DEVELOPMENT AND COMPETITION IN RICE WEEVIL, *Sitophilus oryzae* (L.) AND RED FLOUR BEETLE, *Tribolium castaneum* (Herbst), AND THEIR RESPONSES TO ACTIVE COMPOUNDS IN SELECTED SPICES.

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LIST OF ABBREVIATIONS

gm	grams
mg	milligrams
ml	millilitres
µl	microliters
RH	relative humidity
S. E.	Standard Error
m	meter
cm	centimeters
mm	millimeters
° C	degree Celcius
min	minutes()
h	hour
d	day
wk	week
N	Newton
SI	susceptibility index
PR	Percentage Repellency
ODW	Overall dried weight
TLC	Thin-Layer Chromatography
CC	Column Chromatography
GC/MS	Gas Chromatography/ Mass Spectrum

PERKEMBANGAN DAN PERSAINGAN DALAM KUMBANG BERAS, *Sitophilus oryzae* (L.) DAN KUMBANG TEPUNG, *Tribolium castaneum* (Herbst), DAN RESPONS MEREKA TERHADAP KOMPAUN AKTIF DI DALAM REMPAH TERPILIH.

ABSTRAK

Serangga hasil simpanan menyebabkan lebih daripada 20% jumlah kerosakan pada hasil simpanan di negara membangun dan lebih daripada 9% jumlah kerosakan di negara-negara maju setiap tahun. Keselamatan makanan adalah terancam terutamanya dengan peningkatan pesat dalam populasi dunia. Tesis ini bertumpu pada aspek kelakuan dan kawalan *Sitophilus oryzae* (L.) dan *Tribolium castaneum* (Herbst). Keduanya adalah antara dua serangga hasil simpanan yang paling biasa menyerang hasil simpanan di negara tropika. Satu kajian mengenai kadar perkembangan serangga ini di dalam medium yang berbeza telah dijalankan. *Sitophilus oryzae* didedahkan kepada beras perang, beras tempatan, beras parboiled, beras calrose, beras basmathi, beras merah, beras pulut hitam, dan beras wangi. Kumbang-kumbang tersebut lebih suka medium dengan kandungan karbohidrat yang lebih rendah tetapi kandungan abu dan serat yang lebih tinggi. Lapan jenis tepung dan kanji (tepung atta, tepung gandum, tepung naik sendiri, tepung beras, tepung kastard, tepung jagung, kanji ubi kayu, dan kanji kentang) digunakan untuk membela *T. castaneum* dan kumbang -kumbang itu membesar dengan baik di dalam medium yang mengandungi kandungan protein yang lebih tinggi, manakala kandungan karbohidrat yang tinggi memudaratkan perkembangan mereka. Persaingan intra- dan inter- spesies adalah bergantung kepada ketumpatan populasi permulaan. Dalam kajian intraspesies, fekunditi *S. oryzae* and *T. castaneum* berkurang apabila di atas dan di bawah ketumpatan populasi permulaan yang tertentu. Walau bagaimanapun, kajian interspesies mununjukkan bahawa peningkatan ke atas ketumpatan permulaan *T. castaneum* menyebabkan kesan negatif yang beransur-ansur ke atas perkembangan progeni *S. oryzae* tetapi tidak memberi kesan sebaliknya. Kedua-dua jenis serangga didedahkan kepada lima jenis rempah (*Elettaria cardamomum* (L.), *Brassica nigra* (L.), *Rosmarinus officinalis* L., *Capsicum frutescens* L., dan *Cassia auriculata* L.) dalam kedua-dua bentuk seluruh dan serbuk. Walaupun kadar kematian yang direkodkan tidak tinggi apabila serangga tersebut didedahkan kepada rempah dalam bentuk asal, tetapi *S. oryzae* didapati lebih lemah berbanding dengan *T. castaneum*. Di antara rempah yang diuji, *E. cardamomum* dalam bentuk serbuk adalah paling berkesan terhadap kedua-dua spesies serangga. Akhirnya, kajian untuk mengenalpasti kesan repelensi bahan ekstrak rempah terhadap serangga dijalankan. *Elettaria cardamomum* sekali lagi merupakan calon repelensi yang terbaik. Kehadiran hidrokarbon aromatik, ester, asid ester, asid karbosilik serta beberapa kompaun yang tidak diketahui mungkin menyumbang kepada ciri repelensi rempah tersebut.

DEVELOPMENT AND COMPETITION IN RICE WEEVIL, *Sitophilus oryzae* (L.) AND RED FLOUR BEETLE, *Tribolium castaneum* (Herbst), AND THEIR RESPONSES TO ACTIVE COMPOUNDS IN SELECTED SPICES.

