Research Article

Relative Susceptibility of Rice Variety (D-98) (*Oryza sativa* L.) to Red Flour Beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) in Storage

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Abstract | The commercial availability of rice has been increasing due to consumer demands, which raises new challenges for the management of stored grain insect pests since little is known about the vulnerability of aromatic rice varieties to infestation of *Tribolium castaneum* (Herbst) during storage. In the present study it was investigated the susceptibility of (D-98) *(Oryza sativa* L.) locally known as basmati rice, (its long and broken grains) to *T. castaneum*. In order to find out their quantitative and qualitative losses and pest status of red flour beetle by its growth rate on tested variety. Both host samples were obtained from a local market at Hyderabad, Sindh, Pakistan. The experiment was set-up in a completely randomized design (CRD) in the laboratory at ambient environmental conditions (30ºC and 65% relative humidity) for a period of 90 days. This experiment was replicated by thrice. Five hundred grams of each rice sample were artificially infested with 5 pairs of *T. castaneum* in 2 k.g glass containers with netted lids. ANOVA analysis showed variations among both tested samples in the pre- and post- infestation and it was strongly dependent on the eggs laid, development time of *T. castaneum* and host weight loss. The results showed that broken rice was significantly (P<0.05) more susceptible due to increased progeny growth and decreased developmental period while the long grains were moderately resistant due to decreased progeny growth and increased developmental period of *T. castaneum*. There was significantly (P<0.05) higher mean mortality of adults on long grain (80%) and least on broken rice (25%) after 30, 60 and 90 days of infestation. While mean weight loss percentage was significantly (P<0.05) higher in broken grains (25%) and least in long grains (8.75%). The outcome of this study underscores the need of preventive measures against *T. castaneum* to avoid economic losses to rice destined for long storage duration.

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Introduction

Sindh aromatic rice (*Oryza sativa* L.) also known as basmati rice were the most consumed rice variety across the country and globally. As they are an excellent source of starch, fiber, protein, vitamins, antioxidants and essential minerals (Rajput, 2023). The quality of rice both in the field and stores after harvest is very

important, because it specified on characteristics such as, grain size, width, thickness and physicochemical properties which influence the marketability and their price (Butt *et al.*, 2008). According to Ayub Agricultural Research Institute (2023), Pakistan holds a significant position in the global rice market and is the World's 10th largest producer of rice. Statistically, Pakistan produced 9.3 million metric tons rice and exports over 4 million tons in 2021, which makes rice the major agricultural crop in the country (Ilyas *et al.*, 2022). This crop is cultivated in the fields of Sindh, Punjab and Balochistan regions, widely cultivated rice species at these regions are, (Basmati, Irri-6 and Irri-9) among them Basmati are famous for its flavor and quality, (Pakistan Grain and Feed Annual, 2018).

Despite severe effects of floods and droughts on the agricultural sector in the past decade, Sindh has managed to produce enough rice to feed its population and support the country's foreign exchange earnings. According to Punjab Rice Mills and Cold Storage (2024), the D-98 is one of the best basmati grown in Sindh region, this area containing rich soil and the perfect conditions for sowing. This variety has rich pearl white color grains, their average length is 6.8 mm, has strong aroma and unique taste (Naeem, 2024).

After harvesting rice grains are stored in godowns and stored houses for processing and further import and export to different countries or they might be preserved up to a year for next sowing season, but unfortunately in stores stored grains were susceptible by a number of stored grain insect pests including *Tribolium castaneum* (Herbst), that can damage or destroy the commodity, due to improper management and unclean conditions of godowns, where this pest was already present under cracks and bricks, thus it can easily attacked to stored grains. *T. castaneum* has a broad host range and has been documented to survive on a variety of stored products, including wheat, rice and their flours, (Mckay *et al.*, 2019).

