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Evaluation of Biopesticide Against *Sitophilus Oryzae L.*

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except the excerpts and summaries that I have made clear of the sources.

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Evaluation of Biopesticide Against *Sitophilus Oryzae L.*

ABSTRACT

On a worldwide scale, rice is the third most produced agricultural product, therefore insect pest losses in rice storage might have a considerable influence on food supply for a huge number of people. The *Sitophilus oryzae* L., is one of the most problematic pests in the tropics. Despite the fact that there are few effective fumigants, environmental contamination, non-target organism toxicity, and pesticide residues are all major concerns. Botanicals are a prospective source of pest control chemicals since there are so many plants that contain insecticidal compounds, and they've sparked a lot of attention in recent years as possible natural pesticide sources. The primary goal of this research was to determine the toxicity and repellency of Aloin and citronella oil on *S. oryzae* L. This study was designed using three distinct Aloin extract treatments (5 percent, 10%, and 15%) and two control treatments using ANOVA test. The results revealed that at 15% concentration, the most toxic and repellent effects were observed, followed by 10% and 5%, while insect mortality percentage was found to be directly proportional to time after treatment, and the repellent effect increased proportionally to the level of Aloin extract concentration. Furthermore, the results revealed that 15% concentration had the maximum toxicity and repellency against rice weevil, followed by 10%, and the lowest was found in control (0%) followed by 5% concentration. At the conclusion of the study, we demonstrated that the biopesticide is effective against *S. oryzae* in terms of mortality and repellency. Our agriculture sectors would benefit from research into improving the effectiveness of botanical derivatives as alternatives to synthetic insecticides because these botanicals are not only less expensive but also have a lower environmental impact than insecticides.

Keywords: Toxicity, Repellency, *Sitophilus oryzae L.*, Aloin, Citronella oil, ANOVA, Mortality

Penilaian Racun Perosak Biologi Terhadap *Sitophilus Oryzae L.*

ABSTRAK

Pada skala dunia, beras adalah produk pertanian ketiga yang paling banyak dihasilkan, oleh itu kehilangan disebabkan oleh serangga perosak dalam simpanan beras mungkin mempunyai pengaruh yang besar terhadap bekalan makanan untuk sebilangan besar orang. *Sitophilus oryzae L.*, adalah salah satu daripada perosak yang paling bermasalah di kawasan tropika. Walaupun fakta bahawa terdapat sedikit fumigan yang berkesan, pencemaran alam sekitar, ketoksikan organisma bukan sasaran, dan sisa racun perosak semuanya menjadi kebimbangan utama. Botani ialah sumber prospektif bahan kimia kawalan perosak kerana terdapat begitu banyak tumbuhan yang mengandungi sebatian racun serangga, dan ia telah mencetuskan banyak perhatian dalam beberapa tahun kebelakangan ini sebagai kemungkinan sumber racun perosak semula jadi. Matlamat utama penyelidikan ini adalah untuk menentukan ketoksikan Aloin dan penghalau oleh minyak sitronella pada *S. oryzae L.* Kajian ini direka menggunakan tiga rawatan serbuk ekstrak Aloin yang berbeza (5 peratus, 10%, dan 15%) dan dua rawatan kawalan menggunakan ujian ANOVA. Keputusan menunjukkan bahawa pada kepekatan 15%, kesan paling toksik dan penghalau diperhatikan, diikuti oleh 10% dan 5%, manakala peratusan kematian serangga didapati berkadar terus dengan masa selepas rawatan, dan kesan penghalau meningkat secara berkadar ke tahap kepekatan serbuk ekstrak Aloin. Tambahan pula, keputusan menunjukkan bahawa 15% kepekatan mempunyai ketoksikan maksimum dan penolak terhadap kumbang padi, diikuti dengan 10%, dan yang terendah didapati dalam kawalan (0%) diikuti dengan kepekatan 5%. Pada akhir kajian, kami menunjukkan bahawa racun perosak biologi berkesan terhadap *S. oryzae* dari segi kematian dan penghalau. Sektor pertanian kita akan mendapat manfaat daripada penyelidikan ini untuk meningkatkan keberkesanan derivatif botani sebagai alternatif kepada racun serangga sintetik kerana bahan botani ini bukan sahaja lebih murah tetapi juga mempunyai kesan alam sekitar yang lebih rendah daripada racun serangga.

Kata kunci: Ketoksikan, Penghalau, *Sitophilus oryzae L.*, Aloin, Minyak sitronella, ANOVA, Kematian

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LIST OF ABBREVIATIONS AND SYMBOLS

Bt	Bacillus thuringiensis
cm	centimetre
HAT	Hours After Treatment
IPM	Integrated Pest Management
ITK	Indigenous Technical Knowledge
g	gram
kg	kilogram
LOAEL	Lowest Observed Adverse Effect Level
mg	milligram
nm	nanometre
PIPs	Plant-Incorporated Protectants
°C	degree celcius
&	And
%	Percent

CHAPTER 1

INTRODUCTION

1.1 Introduction

On a worldwide scale, rice is the third most produced agricultural product, therefore insect pest losses in rice storage might have a considerable influence on food supply for a huge number of people. *Sitophilus oryzae*, is one of the most dangerous pests in the tropics. Despite the fact that there are few effective fumigants, environmental contamination, non-target organism toxicity, and pesticide residues are all major concerns. Botanicals are a prospective source of pest control chemicals since there are so many plants that contain insecticidal compounds, and they've sparked a lot of attention in recent years as possible natural pesticide sources (Sekar et al, 2021).

