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Fragment shedding behaviour of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) on pearl millet flour during storage

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Abstract
The insects are the major cause of post-harvest losses of grain that can be a positive cause of many human health issues. Insects infestation in flour can cause significant changes in the quality of Pearl millet flour (PMF). The present study focuses on the fragment shedding behaviour of *Rhyzopertha dominica* and *Sitophilus oryzae* on PMF. The FDA (Food and Drug Administration) has given the DAL (Defect Action Level), i.e. 75 insect fragments per 50 gram of flour. The standard acid hydrolysis method was used to calculate the fragment counts in PMF. The results indicate that the fragments shed by *R. dominica* and *S. oryzae* insects in PMF at 30 ºC with 35 days of storage duration were - *R. dominica* shed 34.00±3.05, 120.00±5.50, and 220.00±53.48 fragments, respectively for 1, 3, and 5 number of infestation. The *S. oryzae* shed 22.00±1.00, 59.00±6.24, and 78.00±10.39 fragments, respectively, for 1, 3, and 5 insect infestations. The *R. dominica* crossed the DAL in 21 and 14 days in 3 and 5 insect-infested PMF. The *S. oryzae* crossed the DAL in 35 days in 5 insect-infested PMF.
The result from the current study indicates that even three insects can degrade the quality of the PMF. A frequent sampling at adequate intervals of time and post-harvest processing within 13 days after harvesting is required (results demonstrated that till 13th day PMF was under the acceptable limit, below DAL) to retain the quality and control the insect infestation and its fragments shredding in PMF.

Keywords: fragment shedding behaviour, defect action level, *R. dominica*, *S. oryzae*

1. Introduction
Pearl millet (*Pennisetum glaucum*) is high in nutritional value compared to other major cereal crops. Pearl millet has a higher protein, fat, fiber, and ash content than other major cereal cultivated crops [1]. Pearl millet is produced nearly half of the world's total millet production. India is number one in millet production compared to other countries with a market share of 41.04% and an annual production of 8.61 million tonnes. The total global millet production is estimated at around 28.45 million tonnes per year [2]. Insect infestation and insect fragments are significant threats for post-harvest grain storage because they are associated with economic losses, create health hazards, product shelf life reduction, etc. This can create qualitative and quantitative damage to the millet. Quantitative and qualitative damage like reduction in the grain or flour weight and nutritional quality of millet is due to feeding of insects [3]. The post-harvest losses of millet were estimated at 11-15% [4]. From the total post-harvest losses, 5% of loss occurs from transportation. 4-5% loss comes from marketing and distribution. Around 2-4.2% loss comes due to storage and infestation, which accounts for the 1300 crores loss per year [4, 5]. The insect infestation and insect fragments create more significant damage to the millet flour; for example - insect faeces contamination (mainly uric acid) increases the flour humidity and creates a favorable condition for fungal and mould growth. Due to Insect contaminations, the products get exposed to quinones which adult insects produce as a defence mechanism [6]. *T. castaneum, R. dominica, S. oryzae* are some of the important insects which create significant changes in the quality of millet flour and decreases the final product quality and consumer acceptability.
Infestation control and insect management are essential to reduce quality losses and improve the product's export value in the international market. The US FDA has sated DAL, i.e. 75 insect fragments/50 g of flour. The current study of insect fragment shedding behaviour on PMF with different insects will help us to know the–Fragment shedding behaviour of insects

& the time required by insects to reach the DAL, so the acceptable time range can be calculated (the time range at which PMF flour will be in the allowable limit) and according to this proper insect management and flour processing decision can be done.

2. Material and Methods

2.1 Sample collection

The experiment was done at the Dept. of Primary Processing Storage and Handling, Indian institute of food processing technology (IIFPT), Thanjavur, Tamilnadu. The pearl millet grain was purchased from the Thanjavur market, and it was cleaned and kept for sun drying to reduce the moisture content for milling suitability. The dried grain (11.2% moisture content) was milled with an electric milling machine (Almtech Enterprise C/15 Industrial Estate). The milled pearl millet flour was stored in an airtight packaging container for further analysis.

2.2 Insect culturing

The *R. dominica* and *S.oryzae* insects were cultured in a laboratory for up to 3 generations. *R. dominica* were cultured in wheat flour sample, and *S. oryzae* were cultured in rice grain sample. All insects were cultured at room temperature at 28 ± 2 °C with RH 65± 5 %. For culturing, random 50 insects were taken of each variety and placed into 500 grams of flour in a 1-litre glass bottle and covered with a muslin cloth.

2.3 Sample preparation

The 50-gram PMF sample was taken into a PET food-grade plastic bottle of 250 ml volume. The female
R. dominica and *S. oryzae* insects of number-1, 3, and 5 were added into 50 gram of flour sample and covered by muslin cloth. The sample was stored at 30 °C in the incubator. The sample was analyzed for insect fragment counts at weekly intervals up to the 5th week of infestation.