ABSTRACT

Stored product insects causes more than 20 % in total damages to stored products in developing countries and 9 % in total damages to stored products in developed countries on a yearly basis. Food security is threatened especially with the rapid increase in world population. This thesis focuses on the behavioural and control aspects of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). Both are two of the most common stored products insect attacking stored products in tropical countries. A study on the insects' development rates in different mediums were carried out. *Sitophilus oryzae* were exposed to unpolished brown rice, local polished rice, parboiled rice, calrose rice basmati rice, red rice, black glutinous rice and fragrant polished rice. The weevils preferred mediums with lower carbohydrate contents but higher contents of ash and fibre. Eight types of flour and starch (atta flour, enriched wheat flour, self-rising flour, rice flour, custard powder flour, corn flour, tapioca starch, and potato starch) were used to rear *T. castaneum* and the beetles developed well in mediums with higher protein contents, while high carbohydrate contents were detrimental to their development. Competition between and within the species is dependent on the initial population density. In the intraspecific study, the fecundity of *S. oryzae* and *T. castaneum* was reduced above and below a certain initial population density. However, the interspecific study showed that an increase in initial density of *T. castaneum* has a gradual negative impact on the progeny development of *S. oryzae* but no effect vice versa. Both insect species were exposed to five spices (*Elettaria cardamomum* (L.), *Brassica nigra* (L.), *Rosmarinus officinalis* L., *Capsicum frutescens* L., and *Cassia auriculata* L.) in both whole and powdered form. Although high mortality was not recorded when the insects were exposed to the whole formed spices, but *S. oryzae* was more susceptible than *T. castaneum.* Among the spices tested, the powdered form of *E. cardamomum* was most effective against both insect species. Finally, a study to determine the repellency effects of extracted materials from the spices against the test insects was carried out. *Elettaria cardamomum* was again found as the best repellent candidate. The presence of aromatic hydrocarbons, esters, acid esters, carboxylic acids as well as some unknown compounds may have attributed to the repellent property of the spice.

CHAPTER ONE

GENERAL INTRODUCTION

About 80% of the food consumed by humans comes from grains (Pimental and Pimental 2008). Pimental (1997) stated that about 40% of world crop losses are attributable to infestation by pests. Insects are the main cause of grain loss (Gwinner et al. 1996) accounting to 15% of the total world crop losses (Pimental 1997). Gomez (2004) stated that the food industry at all levels suffer losses due to pest insects infestation. It is reported that the increase in pest numbers is largely dependent on the type of species that is attacking the product (Aulakh and Regmi 2013) and most of the damages caused by insect pests are results of direct feeding (Santos et al. 1990).

Post-harvest insect pests can be classified into primary pests which attack sound grains, while secondary insect pests infest damaged or broken grains. Grains previously damaged by pre-harvest pests and by primary storage pests enable easier access by secondary pests. Moreover, processed storage products are also susceptible to secondary pest attacks (Rees 2004). Coleoptera (beetles) is one of the main groups of insects that are of economic importance as post-harvest insect pests. Both the larvae and adult stages are destructive and the main culprit for damaging stored products (Sallam 2004).

Buzby and Hyman (2012) stated that food losses can be quantitative, qualitative or both. Quantitative loss refers to a loss in products' weight, while qualitative loss implies a reduction in nutrient value as well as changes to taste, colour, texture or presence of unwanted residues such as insect fragments and frass, and contamination by fungi of the stored product. Consequently, the values of the damaged grains to be sold, consumed or planted will be reduced (Sallam 2004).

A lot of contentions and assumptions over the scale of post-harvest losses, especially damages caused by insects have been made. The continuous increase in world population results in more people to feed, thus a decrease in such losses is vital and can assist in increasing the accessibility of food supply, especially to people in developing countries (Boxall 2001). The phasing-out and subsequent ban of methyl bromide used previously to manage stored product pest insects population has caused a worldwide search for other alternatives (Fields and White 2002). In developing countries such as Malaysia, the phasing out was scheduled in January 2015 (UNEP 2014). Over the years, the demand for novel control methods has intensified as concerns of the effects of control measures towards human health and the environment are surfacing (Mahdi and Rahman 2008).

Therefore, the objectives of this study were;

1. To determine the effects of different mediums on the development of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst).
2. To study the effects of population density on the intra- and inter-species competition of *S. oryzae* and *T. castaneum.*
3. To evaluate the potential of several spices as grain protectants against *S. oryzae* and *T. castaneum.*
4. To ascertain the contact and vapour repellent effect of extracted materials from several spices against *S. oryzae* and *T. castaneum* and isolation of effective and potential repelling compounds from the spice, *Elettaria cardamomum* (L.).

CHAPTER TWO

LITERATURE REVIEW

2.1 World population and world cereal production

In the year 2010, the world population was recorded at 6.9 billion, a 34 percent increase from 2.5 billion in 1950. The world population has grown with an annual rate of 1.1 to 1.2 percent which is about 78 million people per year from 2005 to 2010 (UN 2010). It is estimated that by the year 2050, the world population would reach 10.5 billion (UN 2013). According to the Population Reference Bureau (2012), less developed countries are more likely to have a greater increase in population growth. Therefore, most of the food demands would have come from those in poor parts of the world which is an additional 33 % of the current total world population.

Throughout the years, the upsurge in human population has urged for additional food supply which puts exceptional pressure on the food production industry (Evans 2009, Godfray et al. 2010) which calls for 60 % increase to sustain the food demand in 2050 (Alexandratos and Bruinsma 2012). In 2006, more than 87 percent of the total worldwide grain production consists of maize, wheat and rice (FAO 2006). The world's cereal production was recorded at about 2.475 billion tonnes in 2013 with world production of rice amounting to 745 million tonnes and wheat production at 711 million tonnes (FAO 2013). Nevertheless, food production as well as the distribution systems may not be or are still not sufficient to feed the ever increasing world population which can subsequently lead to food insecurities (FAO 1999). Therefore, the rise in worldwide demand for food is expected for at least another 40 years with the constant increase in population and food intake (Godfray et al. 2010). According to the UN (2001), an average of 2900 kcal per day is required by an adult person for their daily activities. It was reported that an average food of 3500 kcal/day is consumed by people in developed countries, while those in poor countries obtain 2000 kcal/day or less, causing incidents of malnourishment. Gilland (2002) stated that food production in the world can supply about 2800 kcal/day *per capita* and this amount is just about enough to sustain the world population but only if the statistics and calculations are accurate. It is also important to take into account livestock consumption that is recorded at 75 percent of the world cereal production (Alexandratos 1999, Gilland 2002).

