The Effect of Aromatic Materials and Different Storage Conditions of Feedstuffs

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A thesis submitted in fulfilments of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry) with Honours

Faculty of Agro-Based Industry

University Malaysia Kelantan

2022

DECLARATION

I hereby declare that the work embodied in this report is the result of my own research except for the excerpt as cited in the references.

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ACKNOWLEDGEMENT

Foremost, I would like to express my gratitude to Allah SWT for His blessings throughout my study in completing this research successfully. As I am reaching the end of my Final Year Project (FYP), I would like to express my sincere feeling of appreciation to people who help me in this whole journey.

First, I would like to express my deep and sincere gratitude to my one and only FYP supervisor, Dr. Suniza Anis Binti Mohamad Sukri for her commitment, guidance, advice and her time throughout the journey in making sure that I complete this research. Thank you for your support and patience in guiding me to complete this thesis writing.

I also want to express my gratitude to my friends, Nur Syuhada Binti Sazali, Nurin Jazlina Binti Mohamad Fauzi and Nur Nazurah Maryam for their endless support and motivation for me to carry out this whole semester until the end.

Last but not least to my husband, Mior Ahmad Syakir Bin Mior Kasim, the one who help me a lot during this journey through spiritual, motivational, physical and time. I would like to deeply express my gratitude and thankful to Allah for giving me such a wonderful, understanding, loving and caring husband. And also thank you to my family for their endless prayers and support throughout this journey.

The effect of aromatic materials and different storage conditions of feedstuffs

ABSTRACT

A number of studies had proposed on good storage management, however there is not much of experiments that are done in order to find materials that can ward off any insects or pests from the feedstuffs. Thus, this experiment will show either the aromatic materials are effective or not in having good feed storage conditions. Hence, this study is proposed to determine proper storage conditions and also testing several materials to ward off the infestation of insects or pests besides observing any physical changes towards the feedstuffs. The study was carried out for six weeks and self-observation was done weekly to record any presence of pests besides physical changes towards the treatments. The experiments were arranged in a factorial in Randomized Complete Block Design (RCBD) by 5 × 4 × 2 (type of feedstuffs – fish meal, corn meal, *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal; aromatic materials – *pandan* leaves, garlic, cloves and dried chillies; storage conditions – lit room and dark room) to form 40 treatments including Control group and has triplicates, with a total of 120 experimental units. Insects that were present were isolated first before being observed under microscope. Inferential statistics was used for data analysis. From the experiment, it was revealed that 86.7% of the corn meal treatments are infested with insects by nearly half (46.7%) comes from open storage conditions, whereas 6.67% from fish meal in open storage conditions. The addition of cloves as aromatic materials along with the feedstuffs was seen as a way to reduce the rate of infestation as 3 out of 24 containers of cloves treatments were the only infested with the insects. Hence, it was recommended to store feedstuffs in closed storage condition besides inclusion of cloves in certain feedstuffs such as corn meal to ward off any insects while there is no significant difference between treatments of aromatic materials in both *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal in term of insect’s occurrence. Thus, this study can propose the most suitable storage conditions in keeping feedstuffs to those involves in livestock industry especially for small scale farmers that do not have proper feed storage facilities. Besides proposing on how to keep insects or pests away from the feedstuffs kept in regular feed container so that the feedstuffs can last longer without any deterioration effects.

Keywords: storage management, infestation of insects, aromatic materials, animal feedstuffs, deterioration effects.

Kesan bahan aromatik dan keadaan penyimpanan yang berbeza terhadap bahan pakan

ABSTRAK

Beberapa kajian telah dicadangkan bagi mendapatkan pengurusan penyimpanan yang bagus, namun, masih tidak banyak kajian yang dilakukan untuk mencegah kehadiran serangga perosak pada bahan pakan haiwan ternakan. Eksperimen ini dijalankan untuk membuktikan sama ada bahan aromatik dapat memberi kesan dalam pengurusan penyimpanan yang bagus. Justeru, kajian ini dicadangkan untuk menentukan cara penyimpanan makanan yang betul dan juga menguji bahan aromatik bagi mencegah serangan serangga perosak selain memerhatikan sebarang perubahan fizikal terhadap makanan. Kajian ini dijalankan selama enam minggu dan pemerhatian diri dilakukan sekali seminggu untuk mencatat sebarang perubahan terhadap rawatan. Eksperimen menggunakan kaedah faktorial Reka Bentuk Blok Lengkap Rawak (RCBD) dalam susunan 5 × 4 × 2 (jenis bahan pakan – baja ikan, jagung kisar, daun *Trichanthera gigantea* kisar dan daun *Indigofera zollingeriana* kisar; bahan aromatik – daun pandan, bawang putih, bunga cengkih dan cili kering; kaedah penyimpanan – ruang terbuka dan ruang tertutup) bagi membentuk 40 pelakuan berserta kumpulan Kawalan dan mempunyai tiga ulangan, membentuk 120 unit uji kaji. Serangga akan diasingkan terlebih dahulu semasa menjalankan pemerhatian sebelum diperhatikan di bawah mikroskop. Statistik inferensi telah digunakan bagi menganalisis data. Daripada eksperimen ini, sebanyak 86.7% daripada rawatan jagung kisar telah diserang oleh serangga di mana hampir separuh (46.7%) berasal dari kaedah penyimpanan terbuka, manakala 6.67% daripada baja ikan yang juga dalam keadaan penyimpanan terbuka. Selain itu, penambahan bunga cengkih sebagai bahan aromatik bersama bahan pakan dilihat sebagai salah satu cara yang dapat mengurangkan kadar serangan kerana hanya 3 daripada 120 bekas yang dijangkiti serangan tersebut. Maka, penyimpanan bahan pakan di ruang tertutup selain penambahan bunga cengkih dalam bahan pakan seperti jagung kisar dapat mencegah kehadiran serangga perosak. Selain itu, tiada perubahan ketara berlaku di antara rawatan bahan aromatik bagi kedua-dua jenis daun kisar dari segi serangan serangga perosak. Kesimpulannya, kajian ini dapat membantu dalam mencadangkan kaedah penyimpanan bahan pakan kepada mereka yang terlibat dalam industri ternakan terutamanya bagi penternak kecil yang tidak mempunyai fasiliti penyimpanan makanan yang elok. Selain itu, ia dapat memberi idea cara untuk menghindari serangan serangga perosak pada bahan pakan yang disimpan dalam bekas makanan biasa supaya ia dapat bertahan lebih lama tanpa sebarang kesan kemerosotan.

Kata kunci: pengurusan penyimpanan, serangan perosak, bahan aromatik, bahan pakan haiwan, kesan merosot.

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LIST OF SYMBOLS

°C	Degree Celcius
%	Percentage
g	Gram
hrs	Hours
RM	Ringgit Malaysia

LIST OF ABBREVIATIONS

2AP	2-acetyl pyrroline
AM	Aromatic Materials
CM	Corn Meal
CRM	Cereal Mites
FAO	Food and Agriculture Organizations
FM	Fish Meal
IZLM	*Indigofera Zollingeriana* leaf meal
PL	*Pandan* Leaves
RFB	Red Flour Beetle
RW	Rice Weevil
TGLM	*Trichanthera gigantea* leaf meal

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Feedstuffs is one of the important components in livestock industry for both commercial livestock and small-scale farmers. Feedstuffs will contribute in profits or loss of the farmers and may also determine the performance of the livestock. Some farmers tend to ignore the importance of feedstuff storage hence it will cause losses. Losses in feedstuff storage can impacts both farmers and the livestock as it occurred in different categories such as economic loss, quality loss, weight loss, nutritional loss, and health risk (Chow, 1978; Yasothai, 2019).

In livestock industry, the feed cost is generally takes up 60% to 70% of total production cost (Becker, 2008; Zahari and Wong, 2009). In Malaysia, most of the high-quality feeds are made of imported feedstuffs such as corn, soybean meal, tapioca meal, dicalcium phosphate, corn gluten meal and wheat (Chwen, 2017). Farmers tend to have no choice when this high quality of feedstuffs will be the major contributors in the performance of their livestock. According to United States Department Agriculture (USDA), 90% of a feed grain may be accounted for corns while soybean meal is the main oil crop used in the feed (Becker, 2008).

Table 1.1 Most imported of feedstuffs in Malaysia (2013-2015)

Imported feedstuffs	Year 2013 MT ('000)	Year 2014 MT ('000)	Year 2015 MT ('000)
Soybean Meal	1397	1465	1500
Wheat	1684	1545	1625
Corn	3476	3221	3500

Source: USDA (2016); Chwen (2017)

Hence, as the prices for the raw materials of the feedstuffs is increases every year, farmers need to have proper storage to avoid economic loss in order for the farmers to gain more profits.

Quality losses means when the feedstuffs have undergone some changes physically or chemically such as changes in flavour, colour, and nutritive value. There are several factors that can cause losses in feed quality such as the presence of insects, and presence of microorganisms such as mycotoxin which can cause off-flavours and rancidity. The presence of microorganisms such as fungi can cause weight loss, off-flavours, appearance, sizes, discoloration, increases in temperature and moisture and also toxic to humans and also animals (New, 1987).

Weight losses as in losses in feedstuff storage means that the moisture content of the feedstuffs is reduced and it may be due to shrinkage factor. Several factors that can cause weight losses in improper storage management may be from the presence of insects, and rodents, did not handle carefully and also activities of pests such as cockroaches. Insects such as various species of moth, weevils and beetles are the most common insects that are found attracted to the feeds. Not only the insect causes weight losses in the feed, the presence of insects may also cause rancidity where it is developed under a condition where lipid will break down into free fatty acids (New, 1987; Eze, 2011). Improper storage of feedstuffs can cause harm to animals and also humans. This is due to the presence and development of microbial growth such as fungal. Fungal can cause fatal diseases in horses and also swine reported by (Setsetse, 2019).

Therefore, this study is aim to determine a proper storage condition and also testing several aromatic materials to ward off the infestation of insects besides observing any physical changes towards the feedstuffs. The data that are obtained from this experiment have shown which feed storage conditions and also which aromatic materials that can help in warding off insects in order to control the quality of the feedstuffs besides identifying which feedstuffs are more likely to be infested first. Hence, small-scale farmers would have better options in storing their feedstuffs to increase storage life.

