A laboratory-based study on interspecific interactions between two stored-grain insect pests

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Abstract. An attempt has been made to study the interspecific interaction between two stored-grain insect pests. In the laboratory, *Sitophilus oryzae* (Linnaeus, 1763) and *Tribolium castaneum* (Herbst, 1797) were allowed to rear in the same culture box containing healthy wheat grains. *S. oryzae* with a rostrum feeds on whole grains, whereas T. castaneum without a rostrum feeds on grain powder and often enters grooves inside the grains that *S. oryzae* had previously attacked. Multiple culture boxes were maintained for observation in the biological oxygen demand incubator at an optimal temperature range of 28 ± 1°C for 2 months, until the wheat grains were ground into powder. *T. castaneum* was predominantly detected in the grain powder, while *S. oryzae* was mostly found to leave the culture. Subsequently, T-tube experiments with this powder or with whole wheat grains and both insect pest species were carried out, wherein *S. oryzae* was observed to avoid the powder, whereas *T. castaneum* was attracted to it. Additionally, grain powder underwent microbiological examination using the carbol fuchsin method. This study suggests that controlling *S. oryzae* automatically reduces T. castaneum, a secondary pest that feeds on food damaged or broken by the former.

Keywords: stored grain; *Sitophilus*; *Tribolium*; interspecific competition, microbial study

1. Introduction

Attaining food security to meet the demands of the ever-increasing population of today's Earth is a matter of utmost priority for every nation (Majumder, 2023). Approximately 17% of global food production is lost during storage, with insects accounting for 10% and mites, rodents, and diseases for 7%, as per the Food and Agriculture Organization of the United Nations (FAO) (Bouchelos et al., 2018). This constitute a significant issue pertaining to quality assurance (Kumar, 2017). A total of 1,663 insect species have been identified as pests of stored food items, with several species recognized for their increased damage potential and widespread distribution globally (Binseena et al., 2018). An estimated 14 million tons of storage losses occur in India each year, valued at INR 7,000 crore, of which over INR 1,300 crores are attributable to insects (Soujanya et al., 2012). Proper understanding of insect pests' movement and behavior in stored grain bulks aids in the control of infestations (Anukiruthika et al., 2021). The study is focused on the two worldwide distributed pests *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae). *S. oryzae*, popularly known as rice weevil, is considered a major insect pest all over the world considering the damages that it causes both quantitatively and qualitatively in fields, stored grains like rice, wheat, maize, barley, and sorghum, and grain products (Parisot et al., 2021). As they complete their whole development inside the grains, they are known as internal or primary feeders or primary pests (Trematerra et al., 2015; Đukić et al., 2018). On the other hand, *T. castaneum*, commonly known as red flour beetle, is found all over the world as a pest affecting stored grain and grain products like wheat, flour, rice bran, etc. (Kumar et al., 2018). It is a common experimental model organism in several studies as it is easy to handle, has a simple way of maintaining culture, and has a relatively short generation time (Hunt et al., 2007; Deb & Kumar, 2021). *S. oryzae* possesses anteriorly directed long, slender, and stout down curved rostrum adapted for boring into stored grains in addition to mandibular jaws, whereas *T. castaneum* lacks a characteristic rostrum. They prefer to develop outside the grains in damaged grain powder or grain materials. So, *T. castaneum* is known as external or secondary feeders or secondary pests (Trematerra et al., 2015; Đukić et al., 2018). Interspecific interactions in stored insect pests have been documented in a few studies. *Oryzaephilus surinamensis, T. castaneum* and *T. confusum* prefer insect-damaged kernels more than mechanically split or whole kernels (Trematerra et al., 2000). Kernel age and who colonizes first influence interactions between *S. zeamais* and *T. castaneum* (Trematerra et al., 2015). Excavated grains by *S. granarius* support the development of *Cryptolestes ferrugineus* and *T. castaneum* (Vendl et al., 2022). *Prostephanus truncatus* could outcompete *S. oryzae* (Baliota et al., 2022) and *S. zeamais* on stored maize (Mlambo et al., 2026). Competition for progeny production between *S. oryzae* and *S. granarius* depends on the temperature and the nature of stored products (Sakka et al., 2023). Facultative or antagonistic interactions can occur when insect pests coexist (Mlambo et al., 2026). The simultaneous presence of two insect species that consume the same product is likely to cause them to alter some of their key behavioral responses. However, there hasn't been as much research done on the factors that play a critical role in these interspecific interactions and their practical implications. Therefore, an attempt has been made to study the interspecific interactions between two stored-grain insect pests, *S. oryzae* and *T. castaneum*, that share a common food source and to identify major contributors to these interactions as well as to highlight the practical significance of the current study.