In hot and humid tropical region the host range of red flour beetle include a wide range of grains and their products (Aditi *et al.*, 2022). Therefore, found in almost all grocery shops, stored houses and mills. Apart from favorable environmental conditions development of red flour beetle also dependent on diet, (Shweil and Al-Jubouri, 2024). Larvae are the most destructive stage, consuming endosperm of seeds resulting in coagulating consistency, objectionable odour and reduce product quality (Astuti *et al.*, 2020). Since, the deterioration of healthy rice grains in storage mainly due to infestation by *T. castaneum* leads to losses (Ali *et al.*, 2016), which in turn has adverse effects on economy of the country.

The Sindh province has tropical and subtropical regions, where temperature and humidity favor the growth and reproduction of *T. castaneum* on stored rice grains during storage. In light of this, present study aim to investigate the relative susceptibility of (D 98) rice variety to infestation and to assess the pest status of red flour beetle by its growth rate. The assessment of D-98 variety will provide an opportunity to food handlers for planning the proper storage management to avoid cross infestation among susceptible type of rice grains and it will help to assurance food security in Pakistan.

Materials and Methods

The experiments were performed in the laboratory of Department of Zoology, University of Sindh, Jamshoro, (25° 24' 53"N, 68° 16' 22"E and 60 m above sea level), Sindh State, Pakistan. This experiment was conducted under mean 30°C and 65% relative humidity (Skourti *et al.*, 2020) between August-October 2023 and replicate thrice.

Rearing of insect

Wild strain of *T. castaneum* used for the study was established from an infested rice purchased from a local market of Hyderabad, earlier and was maintained afterward on crushed wheat flour under ambient laboratory conditions (Ehisianya *et al.*, 2022).

Preparation of grains for the experiment

Basmati rice (D-98) long and broken grains were obtained from the open market of Hyderabad city. Both samples were carefully wrapped in polyethylene bags labeled separately. After that kept in a deep freezer for 3 days (at temperature below 0°C) to kill any viable eggs, larvae or adults that may be harbored in the rice grains (Ehisianya *et al.*, 2022).

Experimental design and procedure

Five hundred grams each of the rice host were weighed using a digital balance and kept in 2 kg glass jars with mesh lids. Five pairs of 5 days-old adult of *T. castaneum* were introduced into each of the jars and left undisturbed on a work-bench. The experiment was carried out in a completely randomized design

(CRD) (Ehisianya *et al.*, 2022), in which each treatment were replicated three times. Oviposition, developmental period, adult emergence and mortality of red flour beetle were observed; at the same time weight loss and susceptibility index of rice samples were also calculated. Initially ova were counted after 5 days of experimental and then after (30 and 60 days) of experiment, while at the end of (30, 60 and 90 days), the contents of each jar were poured onto a transparent plastic tray and the numbers of adults and immature were counted, also taking a note of living and dead insects.

Weight of the both samples was taken in batches at termination of the experiments using a digital balance and the differences in their weights were recorded, with the help of formula by, (Jehajo and Shah, 2023).

$$\text{Weight loss percent} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

In addition to percent weight loss susceptibility of rice samples to *T. castaneum* was also calculated by using formula by (Jehajo and Memon, 2020).

$$\text{Susceptibility index formula} = \frac{\text{Log y}}{T} \times 100$$

Where y is the number of insects reaching at adult stage and T is the average development period in days.

Table 1: *Categories and its susceptible index.*

Categories	**Susceptible index**
Resistant	1-5
Moderate resistant	6-10
Susceptible	11-15
Highly susceptible	16-21

Statistical analysis

All data were then subjected to ANOVA and differences between means were determined using the Least Significant Difference (LSD) at (P≤0.05) (Ehisianya *et al.*, 2022).

Results and Discussion

Oviposition and T. castaneum development

To observed the oviposition preference, 100 grains from 500g were randomly selected from each sample and the number of ova oviposited were counted after five days of egg laying initially and afterward 60 and 90 days of storage. Once counting, ova were again carefully put into their relevant jars for hatching.

