Volume- 5
Issue- II
December-2020
Rajarata University Journal
ISSN: 2362-0080
www.ruj.ac.lk/journals/

Category: Research Article

Particle Size of Grains and Pulses Affects Infestation by *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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ARTICLE DETAILS

Article History
Published Online:
30th December 2020

Keywords
Stored food, Progeny emergence, *Tribolium castaneum*, broken percentage of food

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ABSTRACT

*Tribolium castaneum*is is a pest of stored rice and other grains. Altered degree of insect infestation in stored food having different particle sizes is reported but such information on local rice varieties and pulses is unavailable. Therefore, we aimed to investigate the relationship between the infestation by *T. castaneum* varies and the grain type and the percentage of fragments during storage, in selected rice varieties and other grains found in the local market. The experiment used the Completely Randomized Design with four replicates. Five local rice varieties *Red Kekulu*, *Red Samba*, *Kuruluthuda*, *Suwandel* and *Red Heeneti*; and other grains cowpea, green gram and black gram were used. From each grain type, samples having brokens as 0%, 10%, 20%, 40%, 60% and 100% were prepared. Twenty *T. castaneum* adults were introduced into a 20 g replicate sample of each grain type, and removed two weeks following the introduction. Every two weeks following the removal of parent adults, the number of progeny adults emerged in each replicate sample was counted and removed. The progeny adult emergence varied with grain type, percentage of broken grain and the duration following initial infestation. Therefore, minimization of grain damage during milling and other handling processes is recommended to ensure better protection of grains from insect infestation during storage.

1. Introduction

Stored product insects are associated with a wide variety of stored commodities. Various types of losses of agricultural produce are occurred due to stored-product insects and are about 10% in temperate areas and 50% in tropical areas [1]. Nearly 80% of the storage grain losses in Sri Lanka occur due to insects [2, 3].

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) has been reported as major pests of stored rice and other cereals in many tropical and sub-tropical countries [4]. It is a major pest found in feed mills, processing plants, warehouses and retail stores [5]. It infests stored grain and other food products including flour, cereals, pasta, biscuits, beans and nuts. There are opportunities that *T. castaneum* colonizes in grains already damaged by other insect species [6].

Stored-product insects can cause significant economic losses such as loss of grain mass, nutrient depletion and effect on public health risk from contamination by allergens, such as uric acid [7]. Insect infestations in stored grains during warm weather can also lead to undesirable taste and off-odors that can render the product inedible [7]. Contamination of stored grains due to the presence of insects can be recognized through live insects, dead insects, larval and pupal exuviae together with fecal matter [8]. Losses can occur in multiple ways such as quality and quantity of the commodities, poor seed germination and viability of market value for commodities due to contaminate of foods through insect's excreta and presence of insect dead bodies These insects can enter the food supply chain at any point such as cleaning, transport, processing, storage [8].

The stored-product insects can damage bulk stored raw commodities. Grains may go through many kinds of handling operations such as processing, milling, manufacturing which can each contribute to grain damage. Broken or cracked kernels are one of the most common forms of grain damage. The cracked or broken particles lead to insect infestation and quick susceptibility to breaking during further handling. Stored product insects are found in stored grains and milled cereals but few insect species cause direct damage to products. The most insect species can feed on processed or damaged grains. *Tribolium castaneum* prefer smaller and finer particle size and structure of substrate particles [9]. Particle size may affect progeny production of stored-product insects. During processing and milling, grains can be cracked in to the different sized particles such as dust, flour, broken and cracked grains. Stored-product insects select cracked and broken grains as their habitats to complete their life cycle. These sizes of particles can determine the feeding preference of the stored product insects. According to the particle size of different commodities, insect infestation may be varied during the storage condition. We aimed to determine if *T. castaneum* progeny adults emerged during storage following initial infestation varies with grain type/variety and percentage of broken grains.

2. Material and Methods

2.1 Rearing of *Tribolium castaneum*

The adults of *T. castaneum* were taken from established cultures in the entomology laboratory, Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura. The adults were collected by using an aspirator connected to vacuum pump (Rocker 300, Rocker Scientific Co. Ltd, New Taipei City).*Tribolium castaneum* 200 adults were introduced to the 250g of wheat flour in a plastic container covered with a piece of cotton cloth material. Both adults were introduced into the growth chamber (FH-1200, Taiwan Hipoint Corporation, Kaohsiung City) maintained at 30°C and 65% RH After 2 weeks, the introduced adults were removed. The progeny adults were taken for the experiment one month following emergence.