The current food crisis situation is reportedly to be extremely alarming given the statistics. It was estimated that in 2010, 925 million people were still undernourished which amounts to approximately 16% of the total population in developing countries (FAO 2010). More recently, FAO (2012) reported that from year 2010 to 2012, around 12.5% of the world population (870 million people) suffers from malnutrition. Carvalho (2006) stated that the divergence between world food production and human population is expected to intensify until the year 2050.

FAO (1999) reported that besides economic and political attributes, environmental factors play a considerable role in upsetting the production of food crops around the world, especially in impoverished places. Agricultural lands are cleared to allow cultivation of plants for biofuels production and to construct housing to accommodate the growing population (Godfray et al. 2010). Crop production is also influenced by the change in climate, where rainfall pattern and temperatures can change the growing season of food crops. Moreover, food crops become more susceptible to pest attacks and diseases. This subsequently disrupts the food production system and also threatens food security (Gregory and Ingram 2000, Gregory et al. 2005, Parry et al. 2005).

Therefore, currently much effort is needed for more food yield and ensure food security to cutback poverty, malnutrition and improve human health and welfare (Carvalho 2006). It is essential to reduce post-harvest losses to maintain future global food security (Alexandratos and Bruinsma 2012). This is required to be carried out in an environmentally and socially sustainable way and one of the major challenges is to revise the approach in producing, storing, processing, distributing and accessing food (Godfray et al. 2010).

2.2 Stored product insect pests

2.2.1 General information

Stored products are materials in a dried form, such as cereals, pulses, dried seeds and root crops, which enables them to be stored for use at other times. One of the major causes for the drop in stored products' quality in places with hot and humid weather is due to the infestation by insects (Chomchalow 2003). Stored product insects have long been associated with human activities dating back to ancient Egyptian times (Rees 2004). A number of stored pests found in the late 20th century have even been reported to be uncovered from the tombs of the pharaohs (Levinson and Levinson 1998). Although many of the pests were initially limited in their distribution, activities of trading over thousands of years have caused them to disperse and spread throughout the world (Rees 2004). Moreover, their morphological, physiological and behavioural adaptations made worldwide infestations possible (Freeman 1973).

According to Rajendran (2002), more than 600 species of beetles and 70 species of moths, 355 species of mites, 40 species of rodents and 150 species of fungi have been recorded and identified to be related to the many types of stored products in existence. The insect pests of stored products have been identified to come mainly from three orders; Coleoptera (beetles), Lepidoptera (moths) and Psocoptera (psocids or booklice) (Rees 2004). Beetles can be categorized into about 110 to 115 families and amongst them, five of the families comprise of more than 20,000 species (Hedges and Lacey 1996). Although beetles are considered beneficial to the environment, some beetles are also pests of crops and stored products (Cramer 2006). Chomchalow (2003) reported that a substantial loss in terms of physical and nutritional value have been reported to be caused by weevils, bruchids and other insects, while Herbert (2009) stated that the main insect pests found to attack or infest stored products were reported to be the lesser grain borer, rice weevils, maize weevils, cadelle beetles, flat grain beetles, rusty grain beetles, sawtoothed grain beetles, foreign grain beetles, mealworm beetles, red flour beetles, confused flour beetles, Indian meal moths, and book lice.

Food security and household incomes can be greatly affected when storage insects cause severe losses to storage products (Belmain and Stevenson 2001, Chomchalow 2003). Besides that, it also causes damaging effects to the economy (Chomchalow 2003). Stored products insect can adjust to food handling processes and storing conditions as these insects have the ability to infest different types of food instead of just one or several food types. Moreover, they can tolerate a broader range of temperature and relative humidity in addition to having relatively long reproduction periods. Some stored product insects can also survive without food for long periods and because of their small size, large populations would have been established before they are detected (Cox and Collins 2002).

Insect pests of stored products can be categorized either as commodity feeders, fungal feeders, predators, parasitoids, foragers and accidentals, or scavengers. Insects that feed directly on the commodity are either primary or secondary pests (Rees 2004). Those that fall into the category of primary pests are internal feeders of kernels that penetrate and feed on commodities that are undamaged and intact (Philips and Throne 2009) or spend a significant amount of time developing within the grain (Mason 2003). On the other hand, secondary pests are external feeders that require the commodity to be damaged or processed (e.g. milled grains and grain-based food products) before they can attack or feed on the broken and fine materials of the commodity (Mason 2003, Rees, 2004, Philips and Throne 2009). Products previously damaged by other pests, mainly by primary pests or products which have been subjected to poor threshing, drying and handling also risks being attacked by secondary pests. These pests are known to attack a wider range of food products than compared to primary pest species (Semple et al. 1992). Meanwhile, storage insects classified as fungal feeders are those that feed on mould and mould spores which give extra nutrients that may not be present from direct feeding of the commodity. Other stored product insects can act as predators by preying on other insects present in the commodity. Parasitoids such as wasps that attack stored product insects can also be a pest itself when present in the commodity. Moreover, stored products may also contain occasional insects categorized as foragers or accidentals such as cockroaches and ants. Some stored product insect pests are also scavengers which include insects that feed on dead insects and other dried material of animal origin (Rees 2004).