Table 2 revealed the data regarding the ovipositional preference, the mean average number of eggs laid on grains was significantly (P≤0.05) different among both samples and were in range of mean (53) in long grains to (131.6) in broken grains after three successive generations, this difference is due to the variation in their physical texture of rice, which have influenced the oviposition site to *T. castaneum*. Furthermore, it was also observed that red flour beetle itself prefer broken grains to the long grains for egg laying. The mean development period (days from egg to adult) was significantly different among the samples and was in the range of (25) in long grains and (18) in broken grains. Thus, the average number of days taken for the insect to develop from egg stage to adult was significantly longest in long grains and shortest in broken grains, this is due to the inability of larvae to get adequate nutrients to feed on them on long grains than the broken grains. Correspondingly, the mean average number of emerged adults after three generations were also significantly low (48) in long rice grains (Table 3), however high (120.3) in broken grains after 90 days of storage, for the reason that number of adult emergence correlates to the number of eggs laid per sample, the higher the eggs the greater the number of emerged adults. Mortality of beetles was highest (80%) on long grains and lowest on broken grains (25%), which was observed after (30, 60 and 90) days of infestation, respectively.

Table 2: *Oviposition of Tribolium castaneum on long and broken rice grains stored for 90 days.*

Rice sample	**Generations**			**Mean**
	F1	**F2**	**F3**	
Long basmati rice	25	61	73	53a
Broken basmati rice	52	120	223	131.6b

Different means in the same column followed by the variable letters are not statistically significant (P ≤ 0.05).

Table 3: *Mean number of Tribolium castaneum adults on long and broken rice grains stored for 90 days.*

Rice sample	**Generations**			**Mean**
	F1	**F2**	**F3**	
Long basmati rice	20	55	69	48a
Broken basmati rice	45	105	211	120.3b

Different means in the same column followed by the variable letters are not statistically significant (P ≤ 0.05).

Grain weight loss and susceptibility index

After completion of experiments, the weight loss of grains from both samples of aromatic rice variety (D-98) was recorded due to infestation of *T. castaneum*. A significant variation (P≤0.05) was found in the weight loss of the grains after 90 days of storage (Table 4). The highest mean weight loss % was (25g) in broken rice and lowest (8.75g) in long rice kernels. During the study it was found that red flour beetle bred less on long grain because of unexposed endosperm of the seeds.

The susceptibility of grains was observed by formula used by (Jehajo and Memon, 2020), as in (Table 1). The number of eggs laid on grains correlated positively to the number of emerged adults, eventually weight loss and susceptibility of rice grains were related with eggs. If more eggs were laid on seeds, more larvae will hatched out which ultimately develop into adult stage, causing sever weight loss to the grains, whereas developmental period of beetles correlated negatively to the measured variables and was strongly negative with susceptibility index. This indicated that as the developmental time increased, the susceptibility index decreased. Hence, broken grains were found susceptible with (11.5) index while long grains found moderately resistant (6.7) index against *T. castaneum*. Although long grains were found moderate resistant but the beetle successfully completed its development on them during long term storage.

Table 4: *Mean weight loss (%) of rice infested with Tribolium castaneum after 90 days of storage.*

Rice host	Mean weight loss %
long rice grains	8.75%a
Broken rice grains	25%b

Different means in the same colums followed by the variable letters are not statistically significant (P≤0.05.)

The results revealed that tested rice grains (both samples) had considerable effects on the oviposition and population emergence; however, both sets were not statistically significant (P≤0.05). The differences in growth rate are likely due to the texture and chemical gustatory composition. Long grains had a slightly low risk of infestation due to distinctly different surface texture and unexposed endosperm of the seed, compared to the broken grains which had high ratio of mean fecundity and population emergence. The development time that red flour beetle took to develop was also significantly different among them. For example, by week 3.4 after oviposition was the highest period on long grains to developed from larvae to adult stage and least 2.4 week on broken grains.

