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CHEMICAL COMPOSITON OF MUSHROOM GLANDS OF ADULT MALE COCKROACHES (*PERIPLANETA AMERICANA*) FROM DIFFERENT MICROHABITATS IN ABEOKUTA, NIGERIA

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Abstract – Mushroom gland is an accessory gland of male cockroaches responsible for sperm enrichment. Its content is affected by sex, age, diet, habitats and others. However, the influence of microhabitats inhabited by cockroaches on the mushroom gland properties had not received much research attention. The goal of this study is to investigate the chemical composition of the mushroom gland of male cockroaches from different locations in Abeokuta. Twenty (20) adult male cockroaches each were collected from four different locations (grocery store, toilet, kitchen, dump site) where they are commonly found in Abeokuta, Nigeria. The mushroom glands of cockroaches were dissected and analyzed for organic (total protein, glucose, cholesterol and triglycerides) and inorganic (Na^+, K^+, Mg^{2+}, Ca^{2+}) contents through standard methods. Data collected were analyzed by One-way Analysis of Variance and significant means were separated by Student-Newman-Kuel (SNK) test. Mushroom gland of cockroaches from the kitchen had significantly higher glucose, cholesterol and triglycerides (5.63, 40.4 and 87.4 mg/dl respectively) than other locations. Similarly, inorganic contents of mushroom gland of cockroaches from kitchen had higher Ca^{2+} and Mg^{2+} concentrations than those of other locations. Triglycerides and Na^+ had the highest concentrations in the mushroom gland. In conclusion, kitchen environment provides its cockroaches the nutrients for better reproductive potential than other locations.

Key words: cockroaches, mushroom gland, metabolites, microhabitats

Izvleček – KEMIČNA SESTAVA GOBASTIH ŽLEZ ODRASLIH SAMCEV ŠČURKOV VRSTE *PERIPLANETA AMERICANA* IZ RAZLIČNIH MIKROHABITATOV V ABEOKUTI, NIGERIJA

Gobasta žleza je pomožna žleza samcev ščurkov, odgovorna za obogatitev sperme. Na njeno vsebino vplivajo spol, starost, prehrana, okolje in drugo. Vendar vpliv mikrohabitatov, ki jih ščurki poseljujejo, na lastnosti gobastih žlez ni bil deležen dovolj raziskovalne pozornosti. Cilj naše raziskave je bil ugotoviti kemično sestavo gobastih žlez samcev ščurkov iz različnih krajev v Abeokuti. Po dvajset odraslih samcev je bilo zbranih na štirih različnih krajih (trgovina z živili, stranišče, kuhinja, smetišče), kjer jih lahko redno najdemo v Abeokuti, Nigerija. Gobaste žleze ščurkov smo izrezali in v njih analizirali organske (skupne beljakovine, glukoza, holesterol in trigliceridi) in anorganske (Na^+, K^+, Mg^{2+}, Ca^{2+}) sestavine s standardnimi metodami. Zbrane podatke smo analizirali z enosmerno analizo variance in značilne vrednosti določili s Student-Newman-Kuel (SNK) testom. Gobasta žleza ščurkov iz kuhinje je imela značilno višje vsebnosti glukoze, holesterola in trigliceridov (5,63, 40,4 in 87,4 mg/dl) kot z drugih lokacij. Podobno je imela vsebnost anorganskih snovi v žlezah ščurkov iz kuhinje višje koncentracije Ca^{2+} and Mg^{2+} kot tiste z drugih lokacij. Trigliceridi in Na^+ so imeli v gobastih žlezah najvišje koncentracije. Sklepamo, da kuhinjsko okolje ščurkom zagotavlja hranila za boljši reproduktivni potencial kot drugi kraji.

Ključne besede: ščurki, gobasta žleza, metaboliti, mikrohabitati

Introduction

There are about 3,500 species of cockroaches found on every continent, except Antarctica, they are among the most primitive and successful pterygote insects. Among the most common species, the American cockroach, *Periplaneta americana* happens to be the largest in size with an average length of 4 cm (Oyebanji *et al.,* 2014). Adults are reddish-brown in appearance with a pale-brown or yellow band around the edge of the pronotum (Oyebanji *et al.,* 2014). They are found majorly in basements, sewers, steam tunnels and drainage systems (Rust *et al*., 1991) and they feed on almost anything they come across, including papers, boots, hair, bread, fish, fruit, dead insects, cloth etc., thereby causing economic loss (Bell and Adiyodi 1981).

