopliae* had LC50 values of 2.78x10 conidia/g and 1.34x10 conidia/g against *S. oryzae* and *R. dominica*, respectively. Hassuba et al. [29] documented the effects of applying varying doses (2.0 × 106, 2.0 × 107, 2.0 × 108, and 2.0 × 109 spores/kg) of *Metarhizium anisopliae* and four species of Trichoderma genius (*T. harzianum, T. citrinoviride, T. viride, and T. asperellum*) on the production of progeny and larval mortality of Trogoderma granarium Everts of wheat grain.

Impact of Entomopathogenic Fungi on the Vigor Index and Seed Germination

Following a 4-month period of storage, the highest percentage of germination was observed when the wheat seed was exposed to *B. bassiana* at high doses (5 and 7 g/Kg of seed), whereas the seed treated with the same fungus at a concentration of 1.0 g/Kg showed the lowest percentage. The seeds treated with *B. bassiana* and *L. lecanii* had the highest vigor index. However, the seeds that were exposed to *M. anisopolae* had the lowest value. The germination percentage and vigor index were found to be lower for the untreated control group. Iqbal et al. [30] provided support for the current findings, stating that after six months of storage, seeds treated with entomopathogenic fungi exhibited over 80% germination, compared to only 20% in control seeds. Similar findings were reported by Abotaleb *et al*. [31], who claimed the germination tests showed no effect from the wheat samples treated with B. bassiana and diatomaceous earth. Theertha et al.'s [32] treatment of cowpea with Metarhizium anisopliae (CFU: 1.0×108) at a dose of 20 g/kg seed + Diatomaceous earth at a dose of 5 g/kg seeds produced similar results.

Conclusions

Based on the findings of the experiment, it was concluded that at higher concentrations, or 5 and 7 g/Kg of grain, *M. anisopoliae*, *B. bassiana*, and *L. lecanii* were the best fungi to inhibit stored grain insects (*S. oryzae, R. dominica*, and *T. castaneum*). These insects were not completely destroyed by the fungus, not even after a full day of exposure. When it came to fungal infections, *R. Dominica* was more prone to *S. oryzae* and *T. castaneum*. With increasing doses, the fungus becomes more effective against these test insects. The viability or germination of seeds was not negatively impacted by the administration of these entomopathogenic funguses. These entomopathogenic fungi have the power to destroy insects and increase the storage life of grain with no sign of microbial growth. This entomopathogenic fungus could offer a safer option than the current dangerous chemical fumigants. These fungi showed no *S. oryzae* adults emerged at higher application levels of 5.0g and 7.0g/Kg of grain. Additionally, with a significance of viability 1.1, about 94% of the wheat samples treated with entomopathogenic fungus germinated.

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Conflict of Interest

The authors declare no conflict of interest.

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