1.2 Research Background

Rice is the most extensively eaten food in the planet. This is why gardeners try to keep unhusked rice grain in a variety of containers so that it may be used throughout the year. However, it is a truth that stored grain pests cause substantial loss or damage under storage settings, posing a severe danger to our national economy. Insect pests cause the loss of 5-8 percent of food grains, seeds, and other stored items each year, with estimates ranging from 5% to 10%. *Sitophilus oryzae L*. (Coleoptera: curculionidae) is a major storage pest that reduces the nutritional value of stored cereal grains all over the world. Around 15% of stored rice is lost to pests, with the *S. oryzae L*., being one of the most damaging pests (Das et al, 2015).

The bug not only destroys the grain, but it also reduces its weight and quality. It harms maize weevils and granary weevils in the same way. *S. oryzae* may cause severe harm to grain under storage settings, either directly by eating the grain or indirectly by causing moisture loss, making the grain more appropriate for their consumption. *S. oryzae* wreak havoc on husked and unhusked stored rice, as well as maize, wheat, and sorghum, especially during the monsoon season. Field damage is less important than storage damage.

To keep the grain safe, a persistent battle against stored grain pests must be undertaken, since they do major harm to the grains. *Sitophilus oryzae* damage has been claimed to be reduced by a variety of preventative techniques. Chemical pesticides, for example, have a variety of major downsides, including unfavourable side effects, hazardous residues, excessive application costs, and, eventually, consumer health risks (human and animal). It also pollutes the air, water, and soil, endangering the entire environment. Furthermore, the prolonged use of chemical pesticides leads to the establishment of cross- and multiple-resistant variants in a variety of key insect species.

In this environment, the development of alternative chemical techniques, such as the quest for novel forms of botanical insecticides and the reintroduction of centuries-old conventional pest control chemicals, is receiving worldwide interest. Because plant-derived insecticides may be used in practical applications in natural crop protection, small-scale farmers profit. Due to their low toxicity to beneficial insects, natural and easily biodegradable crop protection inputs might be an effective component of an IPM approach. Furthermore, botanical pesticides have no or few negative effects and toxic residues that are non-hazardous to humans and other vertebrates, and they can be easily generated by farmers at a cheaper cost, are safer, and easier to apply. The study was undertaken to investigate the repellency and toxicity of natural bio insecticides against rice weevil due to a lack of scientific research in this field in our nation.

1.3 Problem Statement

Environmental contamination, non-target organism toxicity, and pesticide residues are all concerns, despite the fact that there are few effective fumigants. Botanicals are a viable source of pest control chemicals since there are so many plants that contain insecticidal compounds, and they've sparked a lot of attention as a natural pesticide source in recent years. The biopesticide employed to suppress *Sitophilus oryzae L.* in rice, on the other hand, was nearly fatal to humans. From maturation until use, stored grains are vulnerable to loss owing to a range of causes such as physical, sanitary, and nutritional insufficiency. The rice weevil, *Sitophilus oryzae L.*, is a major pest of stored grains, inflicting large quantitative and quality losses as well as economic loss. We can minimise rice weight loss, which results in losses for farmers or storage owners, as well as lower rice aesthetic values, by using an economically feasible pest like aloin extract, which is low in toxicity to people. That is why we have chosen this chemical-free insect control strategy.

1.4 Objectives

The specific objectives of this study are:

I. The toxicity effect of aloin on *Sitophilus oryzae*.
II. The repellent effect of citronella oil on *Sitophilus oryzae*.

1.5 Significant of the Study

This research will also help consumers become more conscious of the usage of biopesticides, allowing them to consume less hazardous rice on a regular basis. As a result, the country's economic growth will be aided, as the less rice lost during storage, the more rice will be produced. Aloin generated from natural sources is a combination of two diastereomers, aloin A (also known as barbaloin) and aloin B (or isobarbaloin), which have similar chemical features but do not damage consumers, which is why aloin was chosen for this study. Aloin is a glycosylated anthraquinone, which indicates that a sugar molecule has been attached to the skeleton of the anthraquinone. Anthraquinones are a collection of yellow, orange, and red pigments found in nature, many of which have cathartic qualities, which aloin also has. Aloin is linked to aloe emodin, a sugar-free compound that shares many of aloin's biological features. This research will also improve customer knowledge of the advantages of low-toxicity goods.

CHAPTER 2

LITERATURE REVIEW

2.1 Life Cycle of *Sitophilus oryzae L.*

Sitophilus oryzae L. is the scientific name for rice weevils. These insects are found all over the world and are believed to have originated in the Far East. They may develop anywhere that has favourable physical conditions for growth and if the grain is left undisturbed for an extended length of time. *Sitophilus oryzae* is a common bacterium found in grain storage and processing plants. Infested grains include wheat, oats, rye, barley, rice, and maize. They've been seen infesting beans, sunflower seeds, and dry maize on occasion. These insects are not biting or wood-destructive. Chewing mouthparts are present in *S. oryzae*. *Sitophilus oryzae* are most easily identified by their comparatively lengthy snout. The adults of the *S. oryzae* are reddish-brown in colour. It has an unevenly shaped thorax and four bright dots on its wing coverings. Rice weevils have the ability to fly. During the larval stage, they are legless, white to creamy white in colour, and have a tiny head. Pupa of *S. oryzae* have snouts, just like adults. A single generation may be completed in 28 days. In the face of predators, *S. oryzae* imitate death by pulling their legs close to their bodies and keeping silent.

Egg: Rice weevil eggs are deposited in the cracks of kernels or dust. A female rice weevil can lay up to four eggs every day. It lays roughly 250-400 eggs during the course of its 5-month lifespan. In three days, the eggs will hatch.

Larvae: They feed for 18 days inside the grain kernel. The larva is the insect's only stage of development. The seed's cuticle hardens and matures inside. It eats several times its own weight and moults to grow in size on a regular basis.

