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Zoological Research

Resistance of Seven Biscuit Types to Infestation by *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: Seven biscuit types namely, Okin, Digestive, Cabin, Peanut, Cream crackers, Hobnobs and Glucose and wheat flour were screened for their resistance to *Tribolium castaneum* (Herbst) at ambient temperature of 28 ± 2 ℃ and 78 ± 2% relative humidity in the laboratory. 50 g of each biscuit sample were infested with four female and two male adult beetles and left for 15 days then remove for a resistant experiment and 70 days for a mortality experiment. Each treatment and the control without beetles were replicated three times. Results showed that there was significant difference ($P < 0.05$) in the mortality rate of *T. castaneum* in the biscuit types and wheat flour samples, while there was no significant difference ($P > 0.05$) in the weight loss of biscuit types. The highest adult mortality of beetles (100%) was obtained from Peanut biscuits at 28 days after infestation while there was only 5.5% adult mortality in wheat flour at 70 days after infestation. The susceptibility index was 0 for all the biscuit types since there was no adult emergence indicating that they were resistant to *T. castaneum* infestation. Resistance in the biscuit types could be due to chemical additives (e.g. sodium chloride and sodium bicarbonate) used in the production of biscuits, which may have inhibitory effect on the development of *T. castaneum* and also the low moisture content of the biscuits. Packages that can easily be perforated or damaged to allow absorption of moisture from the environment should not be used in packing biscuits.

Key words: *Tribolium castaneum*; Infestation; Mortality; Susceptibility index; Weight loss of biscuit

七种糕点对赤拟谷盗(鞘翅目:拟步甲科)感染的抗性

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关键词: *Tribolium castaneum*;感染;死亡率;易感指数;糕点重量损失

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Biscuits are flour – based products, which are consumed by majority of people, both old and young, throughout the world and most especially by children, teenagers and mid-adults (Majumder, 1970). It can be served as a desert or snacks and as a breakfast food when taken with beverages. Its ready-to-serve state and long shelf life contribute to its acceptability by the masses. Wheat as human food is used principally in the form of flour for baked products such as bread, biscuits, pastries and crackers (Kent, 1983). In the manufacture of biscuits, wheat flour, water, fat, sugar and other ingredients are mixed together. Biscuits, which differ from other confectionaries such as bread and cake because of its low moisture content, consist of different nutrients and vary from one biscuit type to another Holl et al (1988).

Insects destroy at least 5% of the world production of all cereal grains after they are harvested and while they are in storage on the farm, in elevators or in warehouses. Likewise, processed and packaged foods are also subjected to attack and unless frequent examinations are made and control measures initiated, serious damage may result in processing plants, wholesale warehouses, retail stores and homes (Davidson & Ly-

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on, 1979). In particular, warehouses and retail shops suffer from insect infestation of processed foods in Nigeria (Odeyemi et al, 2001). The high rate of reproduction and short development period has really enhanced the rapid development of insects from a small number of individual insects to a large mass (Odeyemi & Daramola, 2000). Very few species of insects are able to survive in extreme dry conditions as obtained in low moisture content food products. These insect species include *Oryzaephilus surrinamensis* (L.), *Cryptolestes ferrugineus* (Stephens), *Stegobium paniceum* (L.) and *Tribolium castaneum* (Herbst) (Haines, 1991). Processed flour products are mostly infested by *T. castaneum* in retail stores. The high performances of strains of the beetles in five flour products have been investigated by Via (1991). Ashamo (2002) also investigated the performance of *T. castaneum* in different flours.

In the present study, the level of resistance of different types biscuits to infestation by the flour beetle, *T. castaneum* was investigated.

1 Materials and Methods

1.1 Insect culture and source of biscuits

A laboratory culture of *Tribolium castaneum* (Herbst) was derived from infested wheat flour samples obtained from Oba's market, Akure, Nigeria. The culturing was done in a Kilner jar (1 litre) which was covered with muslin cloth held in place by rubber band to prevent the exit of beetles and to allow ventilation under open laboratory conditions of 28 ± 2℃ and 78 ± 2% relative humidity. Insect culture was maintained by continuously replenishing the devoured and infested wheat flour with un-infested flour. The jar was placed inside a wire mesh cage.

