NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 53 Number 2, August 2022 Pg. 193-198
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Development of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)and Damage to Selected Flours in Storage

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Abstract

Flours from maize, millet, sorghum, wheat and cassava, obtained from a local market in Abia State, Nigeria were evaluated for the development of *Tribolium castaneum* and consequent damage in the laboratory at ambient laboratory conditions of 28-34^{0}C and 58-75% RH for a period of 63 days. Twenty grams each of these flours were artificially infested with 5 pairs of adult *T. castaneum* in a 500 mls glass containers with netted lids. The experiment was set-up in a completely randomized design (CRD) and replicated four times. The results showed variations in the pre- and post- treatment proximate composition of the selected flours which influenced the development of the insect and flour damage. Irrespective of flour type, *T. castaneum* progeny development caused increase in moisture, ash content, and decrease in dry matter, fibre, metabolizable energy, and crude protein after treatment. The tested flours were susceptible to *T. castaneum* at varying degrees. Flours from wheat and sorghum were significantly ($P < 0.05$) more susceptible to infestation due to increased progeny development than others, whereas no progeny developed in cassava flours. There were significantly ($P < 0.05$) higher mean mortality of adult *T. castaneum* in maize flours (1.90) and least in wheat flours (0.25). Mean weight losses were also significantly ($P < 0.05$) higher in maize (25%) and least in cassava and millet flours (8.75%). The outcome of this study underscores the need of preventive measures against *T. castaneum* to avoid economic losses to flours destined for long storage duration.

Keywords: Damage, flours, progeny, storage, Tribolium castaneum

Introduction

Commercially available flours from maize, millet, sorghum, wheat and cassava are highly consumed in many parts of sub-Saharan Africa (Fiedler *et al.* 2014). They are the common food staples in developing countries including Nigeria (Bellotti, 2008). These flours are targeted to improve the vitamin A (Klemin *et al*., 2010), Zinc (Brown *et al*., 2010; Aaron *et al*. 2011) and iron (Ogunmoyela *et al*., 2013) intake of the poor, especially children and women to prevent micronutrient deficiency. They also have no adverse effect on acceptability of complementary foods and breads prepared from these flours and no effect on the utilization of other minerals (Fieller *et al*., 2013; Cardoso *et al*., 2019). In hot humid tropical region like Nigeria, the host range of the rust-red flour beetle (*Tribolium castaneum*) include a wide range of grains and their products (Ajayi *et al*., 2006; Ogedegbe and Edoreh, 2014; Padin *et al.,* 2013; McKay *et al.,* 2019; Aditi *et al*., 2022), such as wheat, millet and acha (Lale and Yusuf, 2001; Bulus, 2008), benniseeds, cowpea, groundnuts, (Odeyemi, 2001; Komolafe and Odeyemi, 2014)), rice, sorghum, maize (Ajayi and Rahman, 2006), and cassava (Zakka *et al*., 2013).

Recent studies on Brazil nuts (*Bertholletia excelsa* HBK) by Pires *et al.* (2017) and Pires *et al.* (2019) considered *T. castaneum* a primary pest, experiencing no difficulty when feeding on the intact product, which contradicts earlier information at *T. castaneum* is a secondary pest due to its inability to infest and damage wholesome grains or products. They however reproduce rapidly in milled or damaged grains and products (Fatime and Ngamo, 2018). The larva is the most destructive stage of the beetle, consuming the endosperm of the seeds resulting in coagulating consistency (Keskin and Ozkaya, 2013), objectionable odour (Johnson *et al*., 2004) and reduce product quality of the flours (Makki *et al*., 2017; Yun *et al*., 2018; Astuti *et al*., 2020). As the variety of flour types for food industry continues to grow, little is known about the ability of stored product insects to develop on these alternative flours and the potential risks these insects might cause to this rapidly growing industry. The deterioration of these flours in storage is mainly due to infestation by *T. castaneum* and other microorganisms leads to losses (Ali *et al.*, 2016), which in turn has adverse effects on the economy of the nation. It is necessary therefore, that such losses be investigated so as to provide baseline information on the susceptibility of these selected flours to *T. castaneum* and to guarantee food security in Nigeria. Hence, this study was conducted to determine the development of the *T. castaneum* and its consequent damage to maize, wheat, millet, sorghum and cassava in storage.