1.2 Problem Statement

Numerous studies have shown on how to carry out proper storage management. However, there is still not much of experiments that are done in order to find a suitable aromatic material that can aid in warding off insects or pests from the feedstuffs. Besides, the usage of imported feedstuffs also links in the problem as they are pricier and if not stored well, it will cause loss for the individuals involved. Moreover, this experiment will also help in determining whether the *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal can substitute common feedstuffs such as fish meal or soybean meal in terms of storage life as they both are known to also have high protein content. Therefore, the impulse to carry out this experiment is strong in order to help small-scale farmers to have better options in choosing the right storage conditions to increase the feedstuffs storage life along with the aid of aromatic materials.

1.3 Research Objective

To determine the proper storage condition and also testing several materials to ward off the infestation of pests such as rice beetles besides observing any physical changes towards the feedstuffs.

1.4 Hypothesis

H_0: There is no significant difference between the storage conditions and the effects of the aromatic materials towards selected feedstuffs.

H_1: There is significant difference between the storage conditions and the effects of the aromatic materials towards selected feedstuffs.

1.5 Scope of the Study

The main focus of the experiment was to observe the effect of aromatic materials. According to Penang Agriculture Department biosecurity officer, rice weevils can be kept out from rice containers by placing dried chillies and also *pandan* leaves reported by Sekaran (2018). Thus, by doing this study, we can prove either the aromatic materials do work in feedstuffs or not. Besides, this experiment also focused on different storage conditions (open and closed area) of the feedstuffs in relationship with increasing its storage life. This is also important in minimizing the operational cost as the feedstuffs are stored well for a longer time. The data of the experiment were collected weekly by observing the treatments by presence of insects, and physical evaluation method that involves sight, smell, and touch. This experiment consists of 40 treatments which has 3 replicates which were observed weekly by self-observation for six weeks starting from 20th November 2021 until 1st January 2022.

1.6 Significance of the Study

Those who involves in livestock industry especially small-scale farmers who do not own silo for feed storage may benefit from the result of the study as the most effective aromatic materials and most suitable storage conditions can be proposed to them in order for their feedstuffs to last longer without any deterioration effects. Besides, in order to cut cost from buying imported feedstuffs, substitution of leaf meal can help the farmers as they can be found locally.

CHAPTER 2

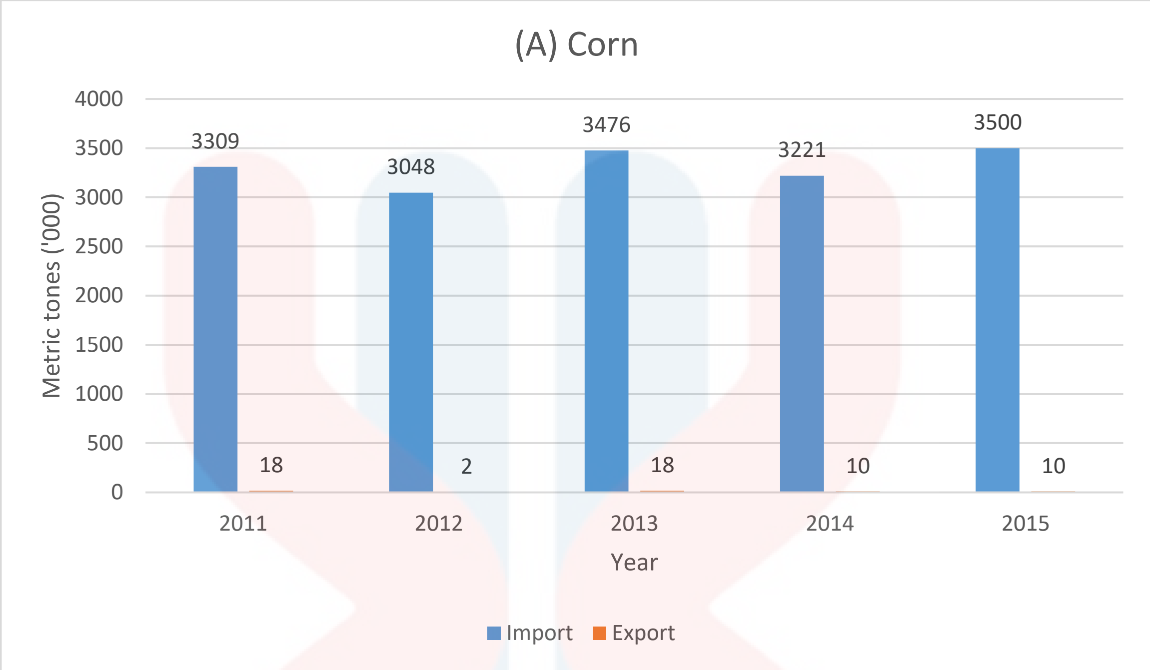
LITERATURE REVIEW

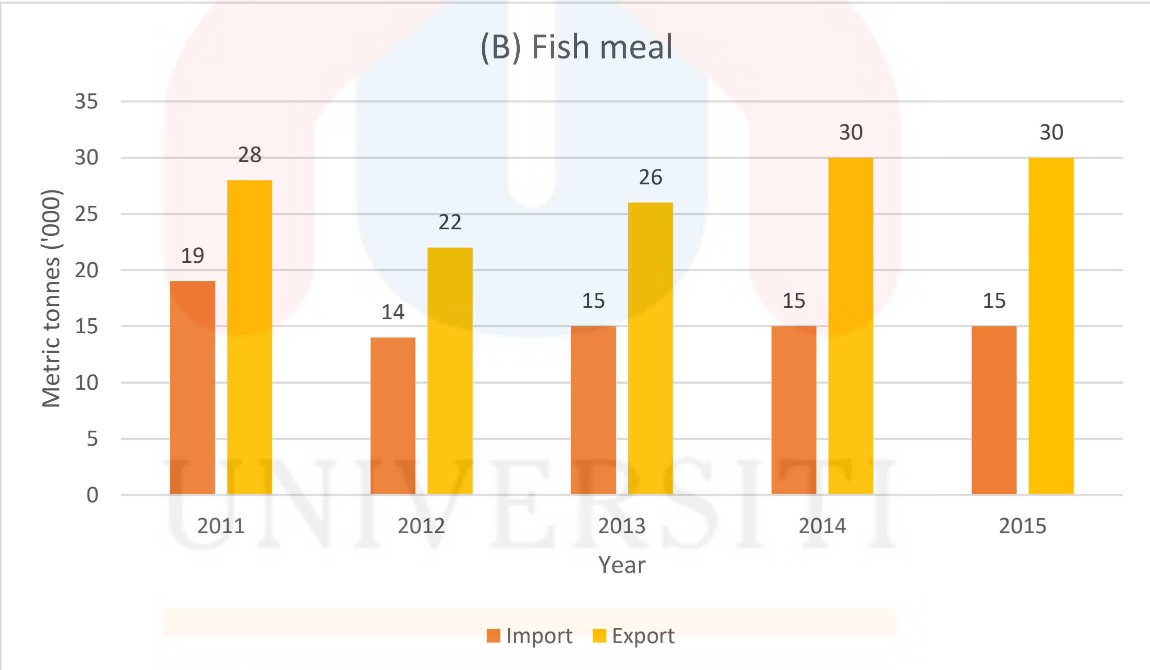
2.1 Animal Feedstuffs

According to The State of Food and Agriculture published from Food and Agriculture Organizations (FAO), developing countries in East and Southeast Asia such as Democratic People's Republic of Korea, Malaysia, Vietnam and especially China has shown significant growth in per capita consumption of livestock products (*The state of food and agriculture*, 2009). As livestock production has increases, so does the feedstuffs especially from protein supplies and sources. Thus, different type of feedstuffs has been widely produced and been used in livestock industry as animal feeds. Most commonly used of feedstuffs in Malaysia is maize which comprised 82% of total feed ingredients used followed by food processing waste which comprised 12% (Zulaiha *et al*., 2012).

Animal nutrition is one of the important aspects for livestock producer in order to have a better production yield. Commercial animal feed would have all the nutrients requirement needed depending on type of the species and also stages of growth. Besides, several factors such as ingredient costs, availability of each ingredient and processing characteristics should be taken into considerations in order to have a better feed formulation besides optimising the feed costs. In order for the animals to have efficient amino acid and protein used, appropriate amount of energy supply is needed from animal feeds and related balanced diet.

Protein comes from different type of sources such as plants, animals, legumes, and oil meal crops. As stated by (Chwen, 2017, p. 11) in his writing, plant protein sources such as wheat, soybean and maize meal are ideal to be used in high performance monogastric diets as they have high crude protein content and balanced amino acid. The dependence of our feed industry to imports feedstuffs is one of the factors that make our production cost higher. Few alternatives have been done in order to overcome this problem by producing local feedstuffs such as palm kernel cake (PKC). However, this oil meal crop has a disadvantage in which it contain high fibre content causing it to have lower nutritive value and also inconsistence in the quality (Zahari and Wong, 2009). Hence, finding several more protein sources as alternatives to replace the existing protein sources is keenly carried out in order to cut the production cost.