Seven biscuits include Okin, Digestive, Cabin, Peanut, Cream cracker, Hobnobs, and Glucose were obtained from supermarkets in Akure, Nigeria, or imported from other countries (Tab. 1). Also uninfested wheat flour was obtained and tested for infestation by *T. castaneum*. All the biscuit types contain using wheat flour as main ingredient, salt, sugar, and sodium bicarbonate while soya, lecithin, rolled oat, yeast, vegetable oil, fat and flavouring may be added. Not all the varieties contain all these additional ingredients but salt, sugar and sodium bicarbonate is present in all.

1.2 Assessment method

The moisture content of the seven types of biscuits and wheat flour samples was determined by placing 5 g of the ground powder of each sample in moisture dishes and drying in a ventilated oven at 105 ℃ for 4 h using the oven-drying method of AACC (1988). The final moisture content was also determined at the end of the experiment.

Test insects used for all experiments were virgin adults obtained from the stock culture and differentiated into sexes using the method of Halstead (1963) and Proctor (1971). Four female and two male adult beetles were introduced into 50 g of each sample in disposable plastic cups (200 mL) and covered with muslin cloth held in place by rubber band for aeration and to prevent exit of insects. This was replicated three times. A control without beetles was also set up and replicated three times. The infested samples were left for 15 days before the beetles were removed and discarded. The number of larvae per sample were then counted and recorded. Each trial replicate was terminated when the larvae died in each biscuit type and until there was no adult emergence in flour samples. At the end of the experiment, the final weight was taken for each sample and recorded.

The developmental period from oviposition to death of larvae was noted in each biscuit type and the period till adult emergence was noted for flour samples. The susceptibility index (SI) for the samples of biscuits and flour was calculated using Howe (1971) for externally developing beetles:

Tab. 1 Morphological characteristics and country of production of the biscuits and wheat flour used in this study

Type of biscuit	Colour	Shape	Mean size (length/diameter)	Country of production
Okin	Light, brownish, yellow	Round with holes on the surface	6.2 cm	Nigeria
Digestive	Light, brownish, yellow	Round with holes on the surface	7.4 cm	United Kingdom
Cabin	Light, brownish, yellow	Rectangular with perforated holes	6.4 cm	Nigeria
Peanut	Deep, brownish, yellow	Ring and rough surface	4.5 cm	China
Cream cracker	Very light, brownish, yellow	Rectangular with holes and swollenness	7.8 cm	Indonesia
Hobnobs	Deep, brownish, yellow	Round and rough surface	6.5 cm	United Kingdom
Glucose	Brownish, yellow	Rectangular	5.0 cm	Dubai
Wheat flour	White	Milled	40 μm diameter	Nigeria

$$SI = \frac{\log n/N}{D} \times 100$$

Where n = Number of F_1 adults, N = Number of adults introduced, D = Time from oviposition to emergence.

1.3 Adult mortality

Six adult beetles (0 – 24 h old, four females and two males) were introduced into 50 g of each seven biscuit type and flour sample contained in disposable plastic cups (200 mL) and covered with muslin cloth held in place by rubber band. This was replicated three times. The experiment was maintained at 28 ± 2 ℃ ambient temperature and 78 ± 2% relative humidity in the laboratory, and left until all the adults died. The mortality of the beetles was observed and recorded on the 14th, 28th, 42nd, 56th and 70th days. The final weight and moisture gain of the samples were calculated and recorded. Insects were presumed dead if they did not respond when probed at the abdomen with a pin.

1.4 Data analysis

All data were subjected to Analysis of Variance (ANOVA) as outlined by Steel & Torrie (1980) and the means which differ at 5% level of probability (P < 0.05) were further classified using least significance difference (LSD) test and Tukey's test.

2 Results and Analyses

In all the biscuit types tested, there was no adult emergence of *T. castaneum* beetles except in the wheat flour with 125 beetles (Tab. 2). Moreover, the wheat flour had the highest mean number of larvae (186) while that of Okin and Digestive biscuits were 5.5 and 3.0 respectively, and the developmental period for larvae varied from 14 days in wheat flour to 28 days in Digestive biscuit (Tab. 2). All the larvae died before pupation.

Since no adult beetles emerged from the biscuit types there was zero SI, but 3.77 from the wheat flour (Tab. 2). This showed that the biscuit types did not favour the development of *T. castaneum* while the flour sample was not resistant to the beetle attack. Results obtained from percentage moisture content shows that there were no significant differences ($P > 0.05$) in the biscuit types.