Cockroaches are found in groups and in warm and dark places and are common in toilets, bathrooms, kitchens, dining and bedrooms. The American cockroach (*Periplaneta americana*) is the largest species which is capable of surviving up to two years, much longer than any other cockroach species (Brenner 1995). Most of the roaches' activities like feeding, mating and locomotion are performed and peaked at night (Iyeh *et al*., 2021).

Mushroom-shaped gland or utricular is a large accessory reproductive gland, whitish in colour and situated at the junction of vasa deferentia with the ejaculatory duct. It has a mass of glandular tubules of three kinds: the peripheral long tubules or utriculi majores, the central tubules or utriculi breviores and the seminal vesicles filled with sperms (Bell *et al*., 2007). Mushroom gland is present in the 6th and 7th abdominal segments of a male cockroach. It acts as an accessory reproductive gland. Its functions include forming a protective layer of the spermatophore and nourishing the sperms.

In Asia and other continents, cockroaches are considered as feed source for livestock due to high nutritive value and ability to culture easily. Ademolu *et al.* (2020) reported a significant difference in the protein and mineral contents of cockroaches from different microhabitats, suggesting that locations where they live and breed influence their nutritive value. Hence, we hypothesized that microhabitats can influence the reproductive capability of its cockroaches inhabitants. As the first step towards testing this hypothesis, the goal of this study is to determine the chemical composition of the accessory mushroom gland of adult male cockroaches from different microhabitats.

Materials and methods

Sample Collection

Adult male cockroaches were captured (at night time) from four different microhabitats (kitchens, toilets, grocery stores and dumpsites) at Camp Area of Abeokuta by hand picking method using sterile gloves and transferred to perforated plastic containers.

Preparation of Sample

The collected samples were taken to the laboratory few hours after collection for further processes. On the arrival of the cockroaches at the laboratory, twenty (20) male cockroaches from each location were put into an envelope and kept in the freezer for 10 minutes to make them immobile. The immobile cockroaches were dissected to extract their mushroom glands according to methods described by Oyebanji *et al* (2014) and the glands were later processed for chemical analysis.

Mineral composition analysis

The mushroom gland samples were carefully extracted from the dissected cokroaches and 0.5g of the gland was homogenised in 5mls of distilled water and the homogenate was used for the mineral analysis. Five µl of the homogenate were wet digested with concentrated nitric and percholoric acids. Calcium (Ca) and magnesium (Mg) contents were determined by Atomic Absorption Spectrophotometer (Model 3030 Perkin Elmer, Nortwalk, USA). Potassium (K) and Sodium (Na) were determined with the aid of Corning 400 Flame Photometer according to the methods of A.O.A,C,(1990).

Determination of Total protein, Cholesterol and Glucose

The protein concentration of each sample was determined using Biuret method as described by Henry *et al*. (1974). The glucose content was determined by the Colorimetric method of Baumniger (1974). The lipids assay was done following the protocols of Grant *et al*. (1987). All the analyses were done in triplicates (three different samples were taken from each microhabitat).

Data from the experiments were analyzed by One-way Analysis of Variance and tested for significance by Student-Newman –Kuel (SNK) test.

Results

The mushroom glands of adult male cockroaches from different microhabitats are rich in organic substances. The results showed the presence of glucose, protein, cholesterol and triglycerides in the mushroom glands, though occurring at different concentrations. Mushroom gland of cockroaches collected from the kitchen had significantly higher glucose, cholesterol and triglycerides than those from other microhabitats. Conversely, mushroom glands of insects from dumpsite recorded least values (Table 1). It is noteworthy that lipids (triglycerides and cholesterol) concentrations in the mushroom gland were significantly higher than that of protein and glucose (Table 1).

