CORRELATION BETWEEN MUNG BEANS *(Vigna radiata)* METABOLITES PROFILES AND RESISTANCE TO STORAGE BRUCHIDS *(Callosobruchus spp)* INFESTATIONS; A POTENTIAL BIOMARKER

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for an award of a diploma or conferment of degree in any institution or University

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DEDICATION

This work is dedicated to my mother Jenesia Gatanka, my wife Edith Nkirote and my children Precious, Victor and Baby Ian for their encouragement during the entire study.

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ABSTRACT

Mung beans (*Vigna radiata* (L.) Wilczek), also known as green grams, is an important legume crop cultivated for food and as a source of income. mung beans seeds are highly susceptible to infestation by bruchids (*Callosobruchus spp)*, which can cause significant post-harvest loses,hence minimizing post-harvest losses is essential for global food security. Bruchids infestation control remains a top priority. The overreliance on chemical pesticides for pest control raises environmental concerns due to the potential harm to non-target species, soil, and water quality. Investigating natural resistance mechanisms in mung beans can contribute to more sustainable and eco-friendly pest management practices. This resistance is believed to be related to the presence of specific metabolites or secondary compounds in the plant biomarkers This study aimed at determining the correlation between mung beans metabolite levels and their resistance to storage bruchids infestations. The susceptibility of twenty-three (23) mung beans varieties both wild and local, obtained from KALRO Katumani were evaluated against pulse beetle, *Callosobruchus malculatus*, under laboratory conditions using 'no choice' test. Fifty (50) seeds of each test sample were placed in a separate petri dish. Five (5) male and female pairs of 0–24-hours old adults of the beetle were released into each petri dish, and then covered well to prevent the insects from escaping and allow air circulation. After 72 hours observations were made and recorded on ovipositioning preference by determining the number of eggs laid, percentage seed damage by counting the seeds with one or more holes from the total. The metabolite profiles were performed on seed samples according to AOAC procedure and using UV-Vis spectrophotometer OD reading for the respective varieties to determine total proteins, carbohydrates and soluble sugar. Total phenol, flavonoids and tannins content were quantified were quantified using gallic acid, catechol and tannic acid as standards respectively Proteins and carbohydrates were quantified using albumin serum and glucose respectively. One way analysis of variance (ANOVA) was used to evaluate the differences in metabolite profiles among 23 mung beans varieties. The ANOVA results ($p \leq 0.05$) indicated that there were significant differences in metabolite profiles across the varieties. F-test was used to compare treatment means, significant F values indicated that the metabolite profile levels among different mung bean varieties were not the same. Pearson's correlation, analysis was used to evaluate the connections between seed damage and metabolite variables. The mung beans variety V100-U exhibited the highest resistance to bruchid (*Callosobruchus maculatus*) infestations, with the seed damage. Other varieties, including AMVU-H, AMVU-A, AMVU-C, and V100-S, also demonstrated high levels of resistance. The ANOVA results, characterized by significant F values and extremely low P values (all < 0.0001), demonstrate that the metabolite profiles (proteins, carbohydrates, phenols, tannins, and flavonoids) among different mung beans varieties are significantly distinct. The study's results implied that the mung beans metabolite profiles are linked to resistance mechanism to bruchid (*Callosobruchus maculatus*) infestation. This study recommends the integration of highly resistant varieties, such as V100-U, into breeding programs to improve overall resistance to bruchid infestations

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AOAC:	Association of Official Analytical Chemists
ANOVA:	Analysis of Variance
ASAL:	Arid and semi-arid areas
IPM:	Integrated Pest Management
KALRO:	Kenya Agricultural and Livestock Research Organization
NACOSTI:	National Commission for Science, Technology and Innovation
ODO:	Optical Density
TIA:	Trypsin Inhibitor Gene
UV/Vis Spectroscopy:	Ultraviolet/Visible Spectroscopy

OPERATIONAL DEFINITION OF TERMS

The following terms have been operationalized as follows in this study.