Materials and Methods

The experiment was carried out in the laboratory of Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike (05°29′N, 07°33′E and 122m above sea level), Abia State, Nigeria to access the susceptibility of maize, wheat, millet, sorghum and cassava flours to *T. castaneum* infestation. Experiment was conducted under (28-34°C and 58-75% RH) between May - August, 2021.

Insect Culture: *T. castaneum* culture used for the study was established from an infested batch of wheat flour purchased from a local market at Umudike earlier and was maintained subsequently on crushed wheat flour under ambient laboratory conditions.

Sexing of Tribolium castaneum: Adults of *T. castaneum* were sexed using their morphological characteristics; the males have a small patch of short bristles (sex patches) on the inside of the first pair of legs (Beeman *et al*., 2022) or hairy punctures on the ventral surface of the anterior femur which is absent in the female species (Dobie *et al*., 1984).

Experimental Procedure: Five hundred grams each of the five different flours (maize, wheat, guinea, millet, and cassava) were obtained from the open market at Umudike. These flours were carefully wrapped in polyethylene bags labelled, and 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 flours.

Infestation: Twenty grammes each of the five different flours were weighed using a digital balance and kept in 500 ml glass jars with netted lids. Five pairs of 3-5 days-old adult *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) in which treatments were replicated four times. At the end of each stage of the experiment (21 and 63 Days After Treatment), the contents of each jar were poured onto a transparent plastic tray and the numbers of teneral adults and immatures were counted taking note of living and dead insects. Weight of the different flours was taken in batches at termination of the experiment using a sensitive balance and the differences in their weights were recorded and percentage weight loss was calculated as

$$PWL = (C-T)/T \times 100$$

Where, C, Initial weight (g); T = final weight (g) (Jackai and Asante, 2003).

Determination of the Proximate Composition of the Flour Types: Proximate composition such as crude protein, moisture, lipid, ash and crude fibre and carbohydrate of maize, millet, sorghum, wheat and cassava flours were assayed using the official methods of the Association of Analytical Chemists (AOAC, 1990).

Statistical Analyses: All data were then subjected to ANOVA and differences between means were determined using the Least Significant Difference (LSD) at $P \leq 0.05$.

Results and Discussion

Results

The pre- and post-treatment proximate composition of maize, millet, sorghum, wheat and cassava, flours are presented in Table 1. There was an increase in moisture (%), a decrease in dry matter contents and metabolizable energy post-treatment in all flour samples. Crude protein (%) and ash contents (%) increased post-treatment in maize flours, but decreased in other samples. Millet flours had the highest crude fibre content (4.54%), while cassava flour had the lowest (0.90%) (Table 1). Table 2 presents the mean number of *T. castenium* larvae that emerged in the treated flours. At 21 DAT, *T. castaneum* larvae developed on all flour types. The mean population of *T. castaneum* larvae on wheat (15.63) and cassava (11.75) flours were not significantly different from each other, but were significantly ($P< 0.05$) higher when compared with the other substrates. The mean number of emerged *T. castaneum* pupae in treated flours in storage is shown in Table 3. The development of *T. castaneum* pupae was observed in all flour types at 21 DAT. Wheat flour (13.88) had significantly ($P < 0.05$) higher population compared to others, while cassava had no pupal stage present. The mean number of adult *T. castaneum* in the infested flours is presented in Table 4. Some beetle emerged on the 21st day as well as on the 42nd and 63rd DAT. On the 63rd DAT, sorghum flours had the highest mean number of adult (7.75) followed by wheat flours (6.88), while cassava flours had no pest (0.00). There was significant ($P <.0.05$) difference between wheat, sorghum, millet and maize flours and cassava flours. The mean percentage mortality of *T. castaneum* in the stored flours is presented in Table 5. There was significantly ($P < 0.05$) higher mean percentage mortality was recorded on maize (1.90%), and the lowest was in wheat flours (0.25%). Irrespective of flour type, significantly ($P < 0.05$) higher mean mortality was observed at 21 DAT and none at 63 DAT.