2.2 Preparation of Culture media

Red Kekulu (red pericarped and raw milled), Red Samba (small round grains), three traditional rice varieties Red Heeneti, Kuruluthuda and Suwandel were used in the study. In addition, cowpea, green gram and black gram were used. Those food commodities used in the experiments were purchased from retailer shop in Anuradhapura, Sri Lanka. Commodities were checked for any impurities, debris and to be free from mold/insect infestation. All the food ingredients were ground using a mix grinder (Mixee-N, Singer India Ltd, New Delhi City). Samples of rice varieties and pulses were prepared by mixing different percentages of broken as 0%, 10%, 20%, 40%, 60% and 100%. For this, rice was ground for 1 minute and sifted through No. 20 (600 µm) and No. 30 (850 µm) sieves. The particles remained on sieve #30 was taken to prepare samples. Cowpea, green gram, black gram were ground for 1 minute and sifted using No. 10 (>850 µm) and No. 20 (600 µm) sieves. The particles that remained on #20 sieve were taken to prepare samples. From each commodity, 20 g was measured and introduced into the plastic vials (60 ml) with perforated lids (Table 1).

2.3 Introduction of adults into different food commodities

Twenty adults of particular a species were introduced into 20 g of each replicate medium separately and allowed for the incubation. The adults were removed from the food commodities after 2 weeks. Rearing media were observed daily to detect the mold development and other insects' infestation in the rearing media. All the cultures were maintained under ambient conditions (30 ºC, 65% RH) inside the incubator. Four replicates were prepared for each treatment. The progeny *T. castaneum* adults emerged in each replicate food medium were counted at every 2 week intervals for six weeks.

Table 1. Composition of different grain samples tested

% Broken	Composition of medium (g)
0	Whole grain 20 g
10	Whole grain 18 g+ Broken grain 2 g
20	Whole grain 16 g+ Broken grain 4 g
40	Whole grain 12 g+ Broken grain 8 g
60	Whole grain 8 g+ Broken grain 12 g
100	Broken grain 20 g

2.4. Experiment design and data analysis

Experiments were set up as Completely Randomized Design with four replicates. Cracked grains were mixed to whole grains as 0%,10%, 20%, 40%, 60% or 100% by volume.

The progeny adults of a particular insect species emerged in one replicate of each grain type and particle size were determined. The success of adult emergence among different broken percentages of a given grain type was compared. This was done at every 2-week intervals for 6 weeks. The data were analyzed using GLM procedure of ANOVA using Statistical Analysis System [10]. The means were separated by Tukey's test at P=0.05.

3. Results and Discussion

In general, the adult emergence differed with the grain type ($F_{7,561}$=23.27, P<0.0001), broken grain percentage in the sample ($F_{5,561}$=16.95, P<0.0001) and duration following infestation ($F_{2,561}$=92.10, P<0.0001). On the whole, all the five rice varieties had higher adult emergence than pulses. Adult emergence increased with the higher percentage of grain particles in the sample. Furthermore, the middle duration (15-28 days) had higher *T. castaneum* adults emerged than first (1-13 days) and last (29-42 days) durations.

At 14, 28 and 42 days following infestation of *T. castaneum* adults in the grains, the progeny adults emerged in a given commodity varied with the percentage of broken grains in the sample (Tables 2 and 3).

Table 2. Percentage (mean±SE) *Tribolium castaneum* adults emerged 14, 28 and 42 days following infestation in different rice varieties *Red Kekulu, Red Samba*, *Red Heeneti, Kuruluthuda* and *Suwandel* (n=4).