2.4 Insect and larva population counts

The insects and larva population number was counted in the weekly interval. The flour was sieved with a 0.5 mm sieve, and insects and larva were collected in a separate box to check the insect's population number. The counting of insects and larva was done manually. After insects and larva counting, it was added again on earlier sieved flour. The insect and larva counts were included in total insect fragments.

2.5 Insects fragment counts

The insect's fragment counts of infested pearl millet flour were analyzed by the acid hydrolysis method (AACC Method 28-41.03) [7]. The infested 50 grams of flour were taken into a beaker containing 500 ml of 5% HCL and digested by autoclaving it for 5 min at 121 °C. After complete digestion of flour, the mixture was transferred to a gravity separation funnel, and 50 ml of mineral oil was added with proper mixing and held for 30 min with gentle stirring in 10 min of intervals. The oil was added to trap the insect fragments on the oil surface. After 30 min the lower layer drained off, and the upper 1 cm layer of the digested mixture was held. Hot water was added to wash and remove the HCL from the mixture. Three-time rinsing with hot water was carried out until the lower layer was visible clearly. After the final rinse, the oil layer with the trapped insect was taken into another beaker and filtered with ruled filter paper and analyzed by Stereomicroscope - Leica Microsystems application suite 2.0 (AACC Method 28-41.03) [7].

3. Statistical analysis

Statistical analysis of data was performed by SPSS (IBM SPSS 25) statistical analysis software. The experiments were conducted in triplicates. The data are shown in mean ± SD (standard deviation). The data were analyzed by one-way analysis of variance, and significance was considered when $p<0.05$, and Tukey Pairwise Comparisons were applied to determine the significance of data.

4. Result and Discussion

4.1 Insects fragment shedding behaviour

An insect is one of the major reasons for the economic and quality losses in grain. The analysis of insect's fragment shredding behaviour in PMF is essential to calculate the acceptable time range. The knowledge of the "acceptable time range" will provide us with an accurate time range of acceptability of flour. The US FDA has given the DAL; if this limit is exceeded, the flour will not be acceptable in the international market. To minimize the losses and improve the quality, the knowledge of the fragment shedding behaviour of insects is essential. The number of fragments sheds by *R. dominica* and *S. oryzae* were counted during the 35 days of storage at 30 ºC temperature. In previous research, we studied on fragment shedding behaviour of *T. castaneum* also, so in this current study, we compared the time range for DAL of three different insects, *i.e*. *T. castaneum*, *R. dominica*, and *S. oryzae*. The result shows that insect fragments increased with an increase in insect's population and infestation days. The zero fragments were observed in control PMF samples. Fragment shedding behaviour of *T. castaneum* was observed in a previous study. It was observed that *T. castaneum* fragment producing behaviour was significantly different ($p<0.05$) from the other two insects crossed the DAL in 14 days by 3, 5 insect-infested PMF. The fragment observed by the standard acid hydrolysis method was mainly larva, adult insects, and insect cuticle (Figure 1). Negi *et al*. 2021 [8] also showed the number of *T. castaneum* fragment counts increased with an increase in insect population with infestation duration. Skourti *et al*. 2019 [9] researched *T. castaneum*, they concluded that the median time period for the better development of *T. castaneum* is 30 ºC. This could be why we also noticed a more significant number of insects and their fragments at 30 ºC storage.

4.1.1 Fragment shredding behaviour *Rhyzopertha dominica*

The result indicates the fragments producing behaviour of *R. dominica* was significantly different ($p<0.05$) from *T. castaneum* and *S. oryzae*. The fragments shedded by *R. dominica* were 34.00±3.05, 120.00±5.50, 220.00±53.48 respectively for 1, 3, and 5 insect-infested PMF in 35 days of storage (Table 1). In 3 insect-infested PMF, the *R. dominica* crossed the DAL in 21 days with 78.00±3.5 fragment counts. In 5 insect-infested flour, the *R. dominica* crossed the DAL in 14 days with 81.00±7.37 fragment counts. The fragment counts were studied by Perez-Mendoza *et al*. 2005 [10] in *R. dominica* infested wheat kernel samples from FTIR method, they found that the 1 infested kernel per 100 g of wheat flour produces 14.0 ± 1.6 fragments, and 4 infested kernel per 100 g produces 94.6±6.4 fragments which is more than DAL. Negi *et al*. 2021 [7] also observed the *T. castaneum* fragment

shedding behaviour in the wheat flour sample; they showed that *T. castaneum* produced more than 75 fragments in 50 grams of sample and crossed the DAL in 15 and 11 days with 3 and 5 insect-infested wheat flour sample, respectively. Our results are in line with the research of Negi *et al*. 2021 [7]. Sanchez-Martinez R. I. *et al*. 1997 [11] studied *R. dominica* infestation and its effects on bread quality. He also observed the rapid increase in the insect population with an increase in infestation days. The insect population observed was 45 and 571 insect/kg in the wheat flour sample at 0-45 and 45-90 days of storage, respectively.