Bioassay	It is a scientific procedure used evaluate the effectiveness of different mung beans metabolites conferring resistance to bruchids.
Biomarkers	Refers to certain metabolites in mung beans that serve as indicators of resistance to bruchid infestations.
Bruchids (*Callosobruchus spp)*	Refers to insects that belong to genus *Callosobruchus* which are common pests that infest mung beans.
Metabolites	They include both primary and secondary chemical compounds that may contribute to the plant's resistance against bruchid infestation.
Metabolomics	It is the comprehensive study of metabolites within a biological sample which includes profiling of mung beans to identify specific compounds that correlate with resistance to bruchid beetles
Mung Beans *(Vigna radiata)*	Mung beans are a type of legume valued for their nutritional content and versatility in cooking commonly known as green grams.
No Choice Test	No-choice tests is an experimental setup used to evaluate the intrinsic resistance of different mung bean varieties to bruchid beetles by observing the pests' survival and reproductive success on each variety.
Primary Metabolites	Primary metabolites are essential compounds involved in basic metabolic processes necessary for plant growth and development, such as sugars, amino acids, and nucleotides.

Secondary Metabolites	They are compounds that are not directly involved in growth and development but often play a role in plant defense.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Mung beans (*Vigna radiata* L.) also known as green grams, is an important legume crop grown throughout Asia and Africa (Prajapati *et al.*, 2022). In Kenya it is commonly grown in various counties, namely; Machakos, Makueni, Kitui, Tharaka Nithi, Embu and Meru, among others (Muchomba *et al.*, 2023). Mung beans play an important role as a food security crop because of its nutritional quality as well as its ability to survive in harsh environmental conditions such as arid and semi-arid lands (ASALs) (Nyongesa *et al.*, 2019). The crop is cultivated in many sub-saharan Africa countries, used for human food sources, as well as a source of income (Kpoviessi *et al.*, 2021). It is a rich source of protein and other essential nutrients and is widely used in traditional cuisines in the form of boiled dry beans, stew, as well as in modern food products (Pataczek *et al*., 2018). However, mung beans crop is often affected by pests and diseases, including the bruchid beetle, which cause significant damage and yield losses (Resources *et al*., 2017).

Bruchids (*Callosobruchus maculatus*) commonly termed as pulse beetle are, storage pests of worldwide importance to mung beans (Mbeyagala *et al.*, 2017). The *C. maculatus* attacks several pulse crops such as mung beans, cow peas and pigeon peas. Severe infestations can lead to grain losses of up to 100 percent within six months of storage (Poornasundari & Thilagavathy, 2015). This pest attacks mung beans both in the field and in storage, with greater losses occurring in stored grains. Bruchid beetles lay their eggs on the mung beans pods and the larvae feed on the seeds, reducing their quality and viability. Several approaches have been used to control bruchids in mung beans crops, including the use of synthetic pesticides, biological control agents and breeding for resistance varieties *(*Harshitha *et al.*, 2022). In Kenya farmers usually apply chemicals to preserve mung beans in storage from bruchid infestation (Wangui, 2021). However, the use of chemical products on food grains in storage has negative effects on human and animal health, and on other organisms leading to biodiversity loss and an extensive environmental contamination (Divekar *et al* 2022; Harshitha *et al*., 2022).

Many developing countries are adopting the use of resistant grain varieties to control stored grain weevils as a popular alternative to the use of chemicals (Yewale *et al*., 2020). Throughout their lifetime, all living organisms encounter environmental and biotic challenges. Darwin's evolutionary theory emphasizes "survival of the fittest" or "natural selection," where the most fit organisms compete, survive and reproduce. The fittest individuals possess diverse genetic potential to defend against stress, allowing them to grow, develop and survive. This adaptive evolution leads to ecological specialization within a species, defining a specific niche and ultimately resulting in speciation (Somta *et al.,* 2008). Plants, being sessile organisms face continuous exposure to various biotic and abiotic stresses in their natural environments, including attacks from herbivores or pathogens, drought, salinity, UV-irradiation, extreme temperatures and nutritional imbalances. Notably, phytophagous herbivores are held responsible for causing approximately one-fifth of the world's total crop production destruction annually (Divekar *et al*., 2022).