2. Materials and methods

2.1.Test organisms and their culture

Fifty adult insects, each of *S. oryzae* and *T. castaneum*, were reared together in multiple culture boxes containing 500 g of healthy wheat grains without any prior exposure to pesticides or pathogens inside a biological oxygen demand (BOD) incubator (LI-BOD-9) at a temperature of 28 ± 0.5°C for two months until the wheat grains were changed into powder due to insect activity. *T. castaneum* was predominantly detected in the grain powders throughout this period, while *S. oryzae* was first contained within whole grains but became scattered later and was finally found to have left most of the culture. In a continuous culture, *S. oryzae* emerged as the pioneer species, with *T. castaneum* as the climax species.

2.2. Study design

The entire design of the present study has been displayed in **Figure 1.**

2.2.1. Behavioral study

The behavioral response of both insect species to the whole wheat grains and insect-infested wheat grain powder collected from culture boxes was studied by using a T-tube apparatus. Each T-tube apparatus has a 45 cm vertical arm and two 28 cm horizontal arms. Each arm has an internal diameter of 3 cm. The openings of the two horizontal arms were plugged with dry cotton. The T-tube was laid horizontally on a table and was left undisturbed during the study for five hours. The photographs of the test organisms, T-tube apparatus, and old, moist, grayish, and insect-infested grain powder are shown in **Figure 2**. For each test organism, two separate tests were performed. For each test, including the control (without any wheat grain powder or whole wheat grains), three replicates were run in parallel. The choice of both insect species was recorded, and the data was analyzed statistically through t-test.

2.2.2. Microbiological study

Initially, the wheat grain powder of the culture boxes, when examined under the microscope revealed no significant finding. Later, two microbiological studies were performed. In one study, wheat grain powder was applied to sterilized cotton and placed on a petri dish, with a small amount of distilled water added to it. The petri dish was then covered with a lid and placed in an incubator for one week of observation. Cotton showed no signs of fungal rust. In another study, when the gram powder was added with deionized water and kept for a day in an incubator, followed by carbol fuchsin staining of the heat dried smears of the suspension, *Bacillus* species were observed in large numbers under a powerful microscope. Further study will be needed to identify the *Bacilli* species growing on moist gram powder in near future.