This study suggested that tested sample sets supported the development and growth of *T. castaneum*, therefore, they must contain all nutritional requirements. Even though red flour beetle might have difficultly to get necessary nutritional components on long grains, thus, it took longer time to accumulate and to trigger adult emergence. On the other hand, broken short grains had quickest emergence time, because of their exposed endosperm which provided readily available nutrition to larvae. Accordingly, these broken grains could be considered as indicators of infestation in a stored houses and grocery shops.

Shweil and Al-Jubouri (2024), also suggested that the development of red flour beetle affected by food substance. A study by Gerken and Campbell (2020), on *T. castaneum* infestation to 18 different kinds of flour, demonstrated that chemical and physical properties of flours have significant effect on development of insects. They revealed that nutrition or chemicals within the flours have impact on oviposition, adult emergence and developmental period. We analyzed that endosperm of the rice grains contain fiber, protein and carbohydrate that tend to lead to high number of eggs and progeny emergence. Skourti *et al.* (2020) worked on different biological parameters of *T. castaneum* on semolina, cracked wheat and maize as feeding commodities, on the basis of values of the intrinsic and finite rate of increase as well as the reproductive value, they found out that red flour beetle was able to increase its population only on semolina and it act as a susceptible commodity. This result also corroborates the findings of Ehisianya *et al.* (2022), they reported that development of *T. castaneum* was depended on type of host it infest, so as the weight loss of host directly proportional to the development of pest. They tested five types of flour (wheat, maize, sorghum, cassava and millet) for 63 days of storage and observed that all the flour were susceptible to *T. castaneum* at varying degrees, however, flour from wheat and sorghum were significantly more susceptible due to increased progeny development than others.

From the result of this study broken grains of basmati rice was the most susceptible to *T. castaneum*

infestation, while the least susceptible/ moderately resistant was long grains of the same variety with low emergence. This may be due to the unexposed endosperm which provides less diet (carbohydrate, protein and fiber) which were responsible for the minimum beetle progeny on them. There were significantly greater mean percentage of weight loss occurred in the broken grains compared to the long grains. It indicated that susceptible grains could be on strong risk of infestation and less on moderately resistant.

Information how beetles choose site for oviposition should provide pest management guide for diverse storage sites where there is a possibility of cross infestation. The assumption that if the broken grains were infested with red flour beetle, the long grains must be infested too and therefore, both types must be treated against the pest. Hence, Information on susceptibility of (D-98) variety of rice can act to either inhibit or promote oviposition and development of *T. castaneum* and applied as risk control tactic. The implication of this result is that merchants, households and food handlers storing large quantities of basmati rice can acquire quantitative and qualitative losses caused by *T. castaneum* infestation without this baseline information.

Conclusions and Recommendations

The findings of present research work revealed that broken grains of D- 98 are susceptible to attack by *T. castaneum*, because this pest could successfully colonize, feed and develop on them, and had high weight loss percentage. Whereas long grains of same variety was found moderate resistant with relatively low weight loss and lowest reproduction of the beetle within given storage time. It was also concluded that *T. Castaneum* experienced no difficulty to infest wholesome grains and it could be considered as primary pest of stored products, which is contradicts earlier information at *T. castaneum* is a secondary pest due to its inability to damage whole grains. Understanding the susceptibility types will also provide pest management information for varieties of rice storage sites. Therefore, it is recommended that high storage protection will be required for both forms of (long and broken grains) aromatic rice variety (D-98) to preserve the grain quality during long term storage.

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Novelty Statement

This is the first study in Sindh, Pakistan to test D-98 rice variety for its relative susceptibility and associated post-harvest losses and to assess the pest status of *T. castaneum* on tested rice grains.

Author's Contribution

Nosheen Jehajo: Conducted the research work, analysed the data and write the paper.
Naheed Shah: Helped in data analysis.

Conflict of interest
The authors have declared no conflict of interest.

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