Table 1: Organic composition of mushroom glands of adult male cockroach (*Periplaneta americana*) from different microhabitats

Location	Total Protein (g/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Grocery Store	1.60 ± 0.17^{a}	4.06 ± 0.60^{b}	25.7 ± 0.55^{b}	71.5 ± 0.86^{b}
Toilet	0.70 ± 0.02^{c}	3.60 ± 0.10^{bc}	23.2 ± 0.45^{c}	32.2 ± 0.86^{c}
Kitchen	0.80 ± 0.10^{c}	5.63 ± 0.25^{a}	40.4 ± 0.45^{a}	87.4 ± 0.55^{a}
Dump Site	1.23 ± 0.15^{b}	3.03 ± 0.15^{c}	22.6 ± 0.20^{c}	22.7 ± 0.36^{d}

Mean values with same superscript across the column are not significantly different ($p>0.05$)

Table 2 shows the inorganic composition of the mushroom gland of adult male cockroaches from different locations. There was significant difference in the concentration of inorganic substances in the mushroom glands from different locations. The mushroom glands of roaches from the kitchen had the highest calcium and magnesium concentrations while the dumpsite had the highest sodium concentration. Sodium recorded the highest concentration of all the minerals analyzed in the mushroom gland while magnesium had the least.

Table 2: Inorganic composition of mushroom gland of adult male cockroach (*Periplaneta americana*) from different microhabitats

Location	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dl)	Magnesium (mg/dl)
Grocery Store	13.7 ± 0.47^{d}	8.76 ± 0.90^{a}	0.52 ± 0.09^{c}	1.23 ± 0.25^{b}
Toilet	23.2 ± 0.25^{b}	3.33 ± 0.41^{d}	3.16 ± 0.40^{b}	1.46 ± 0.35^{b}
Kitchen	22.1 ± 0.40^{c}	4.46 ± 0.32^{c}	8.93 ± 0.25^{a}	2.06 ± 0.20^{a}
Dump Site	46.0 ± 0.20^{a}	5.93 ± 0.15^{b}	3.40 ± 0.26^{b}	1.26 ± 0.35^{b}

Mean values with same superscript across the column are not significantly different ($p>0.05$)

Discussion

The mushroom gland is a male cockroach accessory gland, which is responsible for nourishment of sperms with necessary nutrients transferable to the female cockroach during mating (Chapman, 1990). The present study agrees with this role of mushroom gland as it is rich in both organic and inorganic substances which are highly needed in reproductive activities of insects.

The mushroom gland of cockroaches collected from the kitchen had higher nutrients than those from other habitats. Cockroaches are generally found around food crumbs and leftovers as well as around humid environments (Bell *et al*., 2007). The left over foods and remnants are sources of nutrients to the cockroaches and expectedly the tissues accumulate the nutrients. Ademolu *et al.* (2020) similarly observed that cockroaches found in kitchen areas had higher nutritive values than those collected from other venues. Higher nutrients in the mushroom accessory gland suggests that more metabolites are available in the tissue for reproductive processes and this might explain higher number of cockroaches in the kitchen (apart from being attracted by the crumbs) as the cockroaches lay higher number of viable eggs. The amount of food taken by the grasshopper *Zonocerus variegatus* affected the accessory gland protein content during first week after emergence (Muse, 1993).

Mating is an energy sapping process that costs insect reproductive tissues nourishments (Ademolu *et al*., 2011 and Chapman, 1990). The presence of higher concentrations of triglycerides and cholesterol in the mushroom gland confirm this fact. Cholesterol is the major sterol form synthesized by insects and it's derived from the plant based diets (Jing and Behmer, 2020). Lipids are energy sources, which are highly needed during mating (especially by male insects). The mushroom gland is involved in the secretion of the spermatophore which is discharged during copulation. During mating, the accessory gland protein of *Z. variegatus* dropped by 17.6% (Muse, 1993).

The presence of inorganic substances (minerals) in the mushroom gland and higher concentration of sodium reflects the plant based diets of the cockroaches. Most of the materials consumed by the insects are leftovers from students hostels which are basically plant based (cereals and vegetables). Magnesium and calcium play key roles in oviposition and egg production and being present in mushroom gland makes available in the semen for transfer to the eggs in the female cockroaches during mating.

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