2.2.2 General biology and life cycle

2.2.2.1 The rice weevil, *Sitophilus oryzae* (L.) (Curculionoidea: Dryophthoridae)

Sitophilus oryzae is a primary pest (Rees 2004) and one of the most common pests that cause both quantitative and qualitative losses to stored grains (Park et al. 2003). In warm tropical countries, *S. oryzae* is the main insect pest infesting stored products (Batta 2004). Longstaff (1981a) reported that processed animal foods are also attacked by *S. oryzae* but will only reproduce in whole grains with moisture levels ranging from 10 to 16%. Adult *S. oryzae* are less limited in their diets as the larvae need to develop within grains that are intact even though both adult and larval stages feed on the same foods (White and Leesch 1995).

Measuring from 2.3 to 3.5 mm in length, adult weevils are small with a stout appearance (Plate 2.1). Its forewings have been modified to become hard and protective, known as elytra and are dark-reddish brown in colour with four light-coloured patches (Koehler 1994, Walker 2008). The *S. oryzae* adult has a head which is elongated into a snout where the mouthparts are located (Mason 2003). The snout is as long as the prothorax or the elytra (Koehler 1994) and each adult weevil possess a pair of elbowed antenna (Canadian Grain Commission 2009). Male and female weevils are discerned by the appearance of their snouts. The snout in male weevils is shorter and wider with heavy irregular pitting (Plate 2.2) while female snouts are relatively long and narrow with light regular pitting (Plate 2.3) (Halstead 1963, Rees 2004, Walker 2008b).

Shortly after emerging from kernels, observations showed that adult weevils will begin mating (Singh and Soderstrom 1963). Female weevils will make a small hole (feeding puncture) in a grain kernel and deposits an egg in it (Longstaff 1981a, Rees 2001). The egg is protected when the hole is sealed with a waxy secretion (Longstaff 1981a, Canadian Grain Commission 2009). Campbell (2002) stated that not all holes made by female weevils are deposited with eggs as some grains are left void or becomes their food source. A single female weevil can lay approximately 150 eggs throughout its lifespan (Canadian Grain Commission 2009) and these eggs

Plate 2.1: Adult rice weevil, *S. oryzae.*

Plate 2.2: Male *S. oryzae*.

Plate 2.3: Female *S. oryzae*.

will hatch into larvae with a short and stout ‘C’ shape. Larvae of *S. oryzae* are white or cream in colour with a dark head capsule, legless (Plate 2.4a, 2.4b) and develop inside whole grain kernels (Koehler 1994, Mason 2003). The kernel in which it hatches in will ultimately be hollowed out by the chewing action of the larva as it feeds on it (Alfonso-Rubí et al. 2003). Longstaff (1981a) reported that a single grain may contain more than one egg, which can result in the development of more than one larva. However, cannibalistic behaviours are exhibited by *S. oryzae* larvae if they come across other larvae that are less developed thus making it unlikely for more than one larva to survive, pupate and progress into the adult stage (Danho et al. 2002, Arbogast 1991). After the pupa (Plate 2.5) emerges into an adult weevil, it will stay within the grain for a few days to enable the hardening and darkening of the cuticle layer (Longstaff 1981a). Adult *S. oryzae* will retract their legs towards their body when disturbed and remain motionless to appear dead (Koehler 1994).

The development of *S. oryzae* eggs to pupae can take up to 25 days or more under warm conditions. According to Koehler (1994), the life cycle of *S. oryzae* is completed in 5 to 8 weeks under optimal temperatures (between 27°C and 31°C), the shortest being 25 days at 27°C and 70% RH, while Singh et al. (1974) reported that 30°C and 75% RH is the optimal development conditions for *S. oryzae*. At 27±1°C and 69 ± 3% RH, Sharifi and Mills (1971) reported that a longer average time for the complete development of *S. oryzae* is recorded at around 37 d (range 33-49d). If temperatures drop lower than 17°C, their development will stop (Koehler 1994). Adult weevils are long-lived with a life span that can range from 3 to 6 months (Rees 2004).

Plate 2.4a: *S. oryzae* larvae (Ventral view)

Plate 2.4b: *S. oryzae* larvae (Side view)