Pupa: The pupa stage is 6 days long. The pupa does not consume food. The pupa is encased in a cocoon made by the larva in certain species. Both inside and outwardly, the pupa undergoes significant modifications. Finally, the insect emerges as a fully grown adult.

Adult: Adults range in length from 0.1 to 1.7 cm. Their bodies are separated into three sections: head, thorax, and abdomen. They have three pairs of legs. Adults migrate through the grain, penetrating deep into the mass of the grain and dispersing widely.

Females use a viscous material to cover a small hole in the grain kernel. Inside the grain kernel, the egg hatches into a juvenile larva that migrates toward the grain kernel's centre. The larva eats, matures, and transforms into a pupa now that it is within the grain. As it matures into an adult within the grain, the pupa undergoes major internal and exterior changes. From the emergence hole, the adult emerges. Adults that have emerged are ready to mate and create a family.

Insects grow between 17°C to 35°C. The majority of storage insects like a temperature of approximately 30°C and a relative humidity of 75 percent. The reactions of insects to high temperatures are increasingly important. The majority of insect pests die at temperatures exceeding 40°C. While most stored grain insects can withstand temperatures below freezing for several days, they all perish after being exposed to 65°C for a few minutes. The number of important storage insects is minimal, at approximately 30. The majority of species live for only a few weeks and have a common flavour. Storage insects deposit their eggs in or around fruits and vegetables in large numbers. The larvae settle inside the product after hatching and continue to develop until they reach pupal stage. Other bugs grow and pupate on or in the product as well. Adult storage insects have the ability to survive for long periods of time or for a short amount of time. Bruchids and moths only live for a short time (up to 3 weeks). They deposit the majority of their eggs during their first week as adults. Sitophilus and Tribolium, for example, have a six- to twelve-month lifetime. Deposition of eggs takes place over a long length of time.

Temperature Control: Heating and cooling are obvious ways to pest management since most stored goods insects cannot sustain severe temperatures. To some extent, superheating various commodities for bug control has been a widespread practise. Maintaining temperatures of 55-60°C for 10 to 12 hours is beneficial. In fact, high temperatures swiftly kill most insects, but when it comes to grain and materials, the temperature must be maintained for several hours to achieve thorough penetration.

2.2 Evaluation of Biopesticide

Biopesticides based on live microorganisms and their bioactive chemicals have been studied and advocated as a viable alternative to synthetic pesticides for many years. However, in the majority of situations, their lack of effectiveness, uneven field performance, and high cost have consigned them to niche items. Biopesticides' prospects have improved as a result of recent technology developments and major changes in the external environment. Biopesticides are still a modest component of pest management treatments, despite their substantial market penetration. While improvements in the activity spectrum, delivery methods, durability of impact, and implementation have helped to enhance the use of biopesticides, genuinely disruptive innovations that result in large acceptance are still absent.

Microbial pesticides, entomopathogenic nematodes, baculoviruses, plant-derived pesticides, and insect pheromones, which are used as mating disruption agents, are all gaining attention in scientific journals and the popular press as viable alternatives to chemical pesticides and as critical components of integrated pest management (IPM) systems. Biopesticides, on the other hand, now account for a tiny percentage of the worldwide pesticide business.

Biopesticides are formulations made up of naturally occurring compounds that manage pests in a non-toxic and ecologically acceptable way. They're becoming increasingly popular across the world. Biopesticides are made up of living organisms (natural enemies), their products (phytochemicals, microbial products), or by products derived from animals (e.g. nematodes), plants (e.g. Chrysanthemum, Azadirachta), or microorganisms (e.g. Bacillus thuringiensis, Trichoderma, nucleopolyhedrosis virus) (semiochemicals) The time-tested indigenous technical knowledge (ITK) of employing natural materials to manage pests has proven to be exceedingly successful; yet, as a result of the introduction and widespread use of chemical pesticides, many ITKs have been lost. Conventional pesticides represent a greater hazard to the environment and human health than biopesticides. They are less hazardous than chemical pesticides, are commonly targeted, have little or no residual effects, and may be used in organic farming.

The three basic groups of biopesticides are plant-incorporated protectants (PIPs), biochemical pesticides, and microbial pesticides. Microorganisms (bacteria, fungus, viruses, or protozoans) make up microbial pesticides, which have been employed to successfully manage insect pests. Microbial pesticides are capable of controlling a wide range of pests, despite the fact that each microbial active component is largely unique to a certain insect. Bacillus thuringiensis, or Bt, is a microbial insecticide that is extensively employed. The bacteria produce crystalline proteins and exclusively affects a small number of closely related insect species. The binding of the Bt crystalline protein to the insect gut receptor determines the target insect species.

Biochemical pesticides are naturally occurring compounds that manage pests through non-toxic ways. Insect sex-pheromones (which impede mating and population expansion), aromatic extracts (which lure insect pests to traps), and some vegetable oils are examples. Plant-incorporated protectants are chemicals that are naturally synthesised as a result of genetic alteration in plants. Incorporation of a Bt gene, a protease inhibitor, lectines, or chitinase into the plant genome, for example, causes the transgenic plant to produce its own chemical that kills the targeted pest. Pest-resistant transgenic plants generate naturally occurring biodegradable proteins that have no negative impact on animals or people, decreasing the need for dangerous pesticides. PIPs may be more helpful and cost efficient in underdeveloped nations to aid in the production of safe food, feed, and pasture.

Biopesticides offer a wide range of potential applications in agriculture and public health. Biopesticides do not leave residues on fruits and vegetables, which is a big issue for consumers. Biopesticides can be as successful as conventional pesticides when applied in combination with IPM, especially on crops including fruits, vegetables, nuts, and flowers. Biopesticides combine effectiveness with environmental safety, allowing for more application flexibility and better resistance management. The benefits of biopesticides include the following: I they are inherently less harmful and environmentally safe, (ii) they are target-specific, (iii) they are frequently effective in very small quantities, (iv) they degrade naturally and quickly, and (v) they can be used as part of integrated pest management.