Tab. 2 Mean adult emergence, larval development and susceptibility index of biscuit types and wheat flour subjected to infestation by *T. castaneum*

Sample	Adult emergence (mean ± *SE*)	Number of larva (mean ± *SE*)	Developmental period of larva (d)	Susceptibility index
Okin	0.0 ± 0.0^a	5.5 ± 1.8^b	21	0
Digestive	0.0 ± 0.0^a	3.0 ± 1.3^b	28	0
Cabin	0.0 ± 0.0^a	0.0 ± 0.0^a	—	0
Peanut	0.0 ± 0.0^a	0.0 ± 0.0^a	—	0
Cream cracker	0.0 ± 0.0^a	0.0 ± 0.0^a	—	0
Hobnobs	0.0 ± 0.0^a	0.0 ± 0.0^a	—	0
Glucose	0.0 ± 0.0^a	0.0 ± 0.0^a	—	0
Wheat flour	125.0 ± 9.8^b	186.0 ± 8.9^c	14	3.77

Means followed by the same letter are not significantly different $P > 0.05$ from each other using Tukey's test.

At the end of 70 days, it was found that all the biscuit types absorbed moisture from the environment. The percentage moisture gain in the biscuit types was highest in Okin with a value of 5.91% and least in Digestive with a value of 2.15% (Tab. 3). The highest weight loss of 2.24% was obtained from Okin biscuit while the lowest of 0.01% from Peanut biscuit.

The percentage mortality of *T. castaneum* varied in the biscuit types and flour (Tab. 4). There was significant difference ($P < 0.05$) in the mortality rate of the beetles in the biscuit types and wheat flour. The highest mortality of 100% was recorded for Peanut at 28th days while Hobnobs, Okin and Digestives had mean mortality of 91.66% at the 70th day. Based on the percentage mortality obtained at 70 days of infestation, the order of resistance to *T. castaneum* in the biscuits and flour can be summarized as: Peanut > Cream cracker > Cabin > Glucose > Digestive > Hobnobs > Okin > Wheat flour (Tab. 4).

3 Discussion

Resistance to *T. castaneum* in biscuit types in this study was evaluated on the basis of adult weevil emergence and mortality, larval development, weight loss and index of susceptibility. Using the above parameters, Cabin, Peanut, Cream crackers, Hobnobs and Glucose type biscuits, were very resistance to *T. castaneum* infestation and Peanut appeared to be the most resistance with 100% beetle mortality at 28 days after infestation. The weight loss in Peanut (0.01%) was the

Tab. 3 Weight loss and moisture content of biscuit and flour samples

Sample	Initial moisture content (%)	Moisture gain (%)	Weight loss (mean ± *SE*, %)
Okin	3.52	5.91	2.24 ± 0.12[a]
Digestive	5.51	2.15	1.41 ± 0.22[a]
Cabin	4.38	3.10	0.04 ± 0.01[a]
Peanut	4.28	3.30	0.01 ± 0.01[a]
Cream cracker	4.11	3.26	0.17 ± 0.01[a]
Hobnobs	3.44	3.15	1.59 ± 0.23[a]
Glucose	3.77	5.25	1.22 ± 0.19[a]
Wheat flour	11.49	—	6.32 ± 0.48[b]

Means followed by the same letter are not significantly different $P > 0.05$ from each other using Tukey's test.

Tab. 4 Mortality of adult *T. castaneum* in different types of biscuits and flour samples

Sample	Adult mortality in days (%)				
	14	28	42	56	70
Okin	0.00[a]	0.00[a]	66.66[b]	91.66[c]	91.66[b]
Digestive	0.00[a]	29.17[b]	87.50[d]	91.66[c]	91.66[b]
Cabin	0.00[a]	54.17[c]	87.50[d]	95.83[c]	95.83[b]
Peanut	29.17[c]	100.00[e]	100.00[e]	100.00[d]	100.00[c]
Cream cracker	12.50[b]	70.83[d]	100.00[e]	100.00[d]	100.00[c]
Hobnobs	0.00[a]	8.33[a]	70.83[c]	91.66[c]	91.66[b]
Glucose	0.00[a]	8.33[a]	83.33[d]	87.50[b]	95.83[b]
Wheat flour	0.00[a]	0.00[a]	0.00[a]	0.00[a]	5.53[a]

Means followed by the same letter are not significantly different from each other ($P < 0.05$) using the least significant different (LSD) test.

lowest.