Rice variety	% Broken	% Adult emergence (mean±SE)[a]		
		Duration (days)		
		14	28	42
Red Kekulu	0	0.75±0.25b	0.25±0.25c	0.75±0.48b
	10	3.5±1.3b	38±16.65bc	9.75±3.52a
	20	0b	33.25±12.25bc	3±1.08b
	40	1.25±0.63b	66.75±4.80b	16±5.97a
	60	2.75±0.48b	51.0±11.85bc	15.25±6.38a
	100	29.5±13.48a	166.75±19.77a	0.75±0.48b
Red Samba	0	0.25±0.25b	28.75±1.49bc	13±4.88ab
	10	0.75±0.25b	24±1.29cd	10.5±0.65ab
	20	0.5±0.29b	15±1.78d	5.25±1.55b
	40	0.25±0.25b	14±4.53d	20.0±3.42a
	60	2.25±1.11b	39.75±1.31b	10.0±0.71ab
	100	7.25±0.85a	78.75±3.84a	8.75±1.65ab
Red Heeneti	0	0.25±0.25b	0.25±0.25b	1.0±0.71a
	10	0.5±0.29b	5.25±0.85b	18.75±6.10a
	20	0.5±0.29b	19.5±2.10b	14.5±5.61a
	40	7.75±2.17b	101.75±6.97a	10.0±1.41a
	60	10±1.96a	121.5±25.31a	12.25±3.09a
	100	0b	83.5±2.90a	14±5.07a
Kurulu-thuda	0	1.25±0.75d	23±2.12d	3.5±1.19cd
	10	10.75±0.75c	30.75±0.75d	2±0.71cd
	20	11.25±0.95c	59.75±0.85c	15.25±1.89ab
	40	42.25±1.11b	81±9.55b	11.75±1.75bc
	60	57.25±2.29a	111.75±3.84a	1±0.41d
	100	3.75±1.11d	118.75±2.56a	24.25±4.99a
Suwandel	0	0.25±0.25a	4.25±1.11cd	0.5±0.29b
	10	0.25±0.25a	16.75±4.04bc	4±1.29b
	20	0.75±0.25a	2±0.71d	0.75±0.48b
	40	0a	14.5±0.96bcd	12.0±2.08b
	60	0a	42±3.03a	12.5±1.04b
	100	0.75±0.25a	28.75±5.76ab	33.5±7.4a

[a]For a given duration, means followed by the same letter are not significantly different according to Tukey's test following ANOVA at P=0.05.

Table 3. Percentage (mean±SE) *Tribolium castaneum* adults emerged 14, 28 and 42 days following infestation in cowpea, green gram and black gram (n=4).

Grain	% broken	% Adult emergence (mean±SE)[a]		
		Duration (days)		
		14 days	28 days	42 days
Cowpea	0	0.25±0.25a	2.25±1.11b	0.5±0.29a
	10	0a	2.75±1.38ab	0.25±0.25a
	20	0.25±0.25a	3.75±1.60ab	2.25±0.85a
	40	0a	4±1.29ab	2.0±0.91a
	60	0.25±0.25a	5±1.08ab	4.5±2.22a
	100	0.5±0.29a	9.25±2.06a	11.0±5.96a
Green gram	0	0.75±0.48a	0.25±0.25b	6.75±2.36a
	10	0.25±0.25a	11.75±2.17a	8.5±2.90a
	20	0.25±0.25a	7.75±0.48ab	3.5±1.55a
	40	0.25±0.25a	11.5±2.25a	8.25±2.78a
	60	0a	8.75±2.39a	8.25±2.78a
	100	0a	6±2.08ab	10.25±4.63a
Black gram	0	0a	0a	0.75±0.48a
	10	0.25±0.25a	0a	0.25±0.25a
	20	0.25±0.25a	0a	0.75±0.25a
	40	0a	0a	0.25±0.25a
	60	0a	0a	0.25±0.25a
	100	0a	0a	0.25±0.25a

[a]For a given duration, means followed by the same letter are not significantly different according to Tukey's test following ANOVA at P=0.05.

Tribolium castaneum can develop on milled brown rice and the progeny development [11]. Therefore, it is important to pay attention how the presence of broken grains increase the progeny development as found in the current study. Arthur

[11] also showed that *T. castaneum* progeny development in milled brown rice varies with the temperature. In Sri Lanka, rice is stored throughout the country at different temperature levels. Therefore, future research should be designed to identify how the temperature affects *T. castaneum* development in different rice varieties containing various proportions of fragments.

A recent study has shown that *T. castaneum* infestation in sorghum varies according to the percentage of fraction in the sample [12]. Therefore, the findings in the current study on rice and pulses agree with this previous study. Future experiments need to determine the underlying reason for the increased progeny adult emergence in the samples at 28-42 days following adult removal compared to the other two durations.

In stored-product facilities, the population development in insect species is considered to be resulting from internal/resident population rather than due to infestation from outside [13]. This may lead to have high population levels within a facility during winter months in temperate countries and aggravate grain loss [14,15].

The current study revealed that *T. castaneum* adult emergence increases with the increased broken percentage of grains. Therefore, attention should be paid to minimize grain fragmentation during grain milling process.

4. Conclusion

Progeny *T. castaneum* adult emergence following initial infestation varies between rice varieties, cowpea, green gram and black gram. In general, the emergence of progeny adults increased with the percentage of broken grains in the sample. Future research should explore the effect of grain fragments on the progeny development of other insect species found during grain storage.

Acknowledgement

The authors wish to thank Sri Lanka Council for Agricultural Research Policy (NARP/16/RUSL/AG/01) for the financial assistance provided.

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