Plate 2.5: *S. oryzae* pupae

2.2.2.2 The red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionoidea: Tenebrionidae

Tribolium castaneum is mostly encountered in tropical places and they are often the coloniser of a stored commodity (Rees 2004). The adults are reddish-brown (2.6-4.4 mm) with a pair of antennae that ends with a conspicuous three-segmented club (Plate 2.6) (Bousquet 1990, Rees 2004). This insect species can live for a year (Howe 1962) or more and in some cases for as long as three years as reported by Baldwin and Fasulo (2003). Adult *T. castaneum* females lay white, small and cylindrical eggs amongst food products. They can lay 11 eggs daily at an optimum temperature of 32.5°C and during their entire existence up to 1000 eggs can be laid (Howe 1962, Rees 2004). Larvae possess a pale brown head and their last abdominal segment comprises of a pair of dark upturned and pointed structures (Plate 2.7) (Hill 1975). They are of elateriform, active (Rees 2004) and will remain inside grains while waiting to pupate (Howe 1962, Hill 1975). This insect species spends most of its developmental time (60%) in the larval form (Rees 2004). The pupa is exposed as it is not enclosed in a cocoon or a case (Mason 2003) and can be yellowish white to brown in colour (Plate 2.8) (Hill 1975). At the pupal stage, male and female *T. castaneum* can be easily set apart by examining the genital papillae which is located at the anterior to the urogomphi. The urogomphi is a pair of pointed structure found at the end of each pupa. The genital papillae in female pupae are of finger-like structures and bigger (Plate 2.9) than compared to those of the male pupae which look like fingertips instead of fingers (Plate 2.10) (Beeman et al. 2009).

Under different conditions (temperature and relative humidity) the developmental period of *T. castaneum* can be different. At 30°C, a full life cycle can be completed in 35 days (Hill 1975). When reared in between 20ºC to 37.5ºC, and at

Plate 2.6: Adult *T. castaneum*

Plate 2.7: *T. castaneum* larva

Plate 2.8: *T. castaneum* pupa

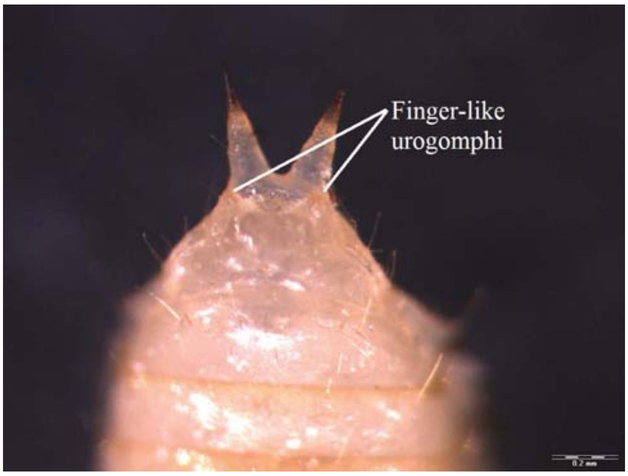

Plate 2.9: Female *T. castaneum*

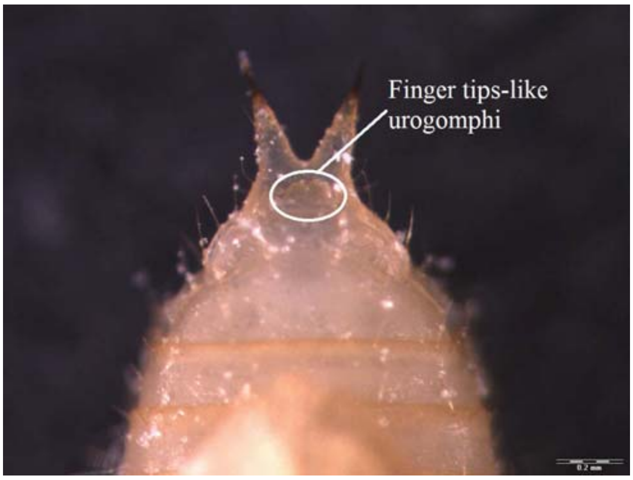

Plate 2.10: Male *T. castaneum*

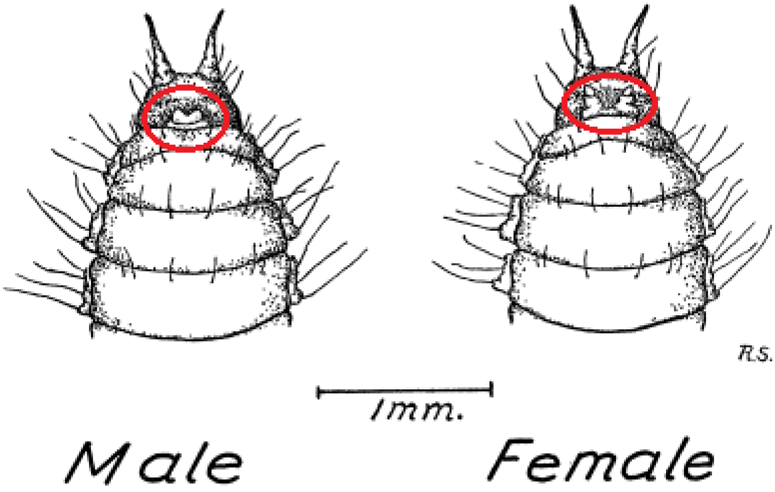

Plate 2.11: Terminal view of male and female *Tribolium* pupae showing the sexual characters (Park 1932).

a relative humidity greater than 70%, a complete life cycle only takes 19 to 20d (Howe 1956). Rees (2001) stated that under optimum conditions of 35°C and 75% RH, the life cycle of *T. castaneum* takes around 20 d.