The resistance of stored foods to attack by insects is influenced by several factors. Some of these factors are presence of toxic alkaloids or amino acids in some products, insect feeding deterrent and digestive enzyme inhibitors (Adesuyi, 1979). In the nutrition of *T. castaneum*, certain organic and inorganic salts are inhibitory to its development (Majumder, 1970). In this study all the biscuit types were resistant to *T. castaneum* development with zero index of susceptibility. This could be as a result of some organic and inorganic salts such as sodium chloride and sodium bicarbonate contained in them as outlined by Holl et al (1988).

In contrast to the biscuit, the wheat flour was found to be highly susceptible to the beetle. Ashamo (2002) observed that *T. castaneum* beetles reared on wheat flour had the highest hatchability of 93.3% and the highest number of adults when compared to beetles reared on sorghum, maize, cassava, and mixture of cassava/sorghum and mixture of maize/sorghum. Chemical compounds that are unlikely to be toxic in human diet at low concentration could be added to the wheat flour during production before their release for sale to consumers so as to prevent or at least reduce the susceptibility to *T. castaneum*. Chemicals or additives such as sodium chloride and sodium bicarbonate used in the manufacturing of biscuits could be used in flour production and in the manufacture of other biscuits that are susceptible to *T. castaneum* infestation.

Low relative humidity could prolong developmental period of storage insects (Haines, 1991). In this study, this seems to have influenced the difference obtained in development of *T. castaneum* between the wheat flour sample and biscuit types. The biscuit types were in dry state with low moisture content (range 3.44 – 5.51%) while the wheat flour had higher moisture content (11.49%) than any of the biscuit types.

In the experiment, it was observed that the biscuit types absorbed moisture. Packages, which can easily be perforated or damaged to allow absorption of moisture from the environment, should not be used in packing biscuits. Maintaining proper hygiene by factory workers in flourmill companies in order to ensure insect pest free products is also very essential (Odeyemi & Daramola, 2000). Warehouses and retail shops should control the storage temperature and prevent condensation in the store, which could favour beetle development.

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PACAP 前体基因在人类起源过程中的快速进化研究取得进展

垂体腺苷酸环化酶激活多肽（Pituitary adenylate cyclase-activating polypeptide，PACAP）是一种广泛表达于中枢神经系统并参与神经发生和神经元信号转导的神经多肽。PACAP 的氨基酸序列在整个脊椎动物中是相当保守的，表明其功能在进化过程中是受到强烈限制的。然而，中国科学院昆明动物研究所宿兵研究员领导的比较基因组学研究组最新的研究结果对这一问题的看法进行了修正。他们的研究结果认为自从人与黑猩猩的共同祖先分离之后，PACAP 前体基因在人类进化过程中经历了快速的进化，导致了人类中 PACAP 前体基因的氨基酸替换速率至少是其他哺乳动物中的 7 倍。另外，他们还注意到从啮齿类到非洲大猿中非常保守的 11 个氨基酸位点上却发生了人类所特有的氨基酸改变。他们认为这种人类特有的氨基酸改变可能是 PACAP 前体基因在人类进化过程中受到强烈的达尔文正向选择的结果。蛋白质结构分析进一步表明，在人类进化过程中可能产生了一种新的并开始在人类大脑中起作用的神经多肽。该项研究结果一经发表，就引起了科学家的高度关注。最新一期的 *Science Nows*（20 April 2005，http：//sciencenow. sciencemag. org/cgi/content/full/2005/420/4）对该项研究结果做了详细报道，并约请美国芝加哥大学的遗传学家 Bruce Lahn 博士和法国卢昂（Rouen）大学的神经多肽学家 Hubert Vaudry 博士对此项研究结果进行了评论。认为宿兵研究组的研究结果是令人鼓舞的，对以前未知蛋白质的激活过程的认识提供了新的视角，对人类大脑进化仅有的几个重要候选基因增添了新的成员。该项研究结果发表在 *Genetics*（published online ahead of print April 16，2005. 10.1534/genetics. 105.040527），论文标题为“Accelerated Evolution of the PACAP Precursor Gene during Human Origin”(论文连接：http：//www.genetics.org/aheadofprint.shtml)。