3. **Results and Discussion**

In the present study, *S*. *oryzae* strongly avoided wheat grain powder of the culture boxes, On the other hand, *T*. *castaneum* was attracted in large numbers to the wheat grain powder kept in one of the horizontal arm of the T-tube **(Figure 3)**. In control, insects passed at random in variable numbers towards the horizontal arm. The powder of wheat grains was infested by *T*. *castaneum*, which caused a persistent and unpleasant odor and turning the powder grayish, which impaired the grain powder's elastic and viscous qualities. This was attributable to the accumulation of quinones released by *T. castaneum* adults and absorbed by the grain powder (Kumari et al., 2011). That's why *S*. *oryzae* avoided infested moist grain powder. Phillips et al. (1993) reported that *S. oryzae* colonizes sound grain and is attracted to volatiles characteristic of fresh grain, while *T. castaneum* utilizes damaged or deteriorated grains and responds best to oils characteristic of damaged or fungus-infested grain. Volatile organic compounds (VOC) in stored grain exert a significant impact on both intra- and interspecific interactions among coleopteran species associated with stored products. *T. confusum* exhibited a positive chemotactic response towards rice that had been infested by the larvae of a primary pest, *S. zeamais*, while demonstrating no such attraction towards grains solely compromised by adult pests (Giunti et al., 2018). Interspecific interaction between or among species sharing the same food resource is often regulated by population density, based on their relative life history strategies. Temperature and relative humidity can influence the population dynamics of insects associated with stored products and the extent of damage to stored-products (Papanikolaou et al., 2018). Moisture in the microenvironment is a major physical factor that influences the population density. That's another reason behind the escape of *S*. *oryzae* from the grain powder; it is not at all suitable for them, as intact whole grains are needed to pass their egg, larva, and pupal stage. Adults *S*. *oryzae* bore intact whole grains with their rostrum and mandibles and laid eggs inside them. On the other hand, *T*. *castaneum* depends on the grains broken by primary pests as they lack rostrum. Such a succession feeding habit allows for the temporal coexistence of both primary and secondary pest species, subsequently influencing community composition, resource accessibility, and frequently resulting in synergistic damage to stored grain (Mlambo et al., 2026). However, it was found that moist grain powder infested by insects favored the growth of certain Bacilli. *T. castaneum* may be attracted to moist grain powder for devouring on such powder that may be decomposed by Bacilli bacteria into simpler and easily digestable compounds. A similar observation was recorded in another study (Trematerra et al., 2000), which reported that whole wheat kernals damaged or broken by primary colonisers like the lesser grain borer, *Rhyzopertha dominica* (Fabricius, 1792) and rice weevil, *S. oryzae*, are preferred by secondary colonisers like the sawtoothed grain beetle, *O. surinamensis* (Linnaeus, 1758), red flour beetle (*T. castaneum*), and confused flour beetle, *T. confusum* (Jaqcquelin du Val, 1868). Secondary colonisers are able to identify changes in the physico-chemical properties of wheat kernel caused by primary colonisers by the release of certain semiochemicals (Trematerra et al., 2000). If phagostimulatory responses are a concern, *Tribolium* spp. is more self-dependent than *Sitophilus* spp. as they have wider food preferences, even processed commodities as well, whereas, *Situphilus* spp. strict on intact grains (Aitken, 1975). Odor of infested or uninfested whole wheat grains and coarse wheat meal, etc. may influence the behavior of insect pests like *Sitophilus* spp. and *Tribolium* spp (Trematerra et al., 2015). Grain volatiles can act as important cues (Phillips et al., 1993; Parisot et al., 2021). The activity of *Tribolium* spp may favor fungal growth in maize flour (Duarte et al., 2021). Another study by Kumari et al. (2011) identified gram positive bacteria, mostly Bacilli and Cocci, and a few fungi capable of producing mycotoxin in *T. castaneum*. The prolonged activity of the *T. castaneum* population and their interactions resulted in increased moisture in the environment, which boosted microbial activity. Bacterial isolates from the gut of

S. oryzae (*Bacillus subtilis*, *Bacillus oceanisediminis* , *Bacillus firmus*, and *Pseudomonas aeruginosa*) can generate volatile organic compounds with antifungal properties that inhibit the growth of *Aspergillus flavus* and break down aflatoxin B1 (Al-Saadi et al., 2024). Further study will be needed to explain how food exploitation pattern of one species repels or attracts other species and it has opened an area of future research.

4. Conclusions

The study concludes that controlling *S. oryzae* automatically minimizes *T. castaneum*, a secondary pest that depends on food damaged or broken by the former to some extent. Mouthparts, food exploitation patterns of interacting insect pest species and the microbes operating there have an impact on the interspecific interactions between two stored-grain insect pests using the same food source: *S. oryzae* and *T. castaneum*. Studying the interspecific interactions between stored grain pests provides valuable insights that will influence pest management strategies and promote sustainable grain storage and production practices. Therefore, in order to ensure the safety and quality of stored grains, effective monitoring and an understanding of the ecology and behaviors of pests affecting stored grains have become essential components of integrated pest management.