(A) Corn
Metric tones ('000)
4000
3500
3000
2500
2000
1500
1000
500
0
3309
18
3048
2
3476
18
3221
10
3500
10
2011
2012
2013
2014
2015
Year
Import
Export


(B) Fish meal
Metric tonnes ('000)
35
30
25
20
15
10
5
0
19
28
14
22
15
26
15
30
15
30
2011
2012
2013
2014
2015
Year
Import
Export

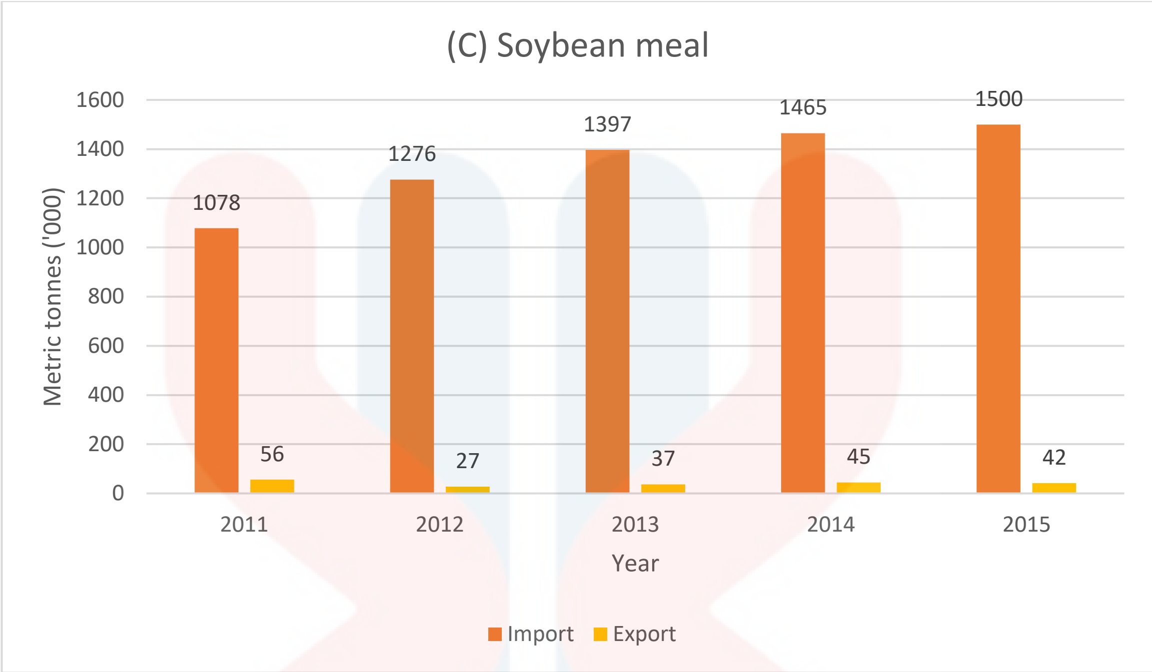


Figure 2.1: Import and Exportation of (A) Corn; (B) Fish Meal; and (C) Soybean meal (2011-2015). Source: (Chwen, 2017)

2.1.1 Fish Meal

Fish meal (FM) is a feedstuff that is made from good quality of whole fish, properly processed and it is proven to have high content of digestible protein and amino acid, vitamins and minerals that are proven to be good for livestock (Kim, 2010). Therefore, FM has become one of the important feedstuffs that are commonly used as source of protein for animal feeds as well as increases productivity and feed efficiency.

2.1.2 Corn Meal

Corn meal (CM) is one of by-products that are used as feedstuffs. It is a dried corn kernels that undergo grinding into three type of textures which are fine, medium and coarse. CM is one of the examples of energy source feedstuff and it has the characteristics of the content of nutritive matters (Milošević *et al.*, 2007). According to an article written by Loh Teck Chwen, maize meal is one of the feed ingredients that is from protein sources that have high crude protein content and balanced amino acid which is ideal in monogastric diets (Chwen, 2017). Malaysia has imported about RM 2.5 billion per year and over 50% of it is for maize (Zahari and Wong, 2009).

2.1.3 *Trichanthera gigantea* leaf meal

Madre de Agua or also known as *Trichanthera gigantea* has also become one of the forages that had been studied on its nutrient composition and its suitability to become livestock feed. Nutrient composition of Madre de Agua in dry matter state for crude protein ranging from 13 % to 22% (Jonna, 2018), 12.5% Crude fibre, 2.66% crude fat, 5% calcium, 0.41% total phosphorus, 21.80% ash and 11.56% moisture (Jaya *et al.*, 2008).

Figure 2.1.3: *Trichanthera gigantea* plant

2.1.4 *Indigofera zollingeriana* leaf meal

Indigofera Zollingeriana had also been proven to have high protein content in its leaves. According to a paper done by (Tistiana, Hartutik, & Djunadi, 2020), nutrient composition of this leaf consists of 28.98% crude protein, 3.30% crude fat, 8.49% crude fiber, 0.52% calcium, and 0.34% phosphorus content.

Figure 2.1.4: *Indigofera zollingeriana* plant

2.2 Repellent Aromatic Materials

2.2.1 *Pandan* Leaves (*Pandanus amaryllifolius*)

Pandan leaves (PL) or its scientific names is *Pandanus amaryllifolius* is a type of shrub that also known for its dwarf fragrant screw pine which is native to Southeast Asia region. PL is a plant that belong to *Pandanaceae* family that is known for its strong sweet smell that are usually used in food for the local people in South-east Asian countries. According to (Sonowal *et al*., 2021), the smell of PL comes from the presence of compound 2-acetyl-pyrroline (2AP). *Pandan* is a vertical, green plant that have leaves in the shape of long, narrow, blade-like that can reach to a maximum of about 4.5 m, have woody aerial roots, and have stems that are two to five cm thick and slender besides decumbent and ascending reaching 1.0 m to 1.6 m tall. In a study, PL is known to act as repellent towards cockroaches and flies (Ismanto *et al*., 2020). However, the usage of PL as insect repellent in feed storage is unidentified either it is effective or not.

Figure 2.2.1: *Pandanus amaryllifolius*

2.2.2 Garlic (*Allium sativum*)

Garlic is a member of onion family which is a part from Lilliceae plant family. Garlic or its scientific name is *Allium sativum* is known to have strong smell. The smell and taste of the garlic is actually coming from a compound that have biological active sulphur-containing known as Allicin [S-(2-propenyl)-2-propene-1-sulfinothioate] (Batiha, et al., 2020). In a study, garlic is proven to be one of the effective materials in anti-viral, anti-bacterial, anti-fungal, antioxidant and have anti-cancer properties (Satyanand *et al*., 2013). Besides, components such as methyl allyl disulfide and diallyl trisulfide has been extracted from garlic to become fumigation agent in order to control stored product pests especially *Tribolium castaneum* (Upadhyay and Ahmad, 2011). However, the usage of whole garlic as an alternative for insect repellent can be proven in this experiment.

Figure 2.2.2: *Allium sativum*

2.2.3 Cloves (*Syzygium aromaticum*)

Cloves is one of the popular spices that are known around the world. Its scientific name is *Syzygium aromaticum* and is known for many medicinal purposes and also used in food preservatives (Diego *et al.*, 2013). As the cloves can acts as food preservatives, this study shall prove either if it can also acts as a preservative for livestock feed. Besides that, cloves is proven to act as mosquito repellent in a study done by (Sabira *et al.*, 2014).

Figure 2.2.3: *Syzygium aromaticum*

2.2.4 Dried chillies (*Capsicum annuum L.*)

Chillies can be one of the insect repellents as it contains an active substance called capsaicin. This substance has hot taste and this makes chilli a strong feeding deterrent to lots of insects (Biao Li *et al.*, 2019).

Figure 2.2.4: *Capsicum annuum L*

2.3 Feed storage

Feedstuffs is one of the important components in livestock industry for both commercial livestock and the small-scale farmers. Feedstuffs will contribute in profits or loss of the farmers or may also determine the performance of the livestock. Feedstuffs need special care during storage in order to avoid any deterioration effects and loss. Some farmers tend to ignore the importance of feedstuff storage hence it will cause losses. Losses in feedstuff storage can impacts both farmers and the livestock as it occurred in different ways such as economic loss, quality loss, weight loss, loss of reputation, and seed loss (Hall, 1970; Chow, 1978; Yasothai, 2019).

As in economic losses, both the farmers and the manufacturers can experience this loss if their feed ingredients had shown deterioration effects. No buyers would buy feeds that had decreased in its quality. Accordingly, through legislation, they are forced to clean up their storage premises and disinfect all the products and the storage to reach hygienic standard. Loss of weight in animal feed ingredients can come from evaporation of moisture from the feed, infestation of insects, rodents, and also birds besides spillage that occur during transportation and handling (Hall, 1970).

Quality loss can be determine by assessing the basis of its appearances such as uniform in sizes, colour, texture, and dirt content, smell and flavour. In order to ensure slight chemical changes in the plant origins feed products, certain precautions is needed to be taken such as products is harvested under optimum conditions to ensure no damage occur followed by immediate and efficient drying until reaching a safe moisture content for storage (Hall, 1970). Improper feed management will cause feed quality to decrease.

If this happen, other problems will also rise in the animals when they are introducing with low quality feeds such as loss of appetite, slow growth, high feed conversion, low survival and high mortality rate (Cruz, 1996).

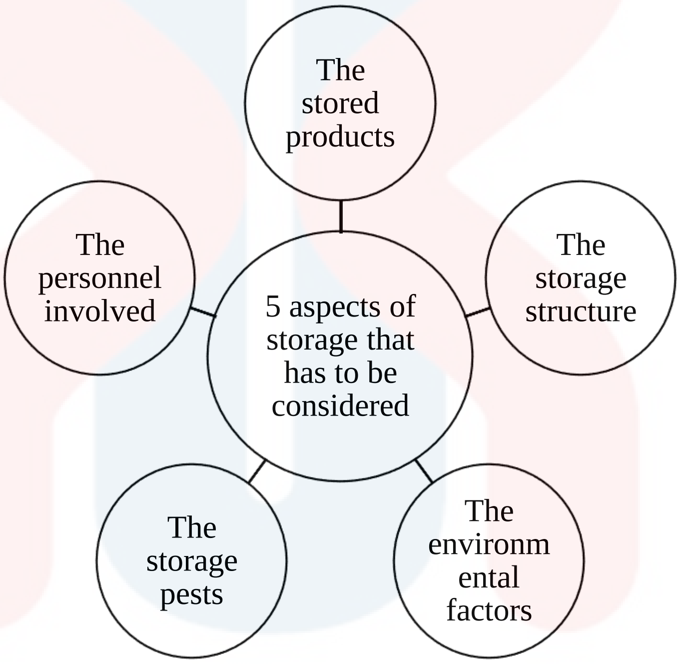


Figure 2.3: Important aspects in storage system (Source: Andales, 1992)

Good storage management will give more benefits to the farmers instead of loss and the value of the feed and performance of the livestock is depend on it.