2.3 Economic importance, damages and impacts of infestation caused by stored product insects with emphasis on *S. oryzae* and *T. castaneum*

Harvested products are usually stored for many reasons including providing a continuous supply of food and feed that can last all year round as well as kept for future planting and sold off when market prices for the particular product is high to acquire more earnings (Demissie et al. 2008). However, storage of products also comes with its consequences as post-harvested products are more susceptible to infestation and damages by stored product insect pests (Rees 2004). Infestation of stored product by insects causes various damages and economic losses. Mpuchane and Siame (1998) stated that with the lack in post-harvest handling procedures, the contamination of food commodities are made worse. Although direct consumption of raw commodities is most common, ingredients and finished products are also attacked (Mason 2003). An estimated 9% of postharvest losses in developed countries and 20% or higher in losses in developing countries were reported to be caused by stored product insects (Philips and Throne 2010). It was reported that total post-harvest losses of crops were accounted at 40% in hot and humid regions while drier regions recorded more than 10% in losses (Appert 1987).

When the quantity and quality of grains are affected, this renders the retraction of products, risks possible discontinued businesses (Cramer 2006) and requires the party at fault to bear the ensuing social and legal costs (Rees 2004). Expenses in control efforts as well as attempts to inhibit further infestations are also increased, while the labour and hardwork that goes into cultivating, managing, manufacturing and storing commodities are made futile with infestation problems (Rees 2004). In the food industry, the presence of arthropod pests should not be taken lightly as some insects can transmit disease-causing bacteria (Olsen 1998b, Foil and Gorham 2000). Individuals who are prone to allergies may develop hypersensitivity towards body parts of insects or waste products such as cast skins, excretory products, and pheromones secreted (Olsen 1998). These by-products will also taint the tastes of food products (Mason 2003). In addition, an increase in commodity moisture levels during and throughout the insect infestation will encourage mould growth (FAO 1985, Rees 2004) and damages to seed embryo by insects will reduce the germination rate of grain seeds (FAO 1985).

Sitophilus spp. attacks whole cereal grains, solid cereal products as well as some pulses (Rees 2004). Each year massive damages are caused by *S. oryzae* to stored cereal grains and products which accounts for hundreds of millions in losses (Reddy 1950). Amongst the locally produced grains, rice in the unhusked form (paddy) and milled form is of utmost concern in Malaysia. These grains are either stored for a short or an extended period of time in different forms and under different conditions (Muda 1985). Several researches have recorded around 40 insect species in Malaysia that attacks stored paddy and rice (Singh 1972, Yunus 1980, Lim et al. 1980, Rahim et al. 1983) with *S. oryzae* as the main species (Rahim 1985). This insect species is a prolific breeder, producing a lot of progeny (Rees 2004). Their infestation not only reduces grains into powder and causes a buildup of heat and moisture in the product (Longstaff 1981a, Kučerová 2002), but the presence of their frass also contaminates the grains (Longstaff 1981a). In a storage area, the presence of *S. oryzae* is mostly due to the relocation of rice stocks (Rahim 1985). Moreover, since *S. oryzae* is capable of flight (Mason 2003), these weevils can infest grains stored at far ends of warehouses (White and Leesch 1995).

Tribolium castaneum is commonly found in flour mills, warehouses, retail stores (Campbell and Runnion 2003) and food-processing facilities (Mills and Pedersen 1990). In Malaysia, this species is one of the main secondary insect species found in significant numbers in warehouses and other storage facilities (Rahim 1985). This species can infest a wide variety of stored grain products such as flour, cereals, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Via 1999, Weston and Rattlingourd 2000). They prefer damaged grains but can also attack intact grains especially if the moisture content is high (Bousquet 1990). According to Smith el al. (1971), bread made from contaminated flour which was previously infested with *Tribolium* spp. has been reported to give a bad taste when consumed. A heavy infestation with this insect species will give flour and other food products a grayish tint which can encourage mould growth and make the products unsuitable for use or consumption (Mason 2003). Thirteen types of quinines have been identified (Howard 1987) from the abdominal glands of *T. castaneum* which gives a foul smell to food products (Rees 2004) These chemicals have been reported to cause jaundice, anaemia, haemoglobinuria and cachexia in humans (Omaye et al. 1981) as well as allergic reactions (Alanko et al. 2000).

2.4 General control of stored product insects

The infestations of insects and their management methods vary (Lim 1988) and **e**ntomologists around the world have long attempted to successfully control stored product insects (Salem et al. 2007), where gaseous, synthetic fumigants and residual insecticides are primarily used in controlling their population (Chomchalow 2003). In developed countries, the use of synthetic pesticides is the main method to protect crops against pests (Fields 2006) and have been applied at a large scale against stored product insect pests in grain storage facilities since the 1950s (Bond 1984). Active ingredients such as deltamethrin, cypermethrin, permethrin and fenvalerate were effective as grain protectants, as sprays on packaging materials and construction materials for warehouses against *Sitophilus zeamais* Motsch., *T. castaneum*, *Rhyzopertha dominica* (F.), *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus* (F.) (Ceballo and Morallo-Rejesus 1986). Insect growth regulators (IGRs) were also tested and were found to be effective with some insects (*Ephestia cautella* (Walker) (Madlanyangbayan and Morallo-Rejesus 1986), *Corcyra cephalonica* (Stainton) (Marbida 1986) and *Plodia interpunctella* (Hubner) (Fajardo and Morallo-Rejesus 1980). Non-chemical methods such as using low or high temperature, low moisture grain, diatomaceous earth, hermetic storage, impact and varietal resistance were also practiced to curb insect infestation (Fields 2006).