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Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

The author declares his consent for publication

Availability of data and material

Data may be shared on valid request.

Competing interests

There is no conflict of interest.

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Authors' Contribution

The author is solely responsible for conceptualization, experiment designing, performing, formal analysis, writing-reviewing-editing and finalization of the manuscript.

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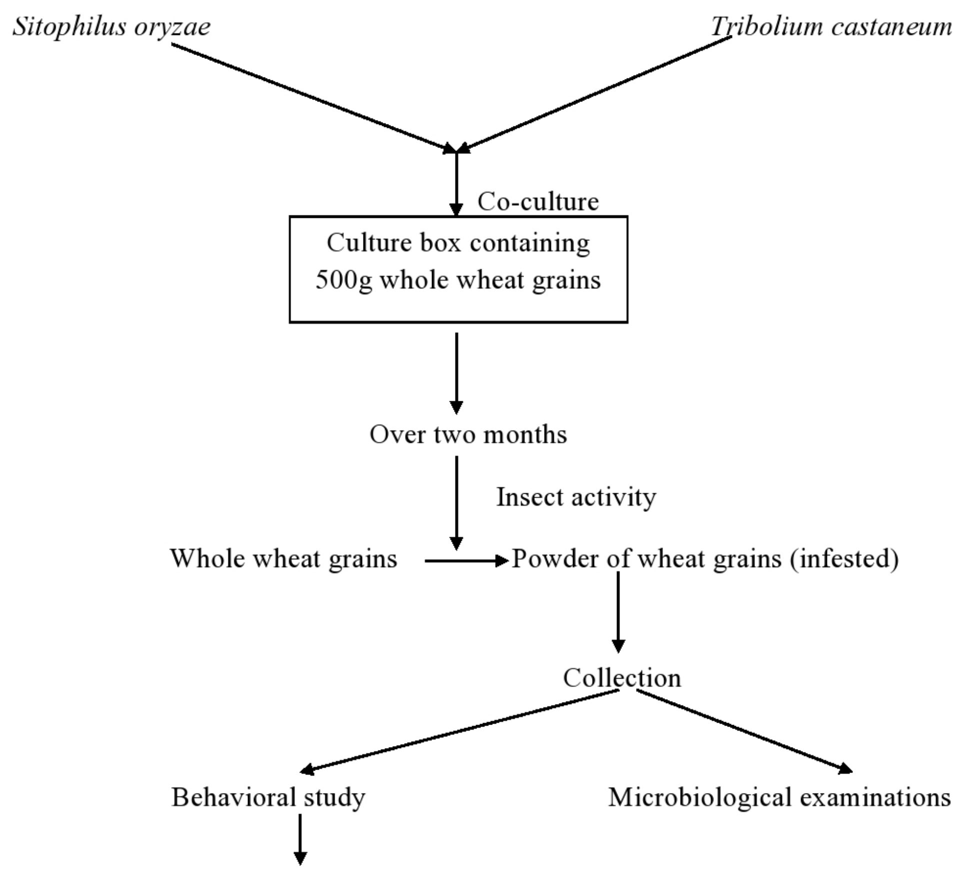

Insect Species	Experiment	To understand insects' preference	
		T-tube apparatus	
Sitophilus oryzae	SET 1	Left arm: Powder of wheat grains (infested)	Right arm: Whole wheat grains (uninfested)
	SET 2 Control	Left arm: *	Right arm: *
Tribolium castaneum	SET 1	Left arm: Powder of wheat grains (infested)	Right arm: Whole wheat grains (uninfested)
	SET 2 Control	Left arm: *	Right arm: *