2.3.1 Types of feed storage

In order to keep feed in good quality for a long time with minimal infestation from insects and microorganism, multiple type of storages have been designed. In a research that was done by Lorini (1993), improper storage structure and inadequate storage practices and handling has causes 20% loss in food grains.

a. Solid-wall bins and silos for bulk storage

A solid-wall bins is a facility that can hold up to several thousand tons of grains. It is a builds in which it can be anything from a small plastered basket to large steel or concrete silos. However, this type of storage have its disadvantages in which it has limitation in durability and protection against pests such as rodents, and insects (*Grain crop drying, handling and storage*, 1994).

b. Metal bins or silos

Commercial storage silo or known as steel bins are much more expensive however, it serves its purpose as it is easily erect and can be moved when in smaller size. It is suggested to use several smaller bins compared to one large bins (Carol *et al*., 2011). In warm climatic regions, it is not preferable due to condensation and humidity effect that can cause microbial growths which will lead to destruction to the stored feed (Vishal *et al*., 2017).

c. Bag storage

Bag storage has many varieties depending on the functions such as the quantity, harvesting method, handling method, moisture content and also costs. It is usually a commonly used facilities. However, this type of storage did not give in purpose against protection against insects in which insecticides need to be used.

d. Storage warehouses

Storage warehouse is a common in most farms as it is not only used for feed storage, but it can also be used to protect physical goods besides storing other materials and farm equipment and also storing chemicals such as insecticides and pesticides.

2.3.2 Factors that affect the quality of the feed

During storage, there are several factors that can affect the quality of the feed. Such factors include are temperature, moisture, light, oxygen (New, 1987), presence of insects and pests, growth of microorganisms such as fungi, using poor containers and storage and also human mishandling of the products (Hall, 1970). Temperature and moisture content are considered as the important factors in storage feed. This is because these two factors can change the quality of the feedstuffs through biochemical reaction, dry matter losses and presence of microorganisms or insects.

Certain fungi species such as *Penicillium* and *Aspergillus* species can grow at optimum temperature of 25-30°C and 30- 40°C. If the temperature of the feed storage facilities is between these temperatures, it will cause growth of the fungi species (Setsetse, 2019). However, if the temperature is monitored and reduced, hence, fungi growth can be prohibited. Mould and infestation of insects during storage can occur easily when the moisture content is detected to be higher than optimum conditions Adebanjo *et al*. (2003); Setsetse (2019). When the products have developed with microbial growth either bacteria or fungi, changes in chemical composition will occur. Mycotoxin which is produced by certain strains of fungi cause death in animals when it is fed with contaminated feed.

Factor such as infestation from the insects can reduce the market value, quality of the feed and also make it unsafe for animal and also human consumption as the insects may carry mycotoxin-producing fungi that are found on plant sufaces (Setsetse, 2019). An FAO consultant, D. W. Hall also agreed on which the occurrence of insects in the feed ingredients will reduce the quality of the feed therefore, he proves the effect of infestation of the red flour beetles (*Tribolium castaneum*) and *Trogoderma granarium* on groundnuts in his research as shown in Figure 2.3.2.

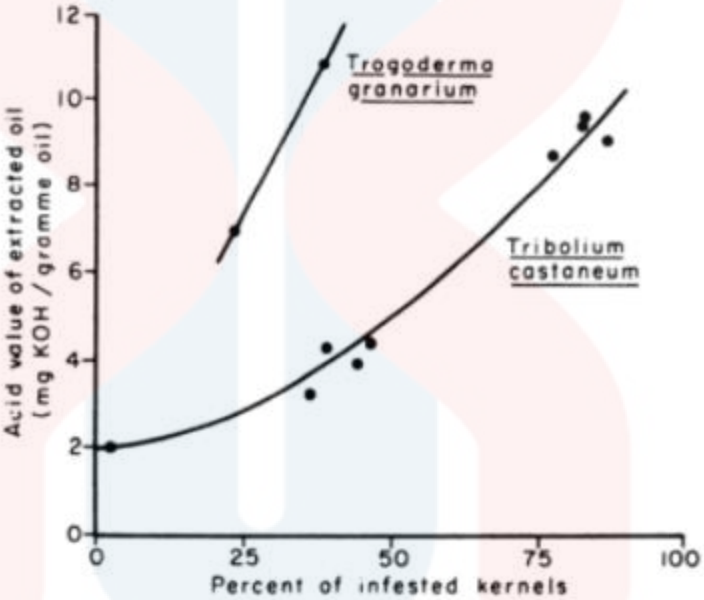


Figure 2.3.2: Infestation by two species of beetles causes incraeses in fat acidity of groundnuts (Source: Hall, 1970)

The storage facility can also be one of the factors that can affect quality of the feeds. This by means, a good storage facility should have a well-ventilated systems that are designed to have minimal condensation effects which is caused by changes in temperature of day and night and also have adequate containment to control pests (Keefe and Campabadal, 2015).

2.4 Infestation of Feedstuffs Caused by Insects and Pests

Insects are arthropod animals that have small sized and comes from large class Insecta. Insects have the characteristics such as having a head, thorax, abdomen, six legs, two antennae, and one or two pairs of wings. According to Johnson (2003), insects are the largest group in the phylum of Arthropoda as there are about 75% of known species of insects (Hickman *et al.*, 2011). Insects can be anywhere around us. This also includes in the facility of storage system. In aquaculture industry, infestation of insects and rodents are one of the problem that are often oversee but it can cause serious damage such that it is not only feeding on the feedstuffs but they also causes feed losses through damaging the feed packaging and also changing the environmental storage condition which can stimulate mould growth (Keefe and Campabadal, 2015). Examples of commonly insect orders that are found in stored products are Coleoptera, Lepidoptera, Hymenoptera, Diptera, Isoptera, Thysanura, Orthoptera, and also Siphonaptera (Semple *et al*., 1992).

There are lots of factors that can cause infestation of insects toward feedstuffs such as placing new feedstuffs side by side with infested one, intestation arise from mills, combine harvesters, field bins, augers and transportation equipment and also grain elevators and related conveyance machinery, climate, and influenece of abiotic environmental factors (temperature, moisture and gas concentrations) (Semple *et al*., 1992).

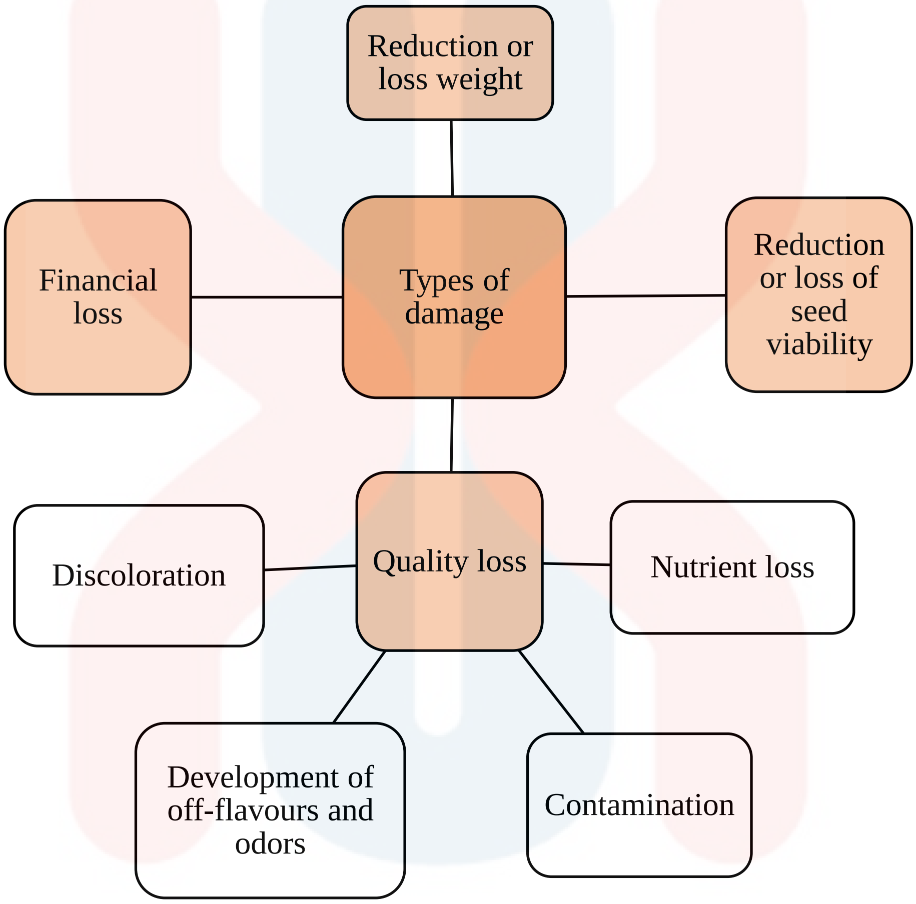


Figure 2.4: Type of damages causes by infestation of the insects (Source: Chow, 1978; Yasothai, 2019).

CHAPTER 3

METHODOLOGY

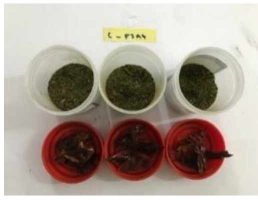
3.1 Samples Preparation

Fish meal (FM) and corn meal (CM) were purchased from local feed supplier while *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal were prepared from scratch. *T. gigantea* leaves and *I. zollingeriana* leaves were purchased from online supplier. The leaves undergo natural drying method before processing it into leaf meal. The leaves were sun dried until it fully dried and become brittle before proceed to grinding it into smaller particles. The leaf meals were kept in zip lock bags before divided into treatments. The aromatic materials (garlic, cloves and dried chillies) were purchased from nearby convenience store where as *pandan* leaves were taken from home garden.

3.2 Experimental Design

The experiments were arranged in a factorial in Randomized Complete Block Design (RCBD) by 4 × 5 × 2 (type of feedstuffs which are fish meal, corn meal, *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal, aromatic materials which are *pandan* leaves, garlic, cloves, dried chillies and control. Storage conditions are lit room and dark room) to form forty treatments. Each of the treatments were replicates into three replicas which makes 120 experimental units. The control group is the group of feedstuffs without adding any aromatic materials. The feedstuffs which are fish meal, corn meal, *Trichanthera gigantea* leaf meal and *Indigofera zollingeriana* leaf meal were labelled as F1, F2, F3 and F4 while aromatic materials which are *pandan* leaves, garlic, cloves, dried chillies and control were labelled as A1, A2, A3, A4, and A5. The storage conditions which were lit room and dark room was labelled as L and C. The designated treatment were as shown in Table 3.2.