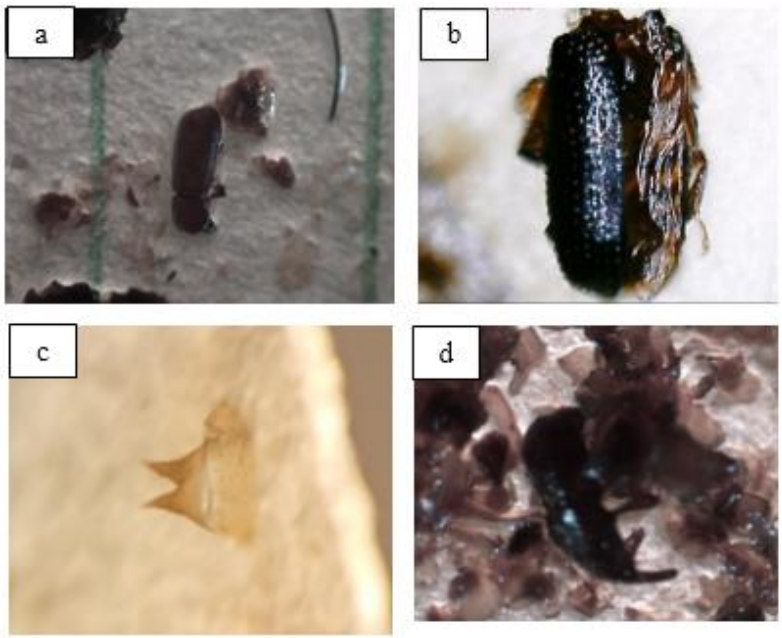
4.1.2 Fragment shredding behaviour *Sitophilus oryzae*

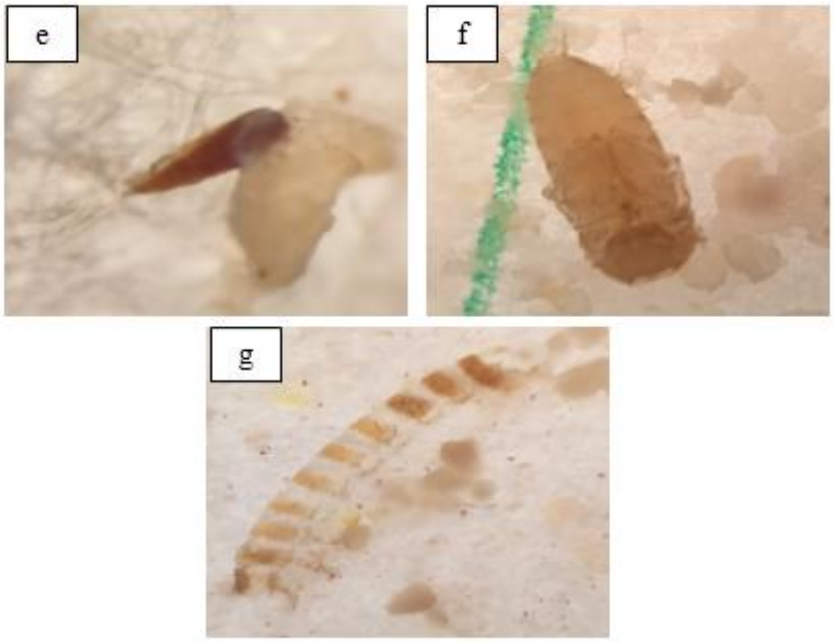
The fragment shedding behaviour of *S. oryzae* was studied. The significance difference (p<0.05) was observed in the fragment-producing behaviour of *S. oryzae*. The result indicates that the fragments produced by *S. oryzae* were 22.00±1.00, 59.00±6.24, 78.00±10.39 respectively for 1, 3, and 5 insect-infested PMF in 35 days of storage (Table 1). *S. oryzae* crossed the DAL in 5 insect-infested PMF in 35 days with 78.00±10.39 fragment counts. Toews MD *et al*. 2007 [12] studied the insect fragment produced by *S. oryzae* in wheat kernel sample. They observed the fragments of the wheat kernel after the milling process; fragments were 37.8±7.4, 265.8±34.1, with one and nine *S. oryzae* adult infested samples, respectively. It was observed by Toews MD *et al*. 2007 [12] that only three infested wheat kernels by *S oryzae* adult crossed the DAL.