*without any wheat grain powder or whole wheat grains

Figure 1. Schematic presentation of the design of the study

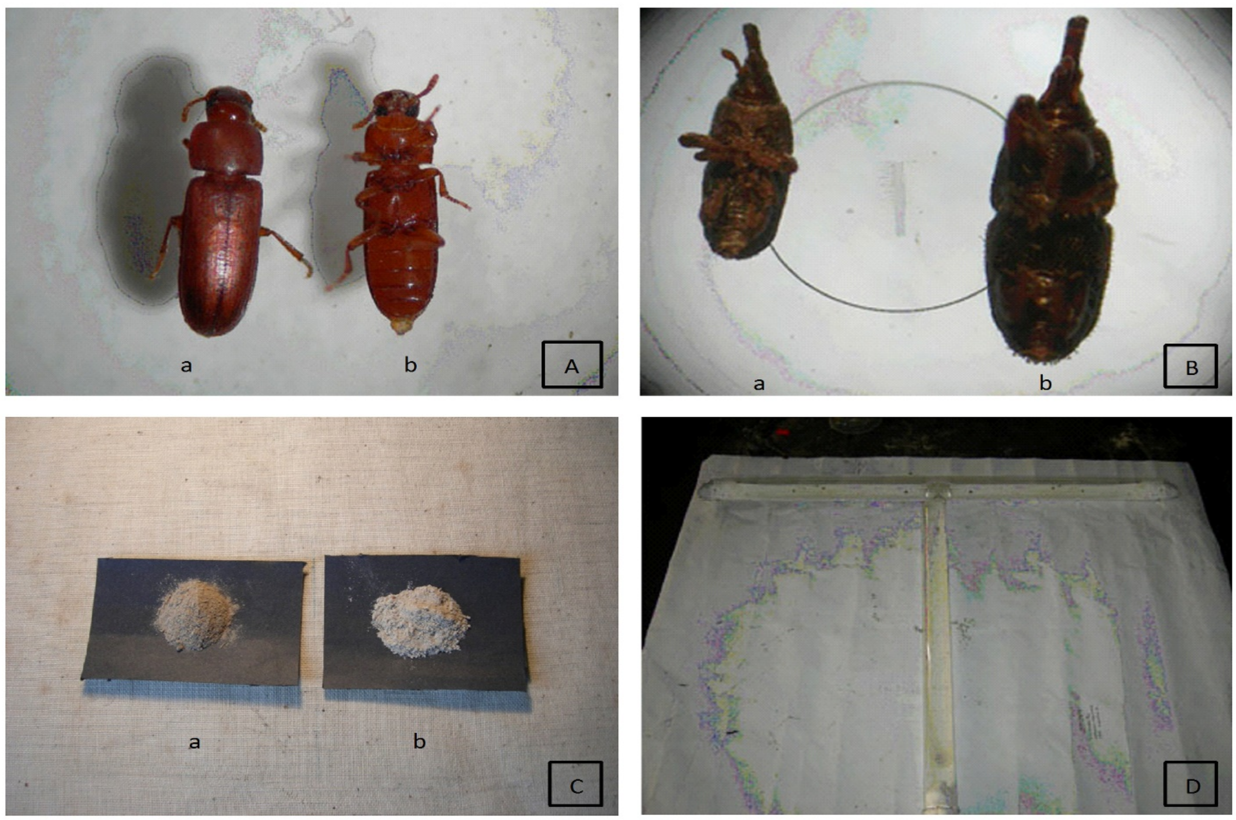

Figure 2. Photographs: A. *Tribolium castaneum*: a. dorsal and b. ventral view; B. *Sitophilus oryzae*: a. ventral and b. dorsal view; C. old, moist and grayish insect-infested grain powder with foul smell (a), dry uninfested grain powder without foul smell; D. T-tube apparatus.

