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Microflora associated with the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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A number of bacteria and fungi were isolated from adults of *Tribolium castaneum* Herbst, (Coleoptera: Tenebrionidae). The investigation of bacteria was isolated more numerous in the insects than in the food from which the insects were taken. The microbes were cultured from the *Tribolium castaneum* on nutrient agar for bacteria and potato dextrose agar for fungi which was incubated at about 37ºC for 48 hours before observation. A variety of microorganisms was found which include the pathogenic bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli, Enterobacter* spp., and pathogenic fungi, *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Penicillium* spp., *Fusarium* spp. *and Rhizopus oryzae*. Moreover, non-pathogenic bacteria were recovered including *Bacillus subtilis*.

Key words: Microorganism, isolation, *Tribolium castaneum*, mycotoxins

Introduction

Tribolium castaneum is a cosmopolitan species causing considerable loss as a storage pest. It is one of the most common tropical beetle pests of stored products and major pest of cereals and a secondary pest of stored wheat (Cotton, 1963). It causes considerable losses as a storage pest in Asian countries (Prakash and Rao, 2003). *T. castaneum* is one of the most common species attacking farm-stored maize in Georgia and South Carolina, U.S.A. (Horton, 1982; Arbogast and Mullen, 1988). *T. castaneum* is a major pest of stored peanut in many parts of the world, it feeds preferentially on the germ and only after the germ is destroyed does it feed to any extent on the cotyledons (Appelbaum, 1969). Cashew kernels are a high value Agricultural commodity. Processed cashew kernels are highly susceptible for infestation by insects like *Tribolium castaneum, Lasioderma sericorne and Ephistia cautella* (Singh,

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1986; Sundararaju, 1993). Of these *T. castaneum* is the most serious pest (Singh, 1986), accounting for nearly 99% insect infestation concerns of the cashew processing industry. Under favourable conditions, it has a short generation time of about 20 days and a high rate of multiplication.

Insect and fungal infestation of food commodities is a common problem due to some agricultural practices that lead to fungal contamination. These spoilage agents lead to the deterioration of the food commodities manifested by loss of weight, nutritional value and toxicity by production of mycotoxins. Allotey and Odamtten (1996) reported that mycoflora such as *Aspergillus, Penicillium* and *Cladosporium*, rest hidden in maize grains that can serve as nutrient sources for insect development. The storage fungi normally accompany or follow insect infestation (Miller, 1995).

Contamination of food commodities is further aggravated by poor practices of post-harvest handling (Mpuchane and Siame, 1998).The generation of metabolic heat and water by insects in stored foods also increases the water activity (aw) and temperature of the maize flour to levels suitable for fungal growth and multiplication (Mills, 1986; Milton and Pawsey, 1988 and Sauer, 1988). The presence and activities of these spoilage agents lead to deterioration of the food commodity, which is manifested by off odours, discolouration, heating, caking, rancidity and toxicity through production of mycotoxins (Sinha *et al.,* 1988). The objective of the study was to isolate and identify the micro-organisms presented in the *Tribolium castaneum*.

Materials and methods

Ten adult beetles *Tribolium castaneum* were taken from the laboratory culture was ground in pre-chilled pestle and mortar with 1 ml of cold distilled water. The insect suspension was streaked on to Nutrient agar (NA) plates for bacterial isolation and potato dextrose agar (PDA), plates for fungal isolation. Nutrient agar plates were incubated at 370C for 48 hours and PDA plates were incubated at room temperature for 3-5 days. After incubation different types of colonies were observed. Bacterial and fungal colonies showing different size, shape, colour, etc. were selected for identification.

Identification of isolates

To identify the bacteria growth on the agar media, tests were carried out to determine their biochemical and morphological characteristics. Gram staining was carried out according as described by Baker (1967). Motility test was according to the technique described by Humphrics (1974). Starch hydrolysis and urease production was according to technique of Harrigan and

McCance(1976). Methyl Red Voges-Proskaeurnd (MRVP), oxidase and catalase test were according to Olutiola (1991), while indole test was according to the specification of Cruickshank *et al.* (1965). Identification of fungi was done according to Gilman *et al.* (1957).

Results and discussion

Adult flour beetles (*Tribolium castaneum)* infested and contaminated the flour giving a persistent and disagreeable odour turning the flour pinkish adversely affecting the viscous and elastic properties of the flour and creates a disgusting task. This was due to the accumulation of the quinones given off by the adults and taken up by the flour. The risk of mold and mycotoxin contamination of a food commodity depends on a complex interaction of several factors, which including moisture content, temperature, fungal species composition, their interactions with insects and previous storage history (Mills, 1986 and Miller, 1995). Moisture has also been found to be an important factor in attracting *T. castaneum* to wheat flour (Willis and Roth, 1950). The interaction of insects with each other and their environment under favourable temperature and grain moisture content usually maximizes biological activity, leading to rapid floral and fauna succession and deterioration of stored products. From this study, prolonged activity of *T. castaneum* population and their interactions generated additional moisture in the environment, which in turn promoted greater microbial activity. Clerk and Badu-Yeboah (1966) stated that kernels are frequently contaminated with the molds especially the storage molds *Penicllium, Aspergillus,* and *Fusarium.*The mold composition changes from "field fungi to "storage fungi" from harvest through processing to storage. In the case of *A. flavus, A. parasiticus, T. castaneum* either promotes its multiplication or is a carrier of its spores and hyphae/ mycelium thus encouraging its dissemination and also reported that *A. flavus* and *A. parasiticus,* are among the *Aspergilli* found in the gut of *T. castaneum.* They also noted that the longer a species of fungi survives in the gut the greater the chance of it being carried over considerable distance before being voided in faecal pellets.