2.3 Aloin

Aloin, also known as barbaloin, is a bitter, yellow-brown coloured compound found in the exudate of at least 68 Aloe species at concentrations ranging from 0.1 to 6.6 percent of leaf dry weight (corresponding to 3 to 35 percent of total exudate) and in another 17 species at indeterminate concentrations. It is used to treat constipation as a stimulant-laxative that induces bowel motions. The substance is present in aloe latex, which is released by cells positioned under the leaf's rind and between it and the gel and is secreted by cells near to the leaf's vascular bundles. It has been employed as a bittering agent in trade when dried (alcoholic beverages).

Aloe Vera consumption has long been recognised as a popular and safe way to receive the many advantages of this versatile plant. Aloe Vera has been called the "plant of immortality" and the "wand of the skies" because of its widespread use in the Americas, Egypt, and Asia. Aloe Vera is safe to eat, but only if you eat the right section of the plant. When people ingest aloin, a naturally occurring chemical found in the skin of the Aloe Vera plant, it functions as a laxative. Aloin can be present in all Aloe vera types. Aloin, which is not to be confused with Acemannan (a therapeutic component found in Aloe Vera), has long been a matter of debate, with some considering it to be useful as a stimulant-laxative for relieving constipation by inducing bowel movements. Aloin's negative effects, on the other hand, considerably exceed its advantages, making its use potentially hazardous.

Aloe vera provides a number of health advantages, including wound healing (first and second degree burns), better digestion and blood glucose levels, and a reduction in weariness and fungal infection, according to (Mallavadhani et al., 2016). Phenolic substances, glycosides (aloins), 1,8-dihydroxy anthraquinones (aloe emodin), 1,4-acetylated mannan, mannose phosphate, and alprogen gluco protein are all abundant in A. vera. Aloe vera's principal ingredient is aloin A (hydroxy anthrone glycoside), which is followed by aloin B. (hydroxy anthrone glycoside). Aloin A has antimicrobial, antifungal, antibacterial, antioxidant, and cytotoxic activities against ovarian tumour cell lines, among other biological features.

Antifungal, antibacterial, anticancer, antioxidant, cryoprotective, immunological modulatory, and insecticidal activities are also found in A. vera leaf exudate and gel. From the gel of A. vera, the phytosterols lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol were extracted; some of these metabolites had a long-term influence on blood glucose management. It has a long history of using naturally occurring plants as efficient biopesticides for the control of storage pests. Plant extracts or fractions, as well as isolated pure chemicals, have all been found to be efficient natural pesticides for a range of storage pests.

Aloin obtained from natural sources is a combination of two diastereomers, aloin A (also known as barbaloin) and aloin B, with comparable chemical characteristics (or isobarbaloin). Aloin is a glycosylated anthraquinone, which indicates that a sugar molecule has been attached to the skeleton of the anthraquinone.

Anthraquinones are a collection of yellow, orange, and red pigments found in nature, many of which have cathartic qualities, which aloin also has. Aloin is linked to aloe emodin, a sugar-free compound that shares many of aloin's biological features.

2.4 Citronella Oil

Citronella oil is an essential oil extracted from the leaves and stems of several Cymbopogon species (lemongrass). Citronellal, citronellol, and geraniol are just a few of the fragrance components found in the oil. These compounds are widely used in the soap, candles, and incense industries, as well as the perfumery, cosmetic, and flavouring sectors across the world. Citronella oil is a natural insect repellent that has been approved for use in the United States since 1948. Citronella oil is classified as a biopesticide having a non-toxic mode of action by the US Environmental Protection Agency. Citronella oil is a natural insect repellant that is widely used. Research has proven its mosquito repellent properties, including its ability to repel Aedes aegypti (dengue fever mosquito). Most citronella repellent solutions must be reapplied to the skin every 30–60 minutes to be effective.

Citronella oil includes a number of aromatic fractions, the most prominent of which are citronellal, geraniol, and citronellol. According to Windholz 1983, Khan 2009 on gas chromatographic study, the Ceylon variety includes a high percentage of monoterpenes (about 27 percent), but the Java variety contains just 1% to 3%, largely in the form of limonene. Wijesekera Sri Lanka, (1973) Geraniol levels are comparable in both forms (18 percent to 21 percent). The Java oil is preferable to the Ceylon oil because it includes 16 percent citronellol and 33 percent citronellal, whereas the Ceylon oil only has 8 percent and 5% citronellal. Essential oils have a wide range of chemical compositions.

Citronella oil also contains citronellyl acetate, beta-bourbonene, geranyl acetate, elemol, L-borneol, and nerol, among other chemicals. Citronella has been genetically modified to produce a geraniol-rich mutant with a geraniol concentration of up to 60%. Ranaweera (1996) The chemical profile of the wild Ceylon variant (often known as mana grass) is considerably different from the two farmed forms.

The efficiency of citronella preparations in avoiding bites from Aedes, Anopheles, and Culex mosquitoes, which transmit dengue disease, malaria, and encephalitis, respectively, was evaluated in a comprehensive review and meta-analysis of 11 controlled laboratory experimental investigations in humans. The assessed outcomes were the difference in mean protection time against bites and percentage of repellency compared to control. If the cage or room approach was utilised, the studies were included. In the systematic review, at least four alternative definitions of "protection time" were utilised. Citronella products differed in terms of dosage type (cream, lotion, solution, spray, wristband), production technique, citronella oil concentration (1.25 percent to 30 percent), and citronella oil quantity employed.