2.4.1 Control of stored product insects using natural products

Today, the control of stored product insect pests demands novel control methods as there are elevated concerns over the deposits of insecticide in processed cereal products, insects developing resistance towards insecticides, higher expenses of insecticide application, and the potential environmental impacts (Aslam et al. 200, Udo 2005, Fields 2006, Salem et al. 2007, Mahdi and Rahman 2008). The search for alternatives has been further intensified with the worldwide phasing out and ban on methyl bromide, a fumigant insecticide effective for killing postharvest insects (Fields and White 2002).

Chomchalow (2003) advised technologists and scientists to develop control practices that are simple and economic as it is vital to reduce unnecessary wasting of stored products in farms and farm storage areas. Moreover, Udo (2005) suggests the need to find organic sources that are easily obtainable, inexpensive, and harmless to animals and the environment. Even without using chemical alternatives, various prevention and control methods that are nontoxic, efficient and simple can be used to control stored-product insect pest populations (Philips and Throne 2010). In recent years, the management of stored product pests focuses on the use of materials of natural origin (Nadra 2006). Scarcely any attention was given in search for plant-based insecticides and repellents as well as determining the efficacy of traditional methods after the discovery of synthetic products (Curtis 1990). Moore et al. (2007) stated that collectively, substances of natural sources do not pose any threat or put consumers at risk as many phytochemicals possess pharmacology properties. Currently, the use of phytochemicals is gaining popularity as the public consider these products to be better as they derive from natural sources (Gerberg and Novak 2007).

Garland (2004) stated that over the years, many researches propose the use of different plants as insecticides that gave very promising results. Botanical sources for example, spices, medicinal and other plants are amongst the many different materials used as protectants of stored products from pests (Chomchalow 2003). According to Liu et al. (2006) and Isman (2006), the use of natural products such as plant extracts are beneficial as they can be target species specific, are locally available, less persistent in the environment and usually possess distinctive modes of action with low mammalian or ecotoxicity. Emeasor et al. (2005) and Nadra (2006) reported that certain oils and extracts of plants as well as the powdered form are highly effective in controlling stored product insects as the constituents of the plants caused high mortality and suppressed the reproduction rate. Moreover, plant products have been found to repel or attract insect pests (Mohan and Fields 2002), where essential oils, powders or distillates have been found to show promise in repelling stored product pests from grains and acts as feeding and oviposition deterrents (Adler et al. 2000). These plant extracts and essential oils show promise in protecting crops as they contain monoterpenoids, diterpenoids, sesquiterpenoids and other compounds that show ovicidal, larvicidal, repellent, deterrent, antifeedant and toxic effects in a wide range of insects (Fields et al. 2001, Pungitore et al. 2003, Boeke et al. 2004, Liu et al. 2006, Isman 2000, 2006).

Plants used for the purpose of biocides development should be easy to grow and plentiful to ensure continuous supply. Besides that, plant parts that show potential repellent activities should be from replaceable parts of the plant, for example the leaves or seeds (Belmain and Stevenson 2001). The production of plant-based compounds that are of top quality may be extremely costly especially when the amount of bioactive compounds obtained is low. Therefore, it is essential that plant extraction methods utilized be uncomplicated (Moore et al. 2007).

Protection of stored products can be achieved with the application of plants, plant parts or extracts that exhibits repellent or toxic properties (Adler et al. 2000). Spices are plant products that can be used to control stored product insects as they are inexpensive, readily obtainable and less toxic (Aslam et al. 2002, Mahdi and Rahman 2008). Spices refers to plant parts used to flavour food which consists of dried seed, fruit, root, bark or vegetative parts of a plant that is used in small quantities (Boning 2010, Mahdi and Rahman 2008). They have many other uses which include preserving food, as medicine, in religious rituals such as incense, as cosmetics, for the perfume industry and even as vegetables (Mahdi and Rahman 2008).

Table 2.1 shows a list of some of the many essential oils (spices/ herbs) tested by numerous researchers against different stored product insects.

Table 2.1: List of essentials oils (spices/ herbs) previously studied against different stored product insects.

Plant name	Insect species	References
Syzygium aromaticum (L.)	*T. castaneum*; *Sitophilus zeamais* Motsch.	Ho et al. (1994)
Illicium verum Hook	*T. castaneum*; *S. zeamais*	Ho et al. (1995)
Myristica fragrans Houtt.	*T. castaneum*; *S. zeamais*	Huang et al. (1997)
Artemisia annua	*T. castaneum*; *Callosobruchus maculatus* (L.).	Tripathi et al. (2000)
Pimpinella anisum L.; *Cuminum cyminum* L.; *Eucalyptus camaldulensis* L.; *Origanum syriacum* var. *bevanii* (Holmes); *Rosmarinus officinalis* L.	*Tribolium confusum* du Val*;* *Ephestia kuehniella* (Zell.)	Tunç et al. (2000)
Elettaria cardamomum (L.)	*T. castaneum*; *S. zeamais*	Huang and Ho (2000)
Curcuma longa L.	*R. dominica*; *S. oryzae*; *T. castaneum*	Tripathi et al. (2002)
Cinnamomum zeylanicum L.; *Etlingera elatior* (Jack); *Etlingera pyramidosphaera* (K.Schum.)*;* *Zingiber officinale* Rosc.; C. longa; *Piper nigrum* L.; *Cymbopogon citratus* L.; *Capsicum annuum* L.	*S. zeamais*	Ishii et al. (2010)
Origanum onites L.; *Satureja thymbra* L.; *Myrtus communis* L.	*Ephestia kuehniella* Zeller; *Plodia interpunctella* Hübner; *Acanthoscelides obtectus* Say	Ayvaz et al. (2010)
Alpinia conchigera Griff; *Zingiber zerrumbet* Smitt.; *Curcuma zedoria* (Berg.)	*T. castaneum*; *S. zeamais*	Suthisut et al. (2011)
Elettaria cardamomum (L.)	*C. maculatus*; *T. castaneum*; *Ephestia kuehniella* Zeller	Abbasipour et al. (2011)