Table 1: Fragment shredding behaviour of *R. dominica* and *S. oryzae*

Number of insect infestation	Infestation duration (days)	Insects fragment counts	
		R. dominica	***S. oryzae***
1	control	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
	7	2.00 ± 0.57^{de}	1.00 ± 0.00^{de}
	14	5.00 ± 1.00^{cd}	4.00 ± 0.57^{d}
	21	8.00 ± 0.50^{c}	8.00 ± 2.51^{c}
	28	22.00 ± 2.00^{b}	13.00 ± 0.57^{b}
	35	34.00 ± 3.05^{a}	22.00 ± 1.00^{a}
3	control	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
	7	3.00 ± 1.00^{e}	4.00 ± 0.57^{d}
	14	39.00 ± 4.50^{d}	6.00 ± 1.00^{d}
	21	78.00 ± 3.51^{c}	24.00 ± 2.00^{c}
	28	96.00 ± 5.56^{b}	41.00 ± 2.00^{b}
	35	120.00 ± 5.50^{a}	59.00 ± 6.24^{a}
5	control	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}
	7	5.00 ± 0.00^{c}	7.00 ± 0.57^{d}
	14	81.00 ± 7.37^{b}	15.00 ± 2.08^{d}
	21	98.00 ± 4.04^{b}	35.00 ± 1.00^{c}
	28	122.00 ± 12.16^{ab}	55.00 ± 9.50^{b}
	35	220.00 ± 53.48^{a}	78.00 ± 10.39^{a}

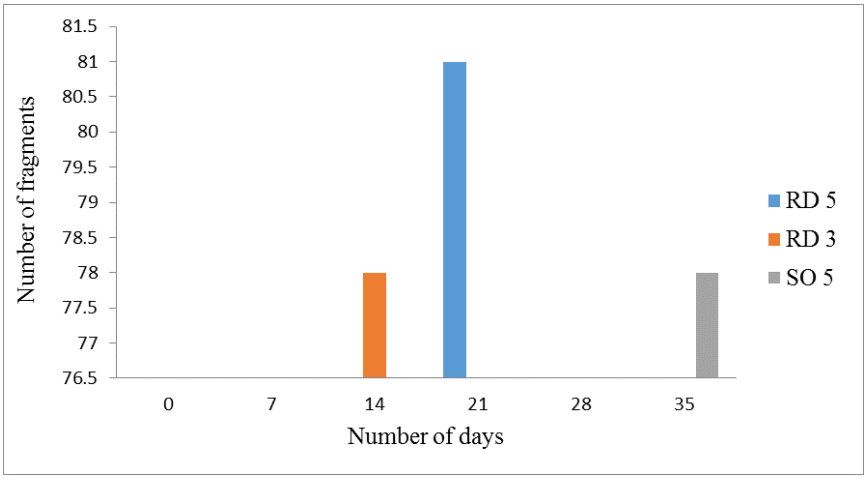
Data are shown in mean ± SD. The means which does not share a letter are significantly different, at p<0.05 by Tukey Pairwise Comparisons.





where 'a' is an image of *R. dominica* adult, image 'b' is *R. dominica* adult elytra fragment, image 'c' is *R. dominica* insect larva urogomphos fragment, 'd' is an image of *S. oryzae* adult insect, 'e 'is *S. oryzae* adult long snout image, 'f' is an *R. dominica* insect pupa image. 'g' is an image of larva abdominal terga. The images were observed by Stereomicroscope-Leica Microsystems application suite 2.

Fig 1: Representing the insect fragments images of three insects i.e. *R. dominica, and S. oryzae*.



Where, 'RD 5' is five *R. dominica* adult infested samples which crossed DAL at 21 days. 'RD 3' is three *R. dominica* adult infested sample which crossed DAL at 14 days. 'SO 5' is five *S. oryzae* adult infested samples, crossed DAL at 35 days.

Fig 2: Comparison of the number of days required by the insects to cross the DAL.

5. Conclusion

The insect fragment counts showing increasing trends with an increase in insect populations. Among two insects, *R. dominica* shows a higher shedding of fragments and crossed the DAL earlier than *S. oryzae* insects. It is important to perform frequent sampling of PMF at regular intervals so the insect fragments can be detected before the crossing of DAL. The proper implementation of the insect management system is required to control the insect infestation. The minimum 13 days acceptable time range (the time till that flour will be under DAL) was observed in the current study; after this time, PMF will be more prone to cross the DAL of 75 insect fragments per 50 g of flour. More systematic techniques of limiting the infestation and fragment counts are required to protect the consumers from food born diseases and allergens. Hence, the present study shows the scope to reduce the post harvest losses of PMF that leads to reduced human health related risks.

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