The majority of cage-method studies employed DEET as a comparison, ranging from 4% to 25%, whereas room-method research compared citronella to DEET ranging from 19% to 50%. In comparison to DEET 4 percent to 25 percent, citronella 10 percent to 30 percent with or without the addition of 5% vanillin provided a shorter mean protection period (19.7 to 390 minutes) against Aedes spp bites (234.4 to 480 minutes). Citronella 20 percent and 25 percent, on the other hand, offered the same mean protection period (480 minutes) against Anopheles and Culex spp. as DEET at the same dose. Furthermore, against Culex spp., 10% citronella had a longer mean protection period (312 minutes) than 7 percent DEET (288 minutes), but not 15 percent DEET (420 minutes).

The addition of 5% vanillin to 25% citronella protected subjects longer (480 minutes) than 25% DEET (360 minutes) against bites from Anopheles mosquitoes. In 2 studies investigating efficacy against Anopheles mosquitoes, citronella at concentrations of 5%, 40%, and 25% plus vanillin 5% provided 100% repellency for at least 3 hours; however, the 25% plus 5% vanillin and 40% citronella oil alone provided continued complete protection for up to 6 hours. By the 6th hour, the repellency of the 5% citronella product dropped to 77.5%.

2.5 Toxicity Effect of Aloin

Aloin and other hydroxyanthracene derivatives, which are abundant in the yellow sap of the aloe leaf, are irritating laxatives. An overabundance of Aloe latex can cause stomach discomfort, spasms, and possibly hepatitis. Electrolyte abnormalities, malabsorption, metabolic acidosis, weight loss, hematuria, and albuminuria may occur with long-term use of Aloe containing Aloin. Aloe gel should not include aloin since it can be contaminated during the filleting process; nevertheless, mechanical separation isn't always complete, and Aloe exudate can get into the gel. The lowest observed adverse effect level (LOAEL) for aloin is 11.8 g/kg body weight. Aloe foods and supplements that are free of aloin and aloe-emodin are thought to be safer than those that include anthraquinones. The aloin content regulation limit in food and drinks in

European nations is 0.1 ppm. A. vera products must be free of aloin with an extreme onset of 0.1 mg/l for human consumption, with the exception of alcoholic drinks, which have a 50 mg/kg limit.

To assess the quantity of aloin in Aloe products, a variety of procedures have been developed. To confirm Aloe quality, the capillary zone method is a simple and cost-effective approach for detecting Aloin and Aloe-emodin. By using an HPLC–UV technique, Bozzi et al. observed concentrations of Aloin A and B in commercial A. vera powders ranging from negligible levels to 16 mg/kg. UV absorbance maxima were observed at 295–300 and 354–360 nm in phenolic compounds from aloe vera.

CHAPTER 3

METHODOLOGY

3.1 Sampling Site

Sitophilus oryzae L. were reared in a 20 cm x 15 cm x 11 cm box with sterile rice for insect feeding, and the box was kept in the room for two months to multiply the insect number, which is 100 of *S. oryzae* for this experiment. To ensure a steady supply of adults, the raising technique was repeated with several batches of insects. *Sitophilus oryzae L.* are red-brown in colour, dull, and have coarse micro sculpting as adults. The lateral elevations of scutellum are generally closer together than their length, and it is clearly more than half the length of scutellum. Females with wider and rounded apical lateral lobes of internal, Y-shaped sclerite and males with median lobe of aedeagus equally convex dorsally in cross section are more narrowly separated (Cao et al, 2014).

3.2 Identification of *Sitophilus oryzae L.*

Taxonomic key were used to identify *S. oryzae* provided by Hong et al. (2018). Adults have a long snout and are between 3 and 4.6 mm long. Although the body looks to be brown/black, careful inspection reveals four orange/red dots grouped in a cross on the wing covers. It's easy to confuse it with the maize weevil, which has a similar appearance. Although the maize weevil is normally larger than the *S. oryzae L.*, rice weevils as large as the largest maize weevils have been discovered, as have maize weevils nearly as small as the tiniest *S. oryzae L*.

3.3 Aloin Extract Preparation

Aloin extract powder was obtained from Xi'an Pincredit Bio-Tech Co., Ltd with 20% of aloin in 40-60g/100ml. We are using the different concentration of aloin extract which is 5 %, 10 % and 15 % follow by (Sarah, 2018). The concentration of aloin is measured based on milligram (mg) to prevent changes from happening to the percentage of aloin extract if we add any substances.

3.4 Instrument

For the instrument that we need to use in this experiment are spatula, beakers, forceps, graduated cylinder and balances. For the beakers is for the preparation of different concentration of the aloin extract powder and the graduated cylinder is use for the measurement of the solution needed in this experiment. The use of spatula and balances of Sartorius with 0.1mg accuracy is for measured the amount of biopesticide needed in the treatment of toxicity test and repellency test. At last forceps use to take the die rice weevil and put aside for observation.

3.5 Treatment

3.5.1 Treatment for Toxicity Test

In the toxicity test, we used three treatments and three replicates with different concentrations of aloin extract powder (5 percent, 10%, and 15%). For the control treatment we use other biopesticide powder as a positive control and the negative control nothing has been put in (Das et al, 2015). In this experiment, five pairs of *S. oryzae* were divided into three replicated treatments by mixing 10 g of rice with three concentrations (5, 10, 15%), as well as two control tests. Following that, the killing ratio was recorded (Sarah, 2018).

3.5.2 Treatment for Repellency Test

In the repellency test, we used three treatments and three replicates with different concentrations of citronella oil: 5%, 10%, and 15%. To prevent citronella oil from changing to citronella oil if mixed with other substances, the concentration of citronella oil was measured in millilitres (mg). In this experiment, we used a different repellent as a positive control and water as a negative control, both of which are expected to have no effect. A container was divided into two groups for the treatment technique we used in this experiment: those who were treated and those who were not. In the half of the rice, a span with citronella oil was placed. Then ten *S. oryzae* were released in the container's centre. The number of insects in each section was then counted at hourly intervals until the third hour (Sarah, 2018).