Even in dry grain, insect activity creates a moist microclimatic within the infested kernel or cotyledon. The inoculums were carried by stored product insects and the epiphytic and endophytic microflora on and in the grains begin to consume the food part of the grain, produce toxins and degrade quality. The adverse condition changed in grain quality that inflicted by various agents. Insects produce uric acid, inoculate fungi and bacteria and leave faecal matter and cast off skins on the grain creating a foul odour. Quinone and other harmful

substances may be produced. Many fungi form dangerous mycotoxins as stated by Majumdar (1970).

The red flour beetle, *T. castaneum* is a major insect pest of stored food. From the present study, it indicated the role and importance of *T. castaneum* in affecting bacterial and fungal population. The isolates of microorganism found in *Tribolium castaneum* as shown in Table 1. The essence was to determine, if these microbes are pathogenic or non-pathogenic. It is therefore necessary to determine the type of microorganisms that can be found around the *T. castaneum*. It was observed that the bacteria isolates recovered were mostly gram-positive bacteria. Only *P.aeruginosa* was gram-negative. The bacteria recovered were found to be mainly rod shaped bacteria (Bacilli) and sphere shaped bacteria (Cocci) and they are all aerobic. They all show acidic response towards glucose, lactose, raffinose, sucrose, maltose and xylose test. Most of these microbes are pathogenic except *B. subtilis, P.aeruginosa, B.cereus, S. aureus*. The fungi recovered were *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus oryzae, Fusarium* species, which are pathogenic that is liable to produce mycotoxin (Table 2).

Some stored–product insects carry fungal spores on their bodies, and these spores may be introduced in to stored food. The infested stored food as feed supplement for livestock is a common practice by local farmers. Microbes such as *S.aureus, P.aeruginosa, Aspergillus* sp.and *B.cereus* are liable and can cause various infectious diseases in livestock. *S.aureus* is capable of causing toxigenic food poisoning and some other infectious diseases which would result in diarrhea (Akonai *et al.,* 1991; Nawigen and Koenig, 1981).

Therefore, to control *T.castaneum* population in stored kernels, maize flour and cereals in general and in turn to reduce the microbial population were shown. *T.castaneum* can be controlled by use of low temperatures. For example, at 90ºC *T.castaneum* has been found to have an LT 50 of 0.9 weeks for non-acclimatised adults and an LT 50 of 3.1 weeks for acclimatized adults which reported by Evans (1983). Exposure at 15ºC was found to kill all newly hatched *T.castaneum* but they were more resistant at 2 weeks with a survival rate of 80%. At four weeks they had a survival rate of 20%. (Howe, 1962).The total insect mortality occurred with an exposure period of 180 minutes at 80 ºC (Mark *et al.,* 2000).

Table 1. Characterisation of microorganisms isolated from *Tribolium castaneum* on Nutrient Agar

Sample No.	Medium	Gram stain	Shape	Motility	Catalase	Oxidase	Campulase	Urease	Methyl red	Voges proskaeur	Starch hydrolases	Oxygen relationship	Indole	Glucose	Lactose	Raffinose	Sucrose	Maltose	Xylose	Probable Identification
1	Nutrient Agar	+	S	_	+	_	_	_	_	_	+	A	_	A	A	A	A	A	A	*S.aureus*
2	Nutrient agar	+	R	+	_	_	_	_	_	_	+	A	_	A	AG	A	A	A	A	*B.cereus*
3	Nutrient Agar	_	R	+	+	_	_	_	_	_	+	A	_	A	A	A	A	A	A	*P.aeruginosa*
4	Nutrient agar	+	R	+	_	_	_	_	_	_	+	A	_	A	A	A	A	A	A	*B.subtilis*
5	Nutrient Agar	_	R	+	_	_	_	_	+	_	+	A	+	A	AG	A	A	A	A	*E.coli*

Key: Positive (+), Negative (-), Sphere (S), Rod (R), Acid (A), Gas (G), Aerobic (A)

Table 2. Fungal identification isolated from the *Tribolium castaneum* on Czapek dox agar and Sabouraud dextrose agar

Sample	Fungi isolated	Description of the isolates
Adult*Tribolium castaneum* on Sabouraud dextrose agar	*Rhizopus oryzae*	Rapidly growing while coloured fungus later colour changed to grey, swarms over entire plate. Aerial mycelium colony and fuzzy.
Sabouraud dextrose agar	*Fusarium* spp	Grow rapidly and produce woolly to cottony, flat, spreading colonies. The colour of the colony may be white, cream,yellow,red,violet pink or purple.
Czapek dox agar.	*Aspergillus niger*	White coloured fungus later colour changed to greyish brown and then brownish to black.
Czapeck dox's agar.	*Aspergillus flavus*	White coloured fungus later colour changed to greenish yellow.
Czapeck dox's agar	*Aspergillus fumigatus*	Showing typical blue green surface pigmentation with a suede like surface consisting of a dense felt of conidiophores.
PDA agar	*Penicillum* spp	Reddish pigmentation can be observed and the culture was flat green with slow growth rate.

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