Table 3.2: Total treatment samples

Feed-stuffs	Pandan	Garlic	Cloves	Dried Chillies
Fish meal				
Corn meal				
T. gigantea leaf meal				
I. zollingeriana leaf meal				

3.3 Experiment Procedure

Feedstuffs and aromatic materials were filled up to only half of the specimen container (ranging five to fifteen g of the feedstuffs). The lid of the container was not seal tightly. All of the samples were labelled and recorded. The treatments were divided into two storage conditions which were open area and close area. The open area is an area where it used a normal daylight room that received 12 hrs of sunlight and 12 hrs of darkness while the close area was inside a drawer which initiates a dark room that do not receive any natural or artificial lights (Alabi *et al.*, 2017). Treatments that were placed in lit room will be labelled as "L" while the treatments that were placed in dark room will be labelled as "C". Temperature and humidity of each condition was also taken and recorded with digital thermometer and hygrometer that were placed in both lit room and dark room. The experiment was carried out for six weeks and self-observation was done once a week.

The method that was used in the experiment was physical method which involves senses of smell, sight, touch and detecting any presence of infestation of insects or moulds in the selected feedstuffs (Teruel, 2002). The physical method was done by using all the senses such that sense of smell can detect any rancidity or off-flavours in the feed, the presence of unrelated materials such as small stones, dirt, pieces of wood, presence if insects and moulds can be detected by senses of sight. By using senses of touch, dryness, wetness or hardness of the feedstuffs can be detected. During the observations of the treatments, if any of the insects were present, they were isolated first before being observed under microscope. The use of microscope can aid in identifying the type and species of insects and moulds present during the observation.

CHAPTER 4

RESULT AND DISCUSSION

From the experiment that was carried out, temperature and humidity of the storage conditions were observed and taken. The results are shown in the Figures 4.1 and Figures 4.2. The average values of temperature and humidity (mean ± standard deviation) of the open storage conditions are 29.01 ± 1.11°C and 82.67 ± 5.38% while in close storage conditions are 29.83 ± 1.10°C and 72.80 ± 5.66%.

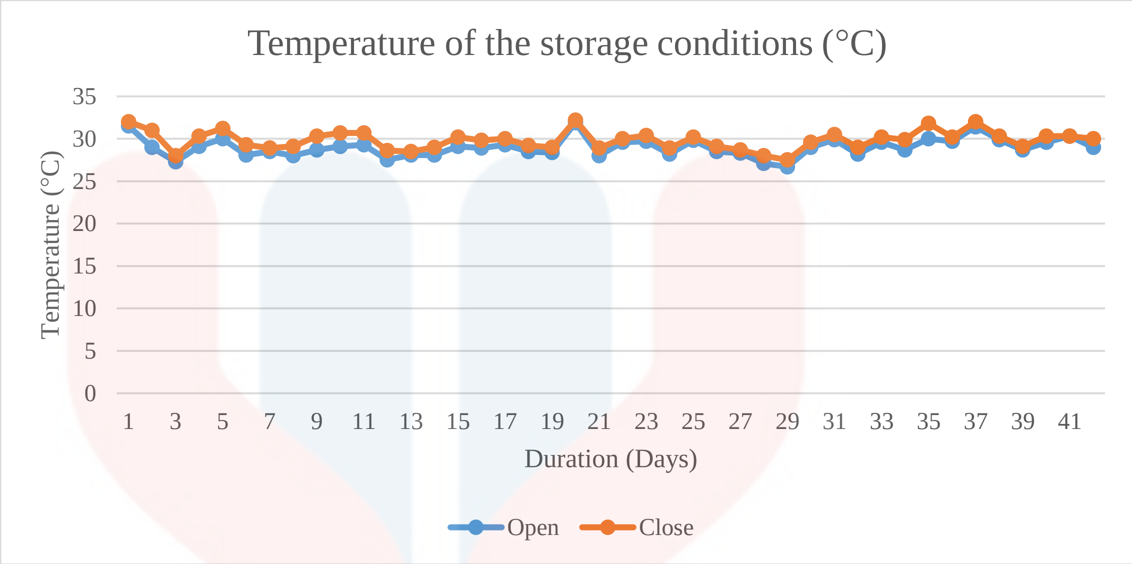


Figure 4.1: Temperature (°C) of the storage condition (open vs close) against duration of storage (days).

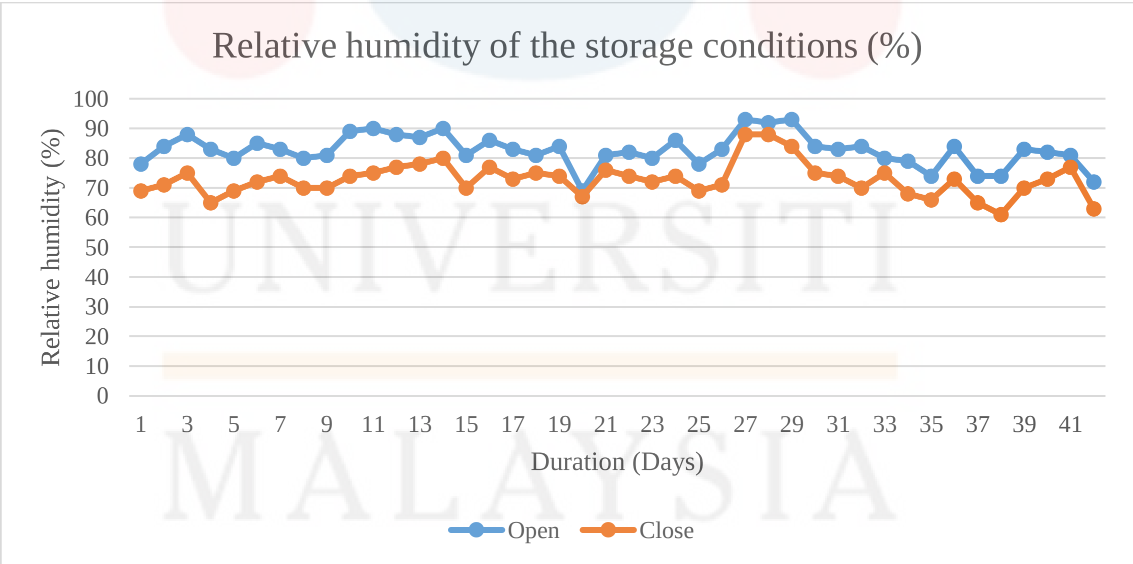


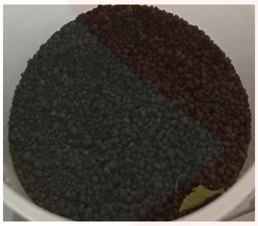
Figure 4.2: Humidity (%) of the storage condition (open vs close) against duration of storage (days).

4.1 Effect of the storage conditions on the feedstuffs

From the experiments, it is found that there is occurrence of insects in certain feedstuffs. Major of insect infestation occurs in corn meal (CM) as shown in Table 4.2 with only difference is that in open storage condition, the infestation starts on week two unlike in closed storage conditions where it already started on week one. This may be due to the differences in temperature and humidity as the closed storage area have slightly higher temperature and lower relative humidity if compared to open storage conditions as shown in Figure 4.1 and Figure 4.2.

According to Fields (1992), 25 to 33°C is the optimum temperature needed for insect infesting stored grain products to grow and reproduce. Thus, this finding can be proven why insect infestation are major in corn meal. Both of leaf meals have shown no signs in insect occurrence while infestation of insects in fish meal (FM) only occurred in open storage conditions starting from week five as shown in Table 4.1.

Table 4.1: Weekly physical observation of the feedstuffs on both storage conditions

Weeks	Observation on the storage conditions	
	Open storage conditions	Closed storage conditions
1	No insects were found. No changes in smell and texture. 	No changes in smell and texture. Rice weevil presents in sample F2A1, F2A2, and F2A4 
2	No changes in smell and texture. Rice weevil presents in sample F2A1, and F2A2 	No changes in smell and texture. Rice weevil presents in sample F2A1, F2A2, F2A4, F2A5 
3	No changes in smell. CM has started to clump. Rice weevil presents in F2A1, F2A2 Larvae of red flour beetle present in F2A5 Adult flour beetle present in F2A1, F2A4	No changes in smell and texture. Rice weevil presents in F2A1, F2A2, and F2A4. Adult flour beetle present in F2A4 Both adult and larvae of red flour beetle present in F2A5

		
4	The smell of the FM and CM had started to incorporated with the aromatic materials. No changes in smell for both leaves meal. Rice weevil presents in F2A1, F2A2 Adult flour beetle present in F2A1, F2A2, F2A3, F2A4, F2A5.	The smell of the FM and CM had started incorporated with the aromatic materials. No changes in smell for both leaves meal. Rice weevil presents in F2A1, F2A2, and F2A4. Adult flour beetle present in F2A1, F2A2, F2A4, F2A5. Larvae of red flour beetle present in F2A1, F2A2
		
5	The smell of the FM and CM had started incorporated with the aromatic materials. No changes in smell for both leaves meal. *Tyrophagus putrescentiae* present in F1A3 Rice weevil presents in F2A1, F2A2	The smell of the FM and CM had started incorporated with the aromatic materials. No changes in smell for both leaves meal. Rice weevil presents in F2A1, F2A2, F2A4, F2A5 Adult flour beetle present in F2A1, F2A2, F2A4, F2A5

	Adult flour beetle present in F2A1, F2A3, F2A4, F2A5 Larvae of red flour beetle present in F2A4, F2A5. 	Larvae of red flour beetle present in F2A1, F2A2 
6	The smell of the FM and CM had incorporated with the aromatic materials. No changes in smell for both leaves meal. However, the leaves meal become clumpy. *Tyrophagus putrescentiae* present in F1A2, F1A3 Rice weevil presents in F2A1, F2A2 Adult flour beetle present in F2A1, F2A3, F2A4, F2A5 Larvae of red flour beetle present in F2A4, F2A5 	The smell of the FM and CM had incorporated with the aromatic materials. No changes in smell for both leaves meal. However, the leaves meal become clumpy. Rice weevil presents in F2A1, F2A2, F2A4, F2A5 Adult flour beetle present in F2A1, F2A2, F2A4, F2A5 Larvae of red flour beetle present in F2A1, F2A2 

Table 4.2: Presence of insects in selected feedstuffs during storage for six weeks.