3.6 Measurement

Toxicity test: The toxic effects of the biopesticide and for the control treatment will takes at every 24 hours after treatment on rice weevil until death. At each replicate the adult mortality was recorded, and the data was adjusted and converted to a percentage from the original data following the Abbott's formula given below (Das et al, 2015).

Corrected mortality=

$$\frac{Observed\ mortality - Control\ mortality}{100 - Control\ mortality} \times 100 \qquad (3.1)$$

Repellency test: Up to the third hour after the treatment, the number of insects in each section was counted at hourly intervals (Das et al, 2015). The following formula was used to convert the data to percentage repulsion:

$$PR = (NC - 50) \times 2 \qquad (3.2)$$

Where,

NC = the percentage of insects present in the control half. Positive (+) values indicated repellency, whereas negative (-) values indicated attraction.

Analysis of variance (ANOVA) was used to examine the data (PR). The average values were then classified into the following classes.

Table 3.1: Repellency rate (%)

Class	Repellency rate (%)
0	>0.01 to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

3.7 Statistical Analysis

This study gathered the results of the toxicity and repellency tests, and the data was statistically analysed using the least significant difference–LSD test (ANOVA) to compare the means.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Toxicity Test

The toxic effects of different concentrations of aloin extract which is concentrations (5%, 10%, and 15%), positive and negative control at every 24 hours after treatment on rice weevil until death was evaluated in this experiment was presented in the graph below.

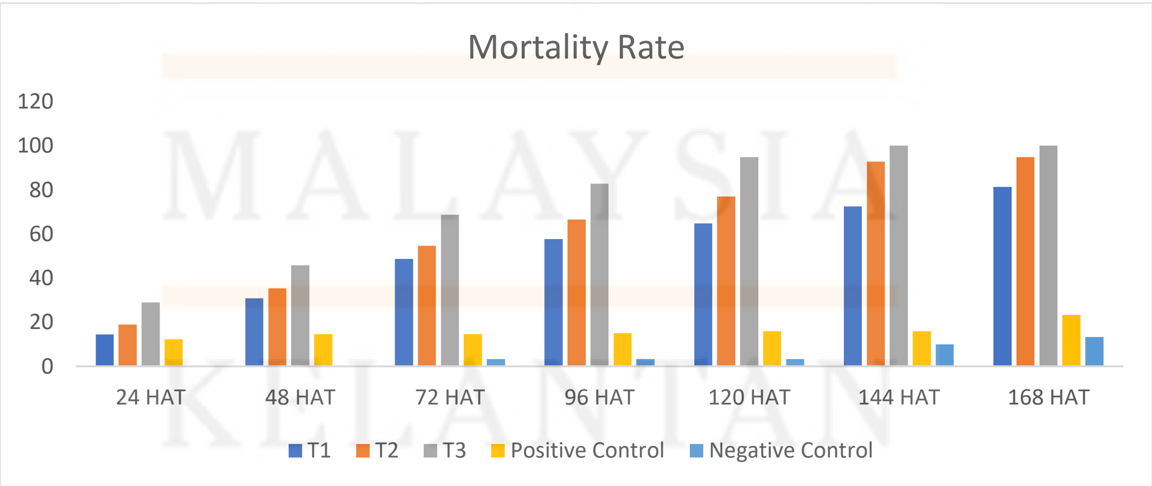

Figure 4.1: Mortality rate of *S. oryzae*

4.1.1 Toxic Effect of Different Treatment of The Aloin Extract Powder on The Mortality of The *S. oryzae*

Based on the graph show above the toxic effect of three different aloin extract on the rice weevil *S. oryzae* at 24, 48, 72, 96, 120, 144 and 168 hours after treatment (HAT) was found statistically insignificant along. But numerically, the results indicated that all of the different concentrations of aloin powder possessed the lowest mortality at 24 HAT and the highest at 168 HAT. At 24 HAT, among three concentrations, we found that 5% claimed the lowest mortality (14.44%) preceded by 10% concentration (18.89%) and the highest mortality (28.89%) was found against 15% concentration. At 168 HAT, treatment 3 caused the highest mortality (100%) followed by treatment 2 (94.81%) due to high toxicity and the lowest mortality (81.63%) against treatment due to low toxic effect.

It was quite evident that all of the concentrations possessed the lowest mortality at 24 HAT and the highest at 168 HAT. At 24 HAT, the lowest mortality (0%) was measured in control (0%) i.e. untreated seed and the highest mortality (33.33%) produced in 15% concentration. At 120 HAT, 3% concentration caused the highest mortality (100%) followed by 2% concentration (76.92%) due to its high concentration and toxicity, and the lowest mortality (16.67%) was measured in control (0%) i.e. untreated seed.

In addition, the highest average daily mortality (17.89%) was observed in case of the treatment 3 and the lowest average daily mortality (3.33%) was observed in case of. So, the order of toxicity for three concentrations and two controls on rice weevil, *S.*

oryzae L. was shown as concentration 15% > 10% > 5% > Positive control > Negative control (0%).

Here, it was worth mentioning that the mortality percentage of the test insects increased with the increase of time until their death. It has been reported that Aloe vera extract cause the highest is reported that the high mortality of the *S. oryzae* cause by the highest mortality at 15% of concentration about 63% mortality of *Sitotroga cerealella* at 24 hours after treatment (HAT) which was much higher than our result (28.89%) obtained against rice weevil at same time after treatment. It might be due to the distinct difference of *S. oryzae*, laboratory environment along with variation in doses of aloin extract.