The mean percentage weight loss in the flours infested with *T. castaneum* is presented in Table 6. There was significantly higher mean weight loss in maize flour (25%), followed by sorghum and wheat flours (10.00%), while millet and cassava flours were 8.75%.

Discussion

Results from this study revealed that all the flour types tested encouraged the growth and development of *T. castaneum*. Kayode *et al.* (2014) reported wide variations between flours with respect to the number of larvae, pupae and F_1 adults that emerged; the proximate analysis of the flours; level of susceptibility; mortality of adult *T. casteneum* and weight loss of the flours together reflect the ability of particular flour to resist pest attack. A similar assertion was made by Alison and Campbell (2020) that the type of flour has a significant effect on the number of eggs laid and progeny produced. At 21 DAT, *T. castaneum* developed on maize, millet, sorghum and wheat flours with the highest progeny on wheat flour and development continued till the 63rd day. According to Booth *et al.* (1990), *T. castaneum* takes about 20 days to develop on a good quality diet with other factors being optimal. This results also corroborates the findings of Mehmood *et al.* (2018), that maize flour infested with *T. castaneum* up to 90 days would be unsuitable for human consumption and animal feed. There was however no insect progeny development in the cassava flours which contradicts the report of Zakka *et al.* (2018). Nutritional contents such as fibre, protein and fat supported high infestation and consequent mortality. In a similar experiment by Cambron *et al*. (2019) on tobacco horn worm (*Manduca sexta)* concluded that high fat diet also led to lower body weight and higher mortality. The increased moisture content of any food is an index of its water activity and is used as a measure of stability and the susceptibility to microbial contamination (Okaraonye and Ikewuchi, 2009). The moisture content of all the samples might be attributed to moisture from the environment (Komolafe and Odeyemi, 2014). Carbohydrates serve as a source of energy and may be converted to fats for storage and to amino acids (Chapman, 1980). Carbohydrates are the major sources of metabolic energy for plant-feeding insects. From the results of this study, the high percentage of carbohydrate in the flours used in culturing *T. castaneum* makes them suitable for the growth and development of *T. castaneum*. Wheat and sorghum were the most susceptible to *T. castaneum* infestation and was followed by millet and maize. The least susceptible was cassava with no emergence. This may be due to the presence of low ether extract (fat and fatty acid), crude fibre and low protein which is responsible for the absence of the beetle progeny in cassava flour when compared to wheat or sorghum flours. Sarwar (2015) opined that the preference, growth and development of *T. castaneum* are greater on high protein content feeds. The mortality of adult *T. castaneum* was higher in maize flours which might be as a result of the high ether extract (fat and fatty acid), unlike in cassava flour which had a low percentage of ash. This assertion is buttressed by Komolafe and Odeyemi (2014) which report that the increase in percentage composition of ash content could be as a result of presence of dead insect parts which constitute the inorganic residue that added to percentage increase.There were significantly greater mean percentage weight losses (damages) occurred in the maize flours compared to wheat, sorghum, millet or cassava flours. A similar study by Turaki *et al*. (2007) in Maiduguri, Borno State, Nigeria showed that significantly higher *T. castaneum* progeny population was recorded in maize compared with millet flours. It is an indication that there could be more risk in storing maize flours, moderate risk in storing wheat and sorghum flours and less risk in storing cassava or/and millet flours. Information of how insect choose site for oviposition should provide management guide for mixed storage sites where there is a possibility of cross infestation. The assumption that if the wheat flour is infested with insects, the cassava flours must be infested too, and therefore, all these flours must be treated against the pest. However, it is likely that the cassava flour will not be infested and does not need to be treated. Information on susceptibility of these flour types can act to either inhibit or promote oviposition and the development of *T. castaneum* in these flours and applied as risk control tactic. The implication of this result is that households, merchants and food handlers storing large quantities of these flours can incur qualitative and quantitative losses caused by *T. castaneum* infestation without this baseline information.

Conclusion

This study has shown that maize, wheat, millet, sorghum and cassava flours are susceptible to attack by *T. castaneum* at varying degrees because the pest can successfully colonize, feed and develop on the flours. Flours from maize, sorghum and wheat had significant weight loss within a relatively shorter time.