Duration (Week)	Open storage conditions				Closed storage conditions			
	F1	F2	F3	F4	F1	F2	F3	F4
1	-	-	-	-	-	+	-	-
2	-	+	-	-	-	+	-	-
3	-	+	-	-	-	+	-	-
4	-	+	-	-	-	+	-	-
5	+	+	-	-	-	+	-	-
6	+	+	-	-	-	+	-	-

+ : insects present; - : insects absent

F1: Fish meal; F2: Corn meal; F3: *Trichanthera gigantea* leaf meal; F4: Indigofera zollingerianan leaf meal.

4.2 Effect of the aromatic materials on the feedstuffs

Inclusion of aromatic materials (AM) in feedstuffs can be significantly seen its changes in the inclusion with corn meal (CM) treatments as shown in Table 4.3. In closed storage conditions, all the inclusion of AM in CM have shown signs of insects present except cloves. Inclusion of cloves in closed storage area is proven that it can be one of the materials to ward off insects from infesting the feed that may be due to strong smell of cloves. However, in open storage conditions, insects infesting cloves treatment in CM starts at week 4 which is similar to the Control group. Both of these treatments have the longest shelf life if compared to others AM inclusion of open storage conditions treatments. It can be proven by research that was done by (Grainge and Ahmed, 1988) that reported that the usage of clove buds in stored products can ward off *Triboleum castaneum* and kill *Ctenocephalides canis* and *Pediculus humanus humanus* while study done by (Ho, 1995) confirms that extraction from this AM is capable in supress the presence of adult *Sitophilus zeamais* and eggs of *T. castaneum.*

Unlike cloves in closed area, other inclusion of AM had started infestation on the first week in the closed area. However, in open area, it takes longer time to be infested which averagely on week three. This may be due to temperature and humidity of the surrounding area. In close area, temperature recorded was ranging from 28.5°C to 32.3°C while its humidity range from 67% to 88% while in open area, temperature ranging from 27.5°C to 31.9°C while the humidity range from 69% to 93%. The temperature is slightly higher while the humidity is slightly lower in the closed area if compared to the open area and this may be the contribution factor for the infestation.

Usage of whole garlic as AM to ward off insects in CM have proven to be ineffective in both opened and closed storage conditions as these treatments have the shortest storage life in terms of insects infestation if compared to others AM as shown in Table 4.3. Thus, whole garlic is not recommended to be used as one of the AM to ward off insects in CM and fish meal (FM) as infestation of cereal mites in FM in open storage conditions had started on week five. However, there is a result from a study published in World Journal of Agricultural Sciences Vol. 7, Issue 5 has shown contradiction from the result obtained from this experiment. In the study, volatile constituent, diallyl sulphaye was extracted from garlic (*Allium sativum*) has shown positive effect as it is toxic when applied as fumigant to ward off adult *Tribolium castaneium* (Upadhyay and Ahmad, 2011). Thus, it showed that the usage of garlic should be extracted and formed essential oil instead of using garlic as whole in order to prevent insects' attack especially red flour beetle as it is proven to has been an established components in fighting against stored gtrain pests.

Infestation of flour beetles inside the corn meal can be explain by a study which state that most of external infesting species such as Indian meal moth and flour beetles like to get its nutrients from processed grain products (Keefe and Campabadal, 2015). Beside, when the temperature is between 20 degree Celcius and 30 degree Celcius, this beetle has the optimum conditions to develop from an egg into an active adult (Keefe and Campabadal, 2015). On physical senses which is smell, FM and CM has incorporated smell with its AM especially the ones with inclusion of *pandan* leaves (PL). However, both leaf meals has no significant changes in smell but all treatments for both of the feed ingredients started to clump on the sixth week although no records of attacks from insects had been observed. Clumping also occurred on CM treatments starting from week three. Other than that, no deterioration effects has occur on both of the leaf meal.

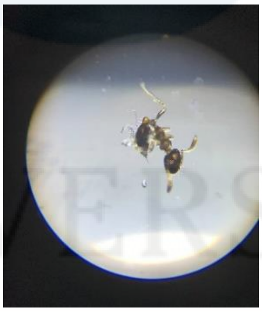
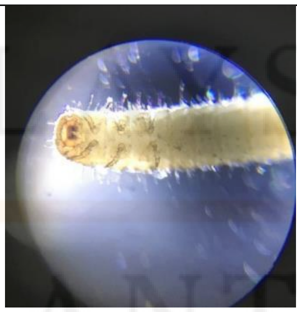
Table 4.3: Observation of the effect on the inclusion of aromatic materials on most infested feedstuff

Aromatic materials		Storage conditions	
		L	C
Pandan leaves		Infestation of insects starts on week 3	Infestation of insects starts on week 1
Garlic		Infestation of insects starts on week 2	Infestation of insects starts on week 1
Cloves		Infestation of insects starts on week 4	No infestation occur
Dried chillies		Infestation of insects starts on week 3	Infestation of insects starts on week 1
Control (No AM)		Infestation of insects starts on week 4	Infestation of insects starts on week 5

4.3 Type of insects and pests that are found in the feedstuffs

There are several types of insects that were found in the feedstuffs during the experiment such as mites (*Tyrophagus putrescentiae*), rice weevil (*Sitophilus oryzae*), pharoah ant (*Monomorium Pharaonis*), red flour beetle (*Tribolium castaneum*). All the insects that were present during the observation is mentioned in Table 4.4 while in Table 4.5 shows the insects with its details. An insects basic life cycle all started from eggs, larvae, pupae and lastly into a beetle. All the eggs that are hatched is either survived or not and it usually takes one to two weeks to hatch under summer conditions. After the eggs hatched, a larvae that looks like a worm will eat, grow and undergo shedding of their skins. After a successful shedding, their heads become larger and by the time they reach full size, they will feed for once and again shed their skins. From stage of larvae, they are now become a pupae or "worms" which is known as a resting stage in which the pupae is inactive and does not eat or move around. After the dormant stages, the pupae turn into an adult beetle which can lives up to one year or more.

Table 4.4: Type of insects and pests infested

Sample	Picture of Insect (under microscope)	Name of the insect
Fish Meal		Mites (*Tyrophagus putrescentiae*)
Corn Meal		Rice Weevil *Sitophilus oryzae*
		Pharaoh Ant *Monomorium pharaonis*
		Red Flour Beetle (Larvae)

		
	 	Red Flour Beetle *Tribolium castaneum*

Table 4.5: List of insects infesting selected feedstuffs

Common name	Other names	Scientific name	Family	Order	Other common stored products attacked
Cereal mites	Forage mite, mill mite	*Tyrophagus putrescentiae*	Acaridae	Astigmata	Cereals, feed, flour
Rice weevil	Black weevil	*Sitophilus oryzae*	Curculionidae	Coleoptera	Rice, maize, wheat, sorghum, pulses
Pharaoh ant	-	*Monomorium pharaonis*	Formicidae	Hymenoptera	Infest almost anything
Red flour beetles	Red weevil, flour weevil, bran bug	*Tribolium castaneum*	Tenebrionidae	Coleoptera	All cereals, starch, pulses, oilseeds, spices, dried fruits

Source (Chomchalow, 2003; Upadhyay and Ahmad, 2011)

4.3.1 Cereal Mites (*Tyrophagus putrescentiae*)

Cereal mites (CRM) are also known as flour or grain mites. They are pale, pearly or grayish white body feature with its leg's colour vary from yellow to reddish-brown. This organisms are often found infesting food and feed products, cereals, cheese, and corns. Female mites will lay eggs that are oval, smooth, white and are 0.12 mm long. After the eggs has been hatched, the larval stage takes place in which the larvae will has only six legs. Next, when they move on to the next life stages which is nymphal stages, they have the same feature as the adult which is they have eight legs. When they are in juvenile stage, their body wall will hardens and suckers will appear on the underside. This stage is called as hypopus (Jacobs, 1988). In this experiment, it is shown that CRM only show occurrence in (FM) under treatments of garlic and cloves as the AM. The infestation is only a minor infestation and it occur at the week fifth of the experiment. Only two out of total treatments had CRM.

4.3.2 Rice Weevil (*Sitophilus oryzae*)

There are two type of weevils that are well-known which are granary weevil (*Sitophilus granius*) and rice weevil (*Sitophilus oryzae*). Both of the species can be found in diverse distribution in which damage are less serious in cooler climates. Rice weevil (RW) often prefer warmer climate compared to granary weevil which are much more tolerant towards low temperature and cold climates (Mason & McDonough, 2012). RW has a dull dark brown with four indistinct light spots unlike the granary weevil which is shiny and reddish-brown beetle, much bigger in size and a bit passive if compared to rice weevil (Shepard, 1938; Mason & McDonough, 2012). These species are known as internal feeders or primary pests that are usually found developing inside kernels, feeding on the inner endosperm, and producing holes in the kernel for adults to exit (Mason & McDonough, 2012). The infestation of RW is most common in CM and it already started infesting the feedstuff during the first week of the experiment.

Figure 4.3.2: Infestation of rice weevil during first week of the experiment

4.3.3 Pharoah Ant (*Monomorium Pharaonis*)

Pharaoh ants has a reddish brown to slightly tan bodies and known to be one of the smallest ants exist (Drees and Jackman 1998). They are an omnivore species which fed on anything as they have broad choices in the diet such as other insects, terrestrial non-insect arthropods, seeds, grains and nuts and also stores or caches food (Morris, 2000). The pharaoh ant only present during the first week of observation. They are gone and are not seen any near the storage for the next observation. This may be due to its foraging behaviour towards stores or supplies food.

4.3.4 Red Flour Beetle (*Tribolium castaneum*)

Red flour beetle (RFB) or its scientific name *Tribolium castaneum* is a species from group Tenebrionidae which is known as darkling beetles. This group has more than 10,000 species which makes them the large and varied group and about 100 species are known to be associated with stored products. This species are most commonly to attack stored products such as maize, wheat, flour and other food ingredients. The life cycle started with white eggs that are small and cylindrical which lay by adult female in the product. Up to eleven eggs can be lay daily at the optimum temperature of 32.5°C. After the eggs was hatched, it turns to a yellowish larvae with a pale brown head that live insides the grain until pupae stages. When reaching the adult stages, this species can live up to a year or more and the female can lay up to a thousand of eggs in her lifetime. The infestation of RFB can be seen starting on week three of the experiment while its larvae has started to be seen on the next week of the observation. This species only be seen infesting CM as stated in Table 4.4 where maize is one of the most common stored products that they infested.