CHAPTER THREE

EFFECTS OF DIFFERENT MEDIUMS IN THE DEVELOPMENT OF *Sitophilus oryzae* (L.) AND *Tribolium castaneum* (Herbst)[2]

3.1 INTRODUCTION

Population growth in stored-product insects are dependent on the food which they consume (Inouye and Lerner 1965). According to Ziegler (1976), the oviposition rate and emergence of new adults are very much dependent on the quality and type of the substrate.

It is known that the main diet of storage pest consists of stored grains (Irshad et al. 1988). However, certain grains or stored products are more prone to insect infestation, while others are less susceptible to attacks. Previous studies have been conducted to evaluate the food preferences for stored product insects in an attempt to ascertain diets that are suitable for their growth and development (Verner 1971). Studies by Baker (1975) found that the development of *Sitophilus oryzae* larvae were improved when the minerals and vitamins supplied by dietary brewer's yeast and wheat germ were replaced with mineral and vitamin mixtures. Certain amino acids were also found to be required for the growth and development of *S. oryzae* larvae (Baker, 1978). Campbell and Runnion (2003) stated that different types of flour have different nutrient constituents and the development of *Tribolium castaneum* is considerably affected when there is a slight difference in quality. *Tribolium castaneum* did not survive when reared on cornmeal (Sokoloff et al. 1965) and their performance was low when reared in white wheat, brown rice and rice, but the addition of yeast improved their development (Sokoloff et al. 1965). Inouye and

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Lerner (1965) suggested studies on the nutritional requirements of *Tribolium* to be carried out in order to understand the nature of their competitive ability.

This study was initiated to understand the nutrient requirements for the development and growth of *S. oryzae* when exposed to different rice types as well as investigate the influence of different diets on the performance of *T. castaneum*. It is hypothesized that the development time for *S. oryzae* and *T. castaneum* on different diets may be related to the nutritional contents of the diet. The damages that these insects can cause to a particular amount of products over a certain period of time are vital in an attempt to ascertain the extent of damages these insects can instigate. Therefore, information regarding the population growth in *S. oryzae* and *T. castaneum* when presented with these diets may be useful in constructing suitable management approaches in an effort to reduce infestations in grain storages.

3.2 MATERIAL AND METHODS

3.2.1 Stored product insects

Adults of *S. oryzae* and *T. castaneum* were collected from subcultures previously reared from the original stock cultures in the Urban Entomology Laboratory, Vector Control Research Unit, Universiti Sains Malaysia, Minden Campus, Penang. The cultures of *S. oryzae* were mass reared on local polished rice (Beras Super Tempatan 15%, Zaa Edar Sdn. Bhd.), while *T. castaneum* was reared on wheat flour (Prestasi Flour Mill (M) Sdn. Bhd.), both under laboratory conditions at 30.0 ± 0.5ºC, 68 ± 2% relative humidity (RH), and 12 h photoperiod in round plastic containers (10 cm in height x 9 cm in diameter).

3.2.2 Medium preparation

The choice of mediums was primarily determined by the availability of these rice grains, flour and starch in Malaysia's commercial outlets. Eight different types of rice grains used for rearing *S. oryzae* are shown in the table below (Table 3.1), while six flour types and two types of starch tested against *T. castaneum* are shown in Table 3.2.

Approximately 20 gm of each rice types (without dust), flour and starch were placed into plastic containers measuring 4.5 cm in diameter and 9 cm in height, respectively. The lids of each container were perforated to allow air movement which helps prevent moisture buildup and inhibit fungal growth.

3.2.3 Assay

3.2.3.1 *Sitophilus oryzae*

Ten pairs of adult weevils (1 to 2-wk old), previously sexed according to Halstead (1963) were placed into a container containing the respective rice types. The experimental setup was replicated five times for each rice type. The weevils were introduced and allowed to feed and oviposit for 7 d before they were removed. They were then left in a cabinet held at 30.0 ± 0.5ºC, 68 ± 2% relative humidity (RH), and 12 h photoperiod. Observations began as soon as the adults of the new generation emerged from any eggs that might have been laid in the rice grains. All newly emerged adults were counted and removed on a daily basis until no more progeny emerged. At the end of the experimental period, the rice in each container was weighed to determine the percentages of weight loss. The weight loss of rice grains refers to emptied grains or any other remaining rice grains that are still intact. Thus, prior to being weighed, the rice was sifted through a 4 mm wire-mesh to remove any debris such as rice dust and frass.

3.2.3.2 *Tribolium castaneum*

Ten adult beetles (5 males and 5 females) of 7-d-old were introduced into the