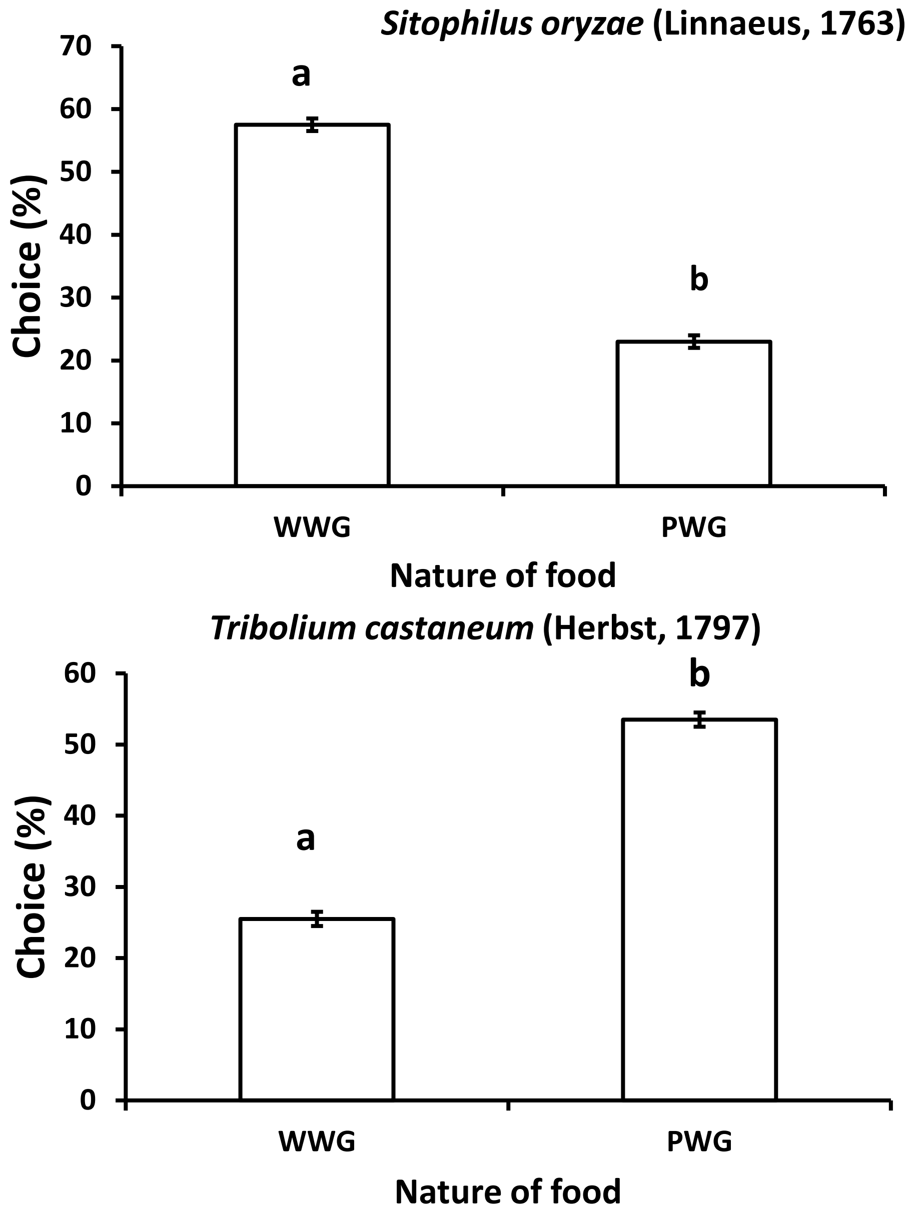

Figure 3. Average percentage of two test insects' choice (% ± SE) towards WWG and PWG recorded through T-tube apparatus after 5 hours of experiment. *Each mean is based on three replicates. Means with dissimilar letter are significantly different at $P = 0.05$. WWG: Whole Wheat Grains (Uninfested); PWG: Powder of Wheat Grains (Infested).