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Table 1: Proximate analyses of cassava, corn, millet, sorghum and wheat flours at Pre- and Post-treatment

Parameter (Time)	Dry Matter (%)	Moisture (%)	Ash/ Mineral (%)	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Nitrogen Free Extract (%)	Metabolizable Energy (Kcal/Kg)
Millet FPre	92.31	7.69	3.53	9.69	2.60	4.56	71.93	2820.96
Millet FPo	90.14	9.86	3.68	9.87	2.73	4.54	69.32	2758.60
Wheat FPre	92.16	7.84	3.06	11.12	2.87	3.00	72.11	2848.58
Wheat FPo	90.07	9.93	1.38	10.29	2.13	3.06	73.21	2801.34
Sorghum FPre	92.28	7.72	4.21	8.68	2.87	2.00	74.52	2795.55
Sorghum FPo	90.10	9.90	1.58	7.04	2.35	2.00	77.13	2775.41
Cassava FPre	91.74	8.26	1.57	1.72	0.00	0.94	87.51	2687.55
Cassava FPo	89.95	10.05	0.79	2.64	0.00	0.90	85.62	2665.07
Corn FPre	92.30	7.70	2.59	6.61	3.03	2.47	77.60	2834.49
Corn FPo	90.11	9.89	3.59	8.33	3.10	2.40	72.69	2756.62

Millet, Wheat, Sorghum, Cassava and Sorghum flour (e.g., CFPre for cassava flour pre-trt.; CFP0 for cassava flour post-trt.)

Table 2: Mean number of emerged *Tribolium castaneum* larvae in maize, millet, sorghum, wheat and cassava flours stored for 0 and 21 days after treatment (DAT)

Crop	Storage Duration (Days)		Mean	LSD
	0	21		
Cassava	0.00	23.50	11.75	
Maize	0.00	19.75	9.88	
Millet	0.00	21.25	10.63	4.60
Sorghum	0.00	20.00	10.00	
Wheat	0.00	31.25	15.63	
Mean	0.00	23.15		
LSD	2.90			

Table 3: Mean number of *Tribolium castaneum* pupae in maize, millet, sorghum, wheat and cassava flours stored for 0 and 21 days after treatment (DAT)

Crop	Storage Duration (Days)		Mean	LSD
	0	**21**		
Cassava	0.00	0.00	0.00	
Corn	0.00	16.50	8.25	
Millet	0.00	18.00	9.00	3.19
Sorghum	0.00	15.00	7.50	
Wheat	0.00	27.75	13.88	
Mean	0.00	15.45		
LSD		2.02		

Table 4: Mean number of adult *Tribolium castaneum* in maize, millet, sorghum, wheat and cassava flours stored for 0, 21, 42 and 63 days after treatment (DAT)

Crop	Storage Duration (Day)				Mean	LSD
	0	**21**	**42**	**63**		
Cassava	0.00	0.00	0.00	0.00	**0.00**	
Corn	0.00	7.00	4.25	4.50	**3.94**	
Millet	0.00	6.50	3.75	7.75	**4.50**	**1.64**
Sorghum	0.00	20.00	3.50	7.50	**7.75**	
Wheat	0.00	12.50	7.50	7.50	**6.88**	
Mean	**0.00**	**9.20**	**3.80**	**5.45**		
LSD			**1.47**			

Table 5: Mean percentage mortality of adult *Tribolium castaneum* in maize, millet, sorghum, wheat and cassava flours stored for 0, 21, 42 and 63 days after treatment (DAT)

Crop	Storage Duration (Day)				Mean	LSD
	0	**21**	**42**	**63**		
Cassava	0.00	1.50	0.50	0.00	**0.50**	
Corn	0.00	8.75	0.75	0.00	**2.34**	
Millet	0.00	1.50	1.00	0.00	**0.63**	**0.60**
Sorghum	0.00	1.00	2.25	0.00	**0.81**	
Wheat	0.00	0.75	0.50	0.00	**0.31**	
Mean	**0.00**	**2.70**	**1.00**	**0.00**		
LSD	**0.54**					

Table 6: Mean weight loss (%) of flours infested with *Tribolium castaneum* at 63 days after treatment (DAT)

Crop	Cassava	Corn	Millet	Sorghum	Wheat
	8.75	25.00	8.75	10.00	10.00
LSD	**6.17**				