4.4 Signs of infestation from microbial activity

In livestock feed industry, no matter if it comes from feed manufacturer or the farmer itself, infestation from microbial activity would likely to happen other than insects infestation. Most of microbial activity comes from mould produced toxins known as mycotoxin which contaminate most foods and feeds (Cetinkaya, 2019) Mycotoxin contamination can be determine based on several major environmental factors which are temperature extremes, humidity, moisture during harvest and storage, harvesting methods, and also infestation from insects (Coulombe, 1993; Cetinkaya, 2019). Mycotoxin contamination can cause several effects as shown in Figure 4.3. There are several analysis that can be done to measure the presence of mycotoxin in feed are high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) or biochemical (immunoassays) techniques (Mueller-Harvey, 2004). In this experiment, no signs of mould or fungi had been observed as shown in Table 4.1.

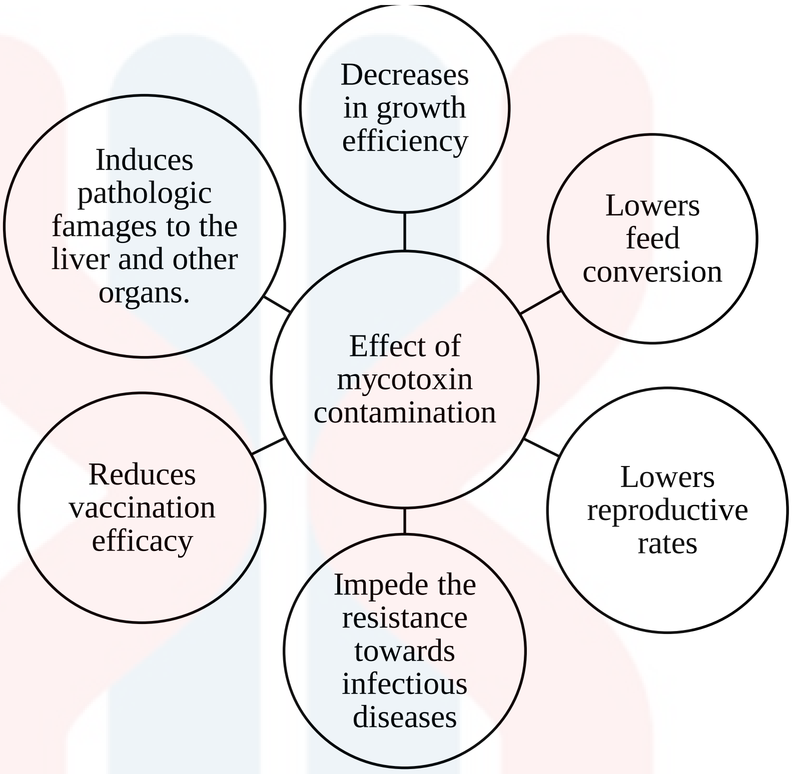


Figure 4.4: Effects of mycotoxin contamination in livestock industry (Source: Coulombe, 1993)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The main objective of this study is to determine how to store feedstuffs properly and testing several materials to ward off the infestation of pests such as rice beetles besides observing any physical changes towards the feedstuffs. From the experiment, it is known that aromatic material such as cloves do have impact in warding off insects if compared with other three aromatic materials in corn meal especially when placing it in closed area making the storage life of the corn meal is longer if compared to corn meal treatments in open area. Thus, small-scale farmers are recommended to use cloves as AM in corn meal in order to store the feedstuff up to a month instead of other AM as a way to ward off insects.

Inclusion of aromatic materials such as *pandan*, garlic and dried chillies in the corn meal placed in open and closed area also had shown difference result as placing in closed area has caused faster infestation of insects which may be due to temperature and humidity of the area that inhibit from insects attacking or reproducing. Fish meal and both *T. gigante*a leaf meal and *I. Zollingeriana* leaf meal has longer shelf life compared to corn meal. Thus, usage of both leaf meals can be heightened as locally produced feedstuffs instead of purchasing import feedstuffs which could cost more towards the farmers in terms of its storage life. However, more study is needed to be done in the future in order to know the palatability and nutrient value of the leaf meals in the livestock diets. If the leaf meals is suitable enough, it can be alternatives of protein source for animal feeds.

As in conclusion, in order to increase the storage life of corn meal, the feed ingredients should be kept inside a closed area in addition with the inclusion of cloves as aromatic materials to ward off any pests during the storage. Next, to increase the storage life of fish meal, the feedstuffs should be kept in a close container in a close area. The inclusion of AM did not significantly give differences if compared to the control group but instead, the feedstuff develop the smell of the AM within it. Besides, the usage of garlic as aromatic materials cannot be used as a whole garlic instead extraction to become essential oil is much more proven to be effective in controlling pest infestation.

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APPENDIX

APPENDIX A

Figure A.1: Grinding of corn to make corn meal

Figure A.2: Collection of experiments materials

Figure A.3: *I. zollingeriana* leaves purchased from local farmer

Figure A.4: T. gigantea leaves purchased from local farmer.

Figure A.5: Drying of the leaves by using sun-drying method

Figure A.6: *T. gigantea* leaves

Figure A.7: *T. gigantea* leaves after complete dry

Figure A.8: *I. zollingeriana* leaves after complete dry

Figure A.9: Before starting grinding procedure

Figure A.10: *I. zollingeriana* leaves after grinding

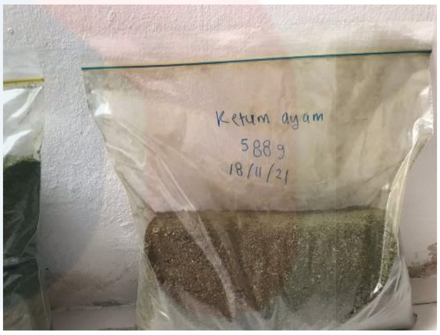


Figure A.11: *T. gigantea* leaves after grinding

Figure A.12: Treatment samples preparation

Figure A.13: Closed area storage conditions (drawer)

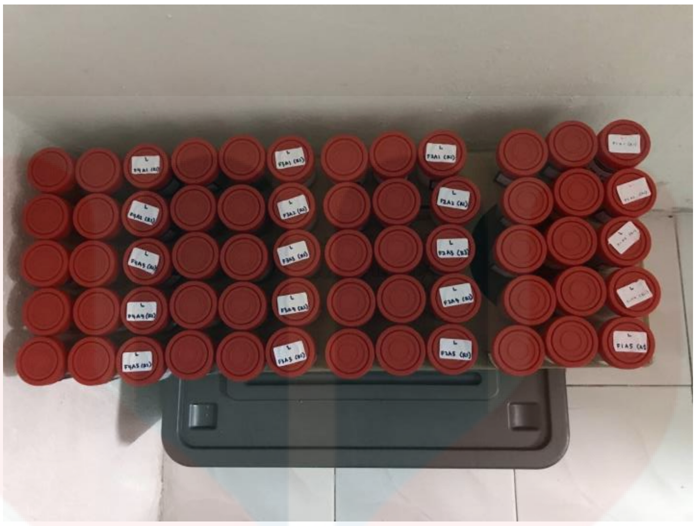
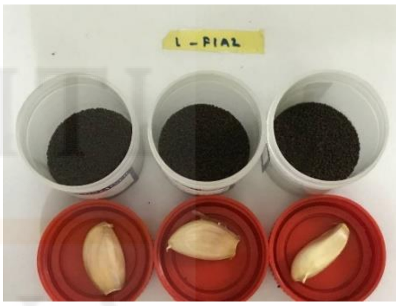
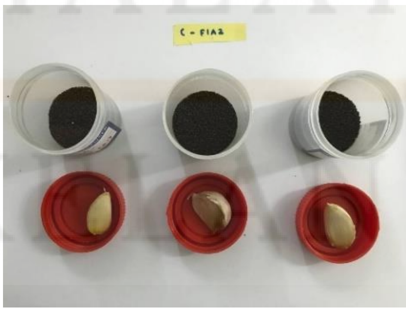


Figure A.14: Open area conditions

APPENDIX B

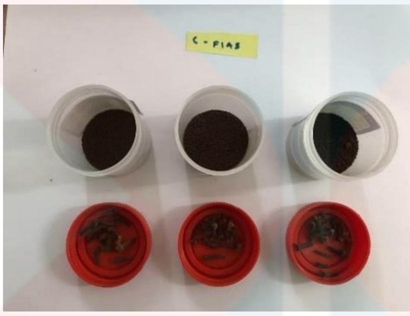
Table B.1: Sample taken during initial and final observation