However, the dissimilar mortality percentage of *S. oryzae* caused by three concentrations might be due to significant variation among the concentrations of the aloin along with disparate composition, presence and functioning of noxious ingredients present in the different concentrations used in the study.

In all, the disparity of mortality percentage of the *S. oryzae* might be owing to variation in degree of presence and functioning of toxic ingredients along with distinct difference in nature and structure of the botanicals used in the experiment. The toxic effect of different concentrations of aloin on the rice weevil, *S. oryzae* at 24, 48, 72, 96, 120, 144 and 168 hours after treatment (HAT) varied significantly from one another.

From the results, it was distinct that mortality of insects increased with the increase of concentration level of aloin; this is supported by where they observed the same phenomenon on *Sitotroga cerealella* by using Aloe vera extract concentrations. The variation in mortality percentage of the *S. oryzae* (rice weevil) might be due to difference in the concentrations (5%, 10% and 15%) of aloin extract having unequal functioning and proportion of toxic ingredients.

4.2 Repellency Test

The toxic effects of different concentrations of citronella oil which is concentrations (5%, 10%, and 15%) and positive and negative control at every 1 hours after treatment on rice weevil until death was evaluated in this experiment was presented in graph below.

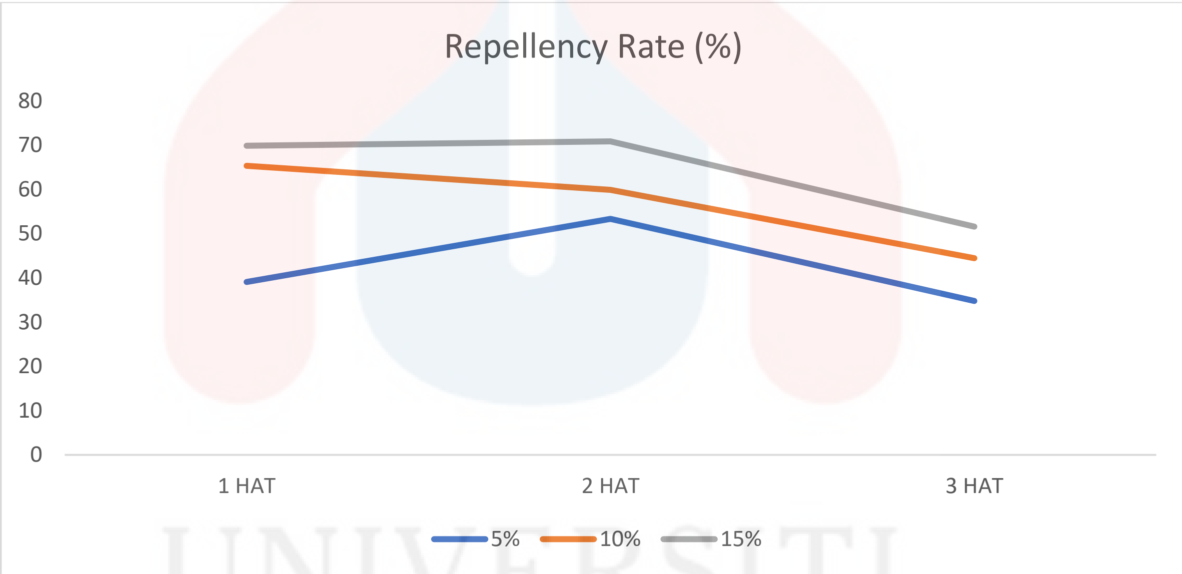

Figure 4.2: Repellency rate of *S. oryzae*

4.2.1 Repellent Effect of Different Concentrations of Citronella Oil on *S. oryzae*

The *Sitophilus oryzae* repellency rate of three treatments at various hours after treatment was statistically significant and insignificant. According to the graph above, treatment 3 had the highest repellent effect (69.85 percent) at 1 HAT, while treatment 2 had the lowest repellent effect (65.33 percent) and treatment 1 had the least repellent effect (39.11 percent). The lowest repellent rate, on the other hand, was discovered at 3 HAT, where the highest (51.58 percent) and lowest (34.78 percent) repellency were found against treatment 3 and treatment 1, respectively.

At different hours after treatment, different concentrations of citronella (5 percent, 10%, and 15%) had a statistically significant repellent effect on *S. oryzae*. The highest number of insects (70.85 percent) were repelled by 15% concentration at both 1 and 2 HAT, and the lowest (34.78 percent) were repelled by 5% concentration at 3 HAT. Treatment 3 (51.58 percent) had the highest average repellency per hour, followed by treatment 2 (56.55 percent), and treatment 1 had the lowest (42.41 percent). Citronella oil had the highest repellent effect on *S. oryzae*, according to (Jaykumar M. et al., 2021), which was significantly higher than our documented result.

It could be due to the fact that the *S. oryzae* are different, the laboratory environment is different, and the botanical formulations are different. The unequal repellent percentage on the *S. oryzae* (rice weevil) in this study could be due to differences in the degree of odour, the presence of repelling compounds, and the activity of citronella oil, as well as their nature and structure.

Again, the highest average repellent intensity per hour (64.18%) caused by high concentration (15%) followed by medium concentration (10%) repelling (56.55%) and the lowest (42.41%) caused by low concentration (5%). The repellent effect of different concentrations of the citronella oil increased with the increase of concentrations which was supported by the study where they observed alike repellent trend on.

According to repellent intensity, the order and repellency class of various concentrations of citronella oil could be expressed as 15%> 10%> 5% and IV>III>III respectively. However, the disparity of repellent effect among three concentrations of citronella oil on the *S. oryzae* (rice weevil) might be owing to difference in the degree of concentrations which regulated their repellent strength as well as persistence of odorous compounds present in the concentrations of the botanicals.

As a result, the researchers discovered that the repellent effect increased as concentrations increased and vice versa. The fact that there were distinct differences in the fluctuation of concentrations (5 percent, 10 percent, and 15 percent) in their quantity, as well as disparate composition, existence, and action of repelling ingredients, could explain the dissimilarity of repellent effect of botanicals and their concentrations on the *S. oryzae* (rice weevil).

CHAPTER 5

CONCLUSION

5.1 Conclusion

This study evaluated the toxicity and repellency effect of aloin and citronella oil as bio pesticides to protect unhusked rice seed damage from the *S. oryzae*. At the conclusion of the study, we demonstrated that the biopesticide is effective against *S. oryzae* in terms of mortality and repellency. Our agriculture sectors would benefit from research into improving the effectiveness of botanical derivatives as alternatives to synthetic insecticides because these botanicals are not only less expensive but also have a lower environmental impact than insecticides. As a result, it can be concluded that this study established the fact that locally available natural bio pesticides can be used as a very effective eco-friendly weapon to protect unhusked unboiled rice from severe *S. oryzae* infestations in storage.

5.2 Limitation of the Study

The lack of existing literature information to support the research was a major stumbling block in the success of this study because aloin is rarely uses as biopesticides. The most significant challenge in this study is the scarcity and high cost of aloin powder sources, as well as the difficulty in rearing *Sitophilus oryzae* L. because the insect died before the study's timeline began, requiring me to rear it again and take time to collect enough samples.

5.3 Recommendation

Based on the result I would recommend for the improvement in future study is; aloin and citronella oil can be use as biopesticide in the combating the store product pest *S. oryzae*. However, in the cost benefit analysis usage of the aloin and citronella oil as biopesticide need to be evaluated. In addition, the sensory test of the rice treated with these aloin to be further tested and its side effects on the human digestive system.

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APPENDIX

Table 1: Mortality rate of treatment 1

		Mortality of the Test Insect (%)						
Treatment	Replicate	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT
1	1	13.33	30.74	51.85	59.26	65.74	75.65	89.26
	2	13.33	30.74	45.93	56.02	63.89	70.93	77.89
	3	16.67	31.11	48.15	57.87	64.88	70.93	76.74
Control	Positive	12.31	14.65	14.65	15	15.89	15.89	23.33
	Negative	0	0	3.33	3.33	3.33	10	13.33

Table 2: Mortality rate of treatment 2

		Mortality of the Test Insect (%)						
Treatment	Replicate	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT
2	1	23.33	41.11	58.52	70.37	80.56	88.67	91.76
	2	16.67	30.74	53.33	68.06	72.22	100	100
	3	16.67	34.07	51.85	61.11	77.98	89.87	92.67
Control	Positive	13.45	13.45	15.54	16.66	18.67	16.67	23.33
	Negative	0	0	3.33	3.33	6.67	10	10

Table 3: Mortality rate of treatment 3

		Mortality of the Test Insect (%)						
Treatment	Replicate	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT
3	1	33.33	44.81	72.22	85.19	95.64	100	100
	2	23.33	44.44	64.07	76.39	90.89	100	100
	3	30	48.15	69.67	86.85	97.89	100	100
Control	Positive	15.76	15.76	22.46	27.33	30.45	30.45	33.33
	Negative	0	0	0	3.33	3.33	10	13.33

Table 4: Mean of Mortality rate

	Mortality of the *S. oryzae* (%)						
Treatment	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT
1	14.44333	30.86333	48.64333	57.71667	64.83667	72.50333	81.29667
Positive	12.31	14.65	14.65	15	15.89	15.89	23.33
Negative	0	0	3.33	3.33	3.33	10	13.33
2	18.89	35.30667	54.56667	66.51333	76.92	92.84667	94.81
Positive	13.45	13.45	15.54	16.66	18.67	16.67	23.33
Negative	0	0	3.33	3.33	6.67	10	10
3	28.88667	45.8	68.65333	82.81	94.80667	100	100
Positive	15.76	15.76	22.46	27.33	30.45	30.45	33.33
Negative	0	0	0	3.33	3.33	10	13.33

Table 5: Repellency rate of treatment 1

	Repellency Rate			
Replicate	1 HAT	2 HAT	3 HAT	Repellency per hour
1	34.44	44.44	23.44	34.11
2	48.44	57.78	40	48.74
3	34.44	57.78	40.89	47.03

Table 6: Repellency rate of treatment 2

	Repellency Rate			
Replicate	1 HAT	2 HAT	3 HAT	Repellency Per Hour
1	59.65	48.89	26.67	45.07
2	66.67	60	48.89	58.52
3	69.67	70.72	57.78	66.06

Table 7: Repellency rate of treatment 3

	Repellency Rate			
Replicate	1 HAT	2 HAT	3 HAT	Repellency Per Hour
1	66.67	68.44	44.44	58.52
2	72.77	72.77	53.67	66.4
3	70.11	71.33	56.65	66.03

Table 8: Mean of repellency test and repellent class

	Repellency Rate				
Treatment	1 HAT	2 HAT	3 HAT	Repellency Per Hour	Repellency Class
1	39.11	53.33	34.78	42.41	III
2	65.33	59.87	44.45	56.55	III
3	69.85	70.85	51.58	64.18	IV

Table 9: Anova for Toxicity Test

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7208.543	3	2402.848	1.983087	0.143402	3.008787
Within Groups	29080.08	24	1211.67			
Total	36288.63	27				

Table 10: Anova for Repellency Test

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6701.761	3	2233.92	20.97796	0.00038	4.066181
Within Groups	851.9115	8	106.4889			
Total	7553.672	11				

APPENDIX

Thesis

ORIGINALITY REPORT

20%	16%	6%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

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