List of treatments		Initial (week 1)	Final (week 6)
F1A1	L		
	C		
F1A2	L		
	C		

F1A3

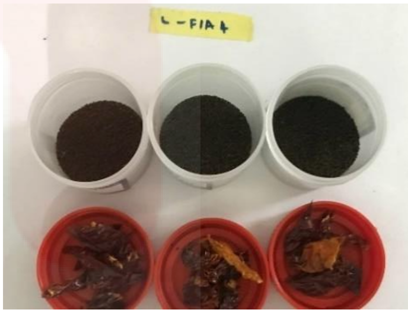
L

C

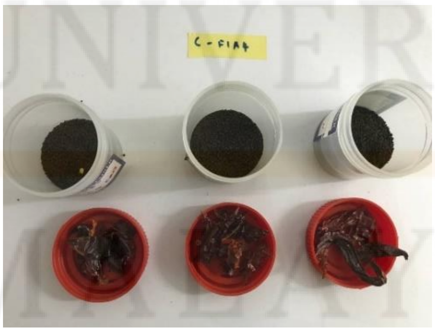


F1A4

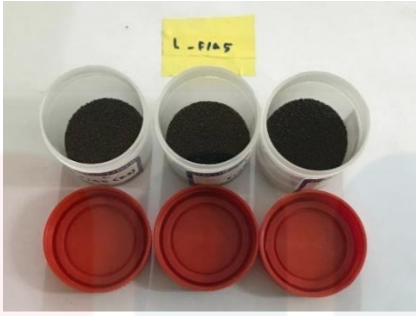
L

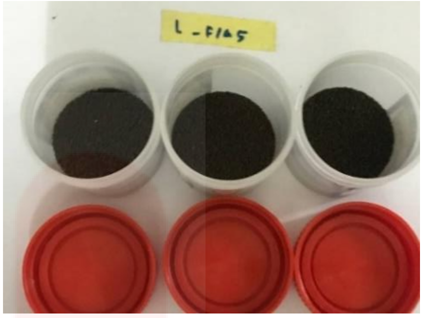


C



F1A5 L





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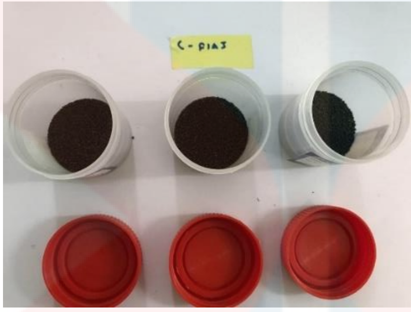
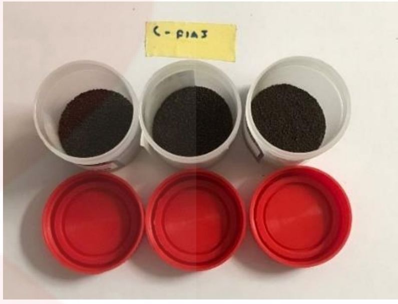
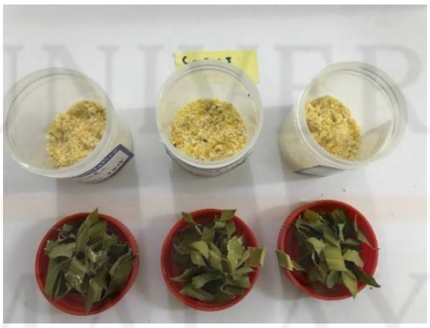




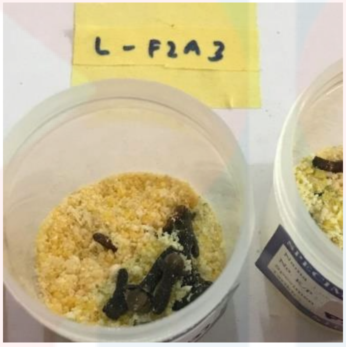
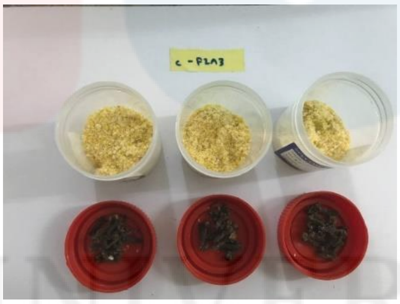
L

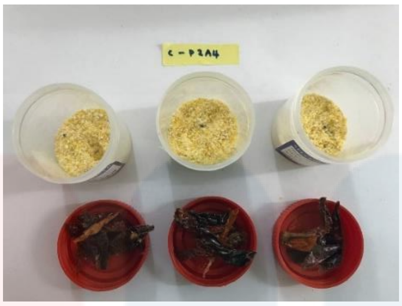
F2A1

C




F2A2 L

	C		
F2A3	L		
	C		
F2A4	L		
	C		



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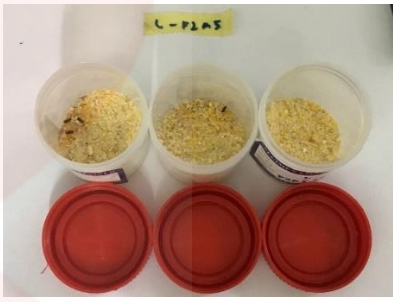
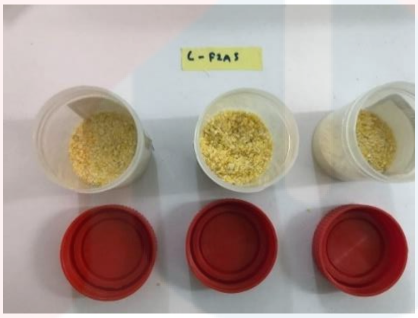
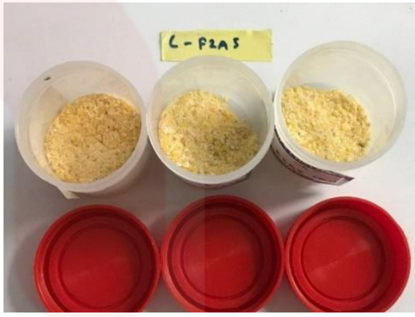


F2A5

C





L


F3A1

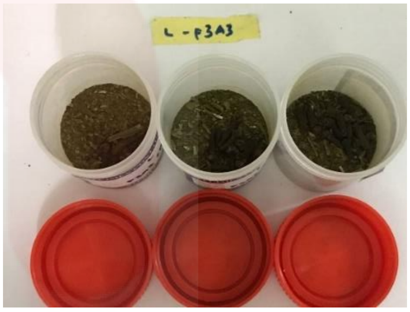
C

L

F3A2

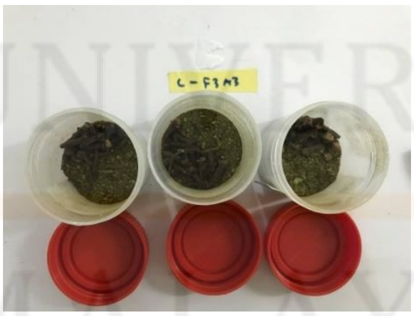
C

L

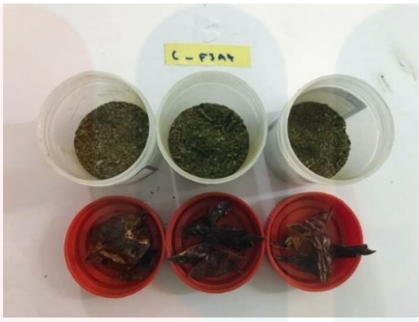
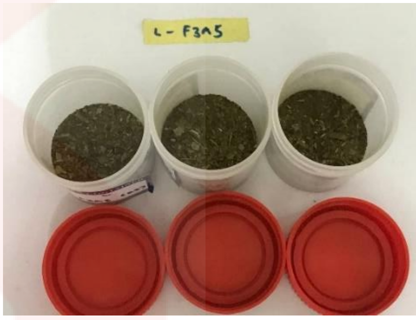
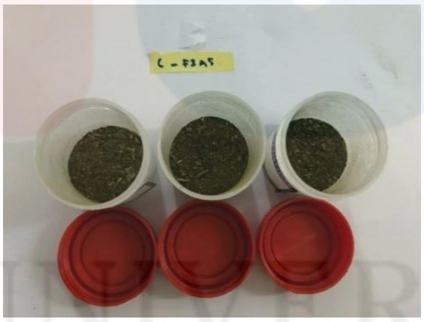
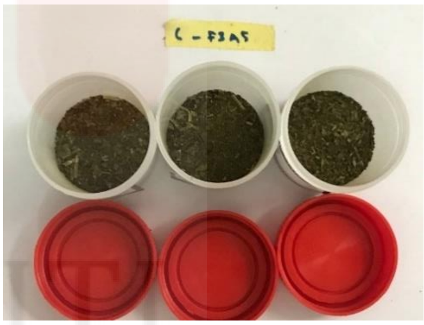


F3A3

C



F3A4 L

	C		
F3A5	L		
	C		
F4A1	L		
	C		

F4A2

L

C

F4A3

L

C

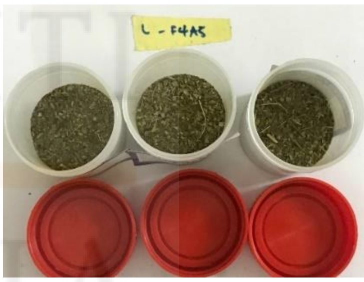
F4A4

L

C

F4A5

L



C

Table B.2: Infestation of insects or pests towards the feedstuffs

List of treatments		Replicates	After 7 days in storage	After 14 days in storage	After 21 days in storage	After 28 days in storage	After 35 days in storage	After 42 days in storage
F1A1	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F1A2	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	Yes
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F1A3	L	(R1)	No	No	No	No	Yes	Yes
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F1A4	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F1A5	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F2A1	L	(R1)	No	No	Yes	Yes	Yes	Yes
		(R2)	No	No	Yes	Yes	Yes	Yes
		(R3)	No	Yes	Yes	Yes	Yes	Yes
	C	(R1)	Yes	Yes	Yes	Yes	Yes	Yes
		(R2)	Yes	Yes	Yes	Yes	Yes	Yes
		(R3)	Yes	Yes	Yes	Yes	Yes	Yes
F2A2	L	(R1)	No	No	No	Yes	Yes	Yes
		(R2)	No	Yes	Yes	Yes	Yes	Yes
		(R3)	No	Yes	Yes	Yes	Yes	Yes
	C	(R1)	Yes	yes	Yes	Yes	Yes	Yes
		(R2)	Yes	Yes	Yes	Yes	Yes	Yes
		(R3)	Yes	Yes	Yes	Yes	Yes	Yes

F2A3	L	(R1)	No	No	No	Yes	Yes	Yes
		(R2)	No	No	No	Yes	Yes	Yes
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F2A4	L	(R1)	No	No	Yes	Yes	Yes	Yes
		(R2)	No	No	No	No	Yes	Yes
		(R3)	No	No	No	No	Yes	Yes
	C	(R1)	Yes	Yes	Yes	Yes	Yes	Yes
		(R2)	Yes	Yes	Yes	Yes	Yes	Yes
		(R3)	Yes	Yes	Yes	Yes	Yes	Yes
F2A5	L	(R1)	No	No	No	Yes	Yes	Yes
		(R2)	No	No	Yes	Yes	Yes	Yes
		(R3)	No	No	No	Yes	Yes	Yes
	C	(R1)	No	No	No	No	Yes	Yes
		(R2)	No	No	No	No	Yes	Yes
		(R3)	No	Yes	Yes	Yes	Yes	Yes
F3A1	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F3A2	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F3A3	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F3A4	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F3A5	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No

F4A1	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F4A2	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F4A3	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F4A4	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
F4A5	L	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No
	C	(R1)	No	No	No	No	No	No
		(R2)	No	No	No	No	No	No
		(R3)	No	No	No	No	No	No