Plants have evolved several types of secondary metabolites such as alkaloids, terpenes, amines, glucosinolates, cyanogenic glucosides, quinones, phenolics, peptides and polyacetylenes as a defensive shield to protect themselves from phytophagous herbivores (Divekar *et al.*, 2022). Plant defence involves both mechanical and chemical factors. Understanding these factors helps in developing adequate breeding strategies. In cow peas, reports showed that seeds colour, size, hardness, texture and biochemical compounds are usually involved in the resistance pathways; however, it has been reported that physical or mechanical characteristics of cow pea seeds were less significant than the biochemical components of cow pea seeds for conferring resistance to *Callosobruchus maculatus*. Secondary metabolites have been revealed to be important in conferring the cow pea seed resistance to bruchid (Kpoviessi *et al*., 2021). For example high levels of phenol and tannins showed a detrimental effect on the growth and development of *C. maculatus* in cow peas whereas high levels of total soluble sugars favoured them (Prajapati *et al.,* 2022). There is need to understand the mechanisms underlying mung beans resistance to bruchids in order to develop more effective and sustainable pest control strategies. One approach is to analyse the metabolite profiles of different mung beans varieties and identify potential biomarkers of resistance (War *et al.,* 2017).

Metabolites are small molecules produced by cellular metabolism and they play essential roles in various biological processes including defence against pests and diseases (Profile, 2020). By analysing the metabolite profiles of different mung beans varieties, researchers can identify the specific metabolites associated with bruchid resistance and develop potential biomarkers that can be used to screen and select more resistant varieties (Padhiyar *et al.*, 2022). This research contributes to the identification of resistant varieties and promote the production of more resilient and high-quality mung beans varieties (Prajapati *et al.*, 2022). In this study, metabolomics tools and approaches were applied in analysing and relating mung beans secondary metabolite profiles with resistance to storage bruchids as a potential breeding biomarker.

1.2 Statement of the Problem

Mung beans (*Vigna radiata*) are a vital source of protein and nutrition for many communities, particularly in regions where they are a staple food. However, these legumes are highly susceptible to infestations by storage bruchids (*Callosobruchus spp*), which cause significant post-harvest losses. Current methods of pest control often rely on chemical pesticides, which have adverse environmental and health effects. Efforts to develop more sustainable and eco-friendly pest control strategies, such as exploiting the inherent resistance of certain plant varieties, have gained attention. Previous studies have indicated that mung beans varieties exhibit varying levels of resistance to storage bruchids. This resistance is believed to be related to the presence of specific metabolites in the plant. There is a need to further investigate and understand the correlation between mung beans' metabolites and the resistance to storage bruchid infestations. This correlation, if established, could provide valuable insights into potential breeding strategies for developing pest-resistant mung beans varieties hence reducing reliance on chemical pesticides, and minimizing post-harvest losses.

1.3 Main Objective

To determine the correlation between the mung beans (*Vigna radiata*) metabolite profiles and resistance to storage bruchids *(Callosobruchus spp)* infestations.

1.3.1 Specific Objectives

i. To identify mung beans varieties resistant to storage bruchids infestations using “No choice” test by determining seed damage

ii. To determine the metabolite profiles of mung beans varieties using UV Vis spectroscopy.
iii. To determine the relationship between mung beans metabolite profiles and resistance to storage bruchids infestations.

1.4 Hypothesis

H_{01} There is no significant difference in mung beans varieties resistance to storage bruchids.

H_{02} There is no significant difference in metabolite profiles in mung beans varieties.

H_{03} There is no correlation between mung beans metabolites profiles and resistance to storage bruchids infestations.

1.5 Justification

Mung beans (*Vigna radiata*) are a crucial source of dietary protein and essential nutrients for millions of people, particularly in regions where they are a dietary staple. Ensuring their productivity and minimizing post-harvest losses is essential for global food security. Infestations of mung beans by storage bruchids (*Callosobruchus spp*) result in substantial economic losses for both small-scale and large-scale agricultural producers. These losses have a direct impact on livelihoods, food security and economic implications. The overreliance on chemical pesticides for pest control raises environmental concerns due to the potential harm to non-target species, soil, and water quality. Investigating natural resistance mechanisms in mung beans can contribute to more sustainable and eco-friendly pest management practices. Reducing the use of chemical pesticides not only benefits the environment but also minimizes potential health risks to farmers and consumers who may be exposed to pesticide residues. Understanding the correlation between mung beans' metabolites and resistance to storage bruchids can inform targeted breeding programs. By developing resistant varieties, we can minimize post-harvest loses and reduce the need for chemical interventions. This research offers an opportunity to advance understanding of the correlation between mung beans secondary metabolites profiles on resistance to bruchid infestations using Ultraviolet visible spectroscopy (UV-Vis spectroscopy) a cost effective, accessible technique that is sensitive to specific absorption characteristics of different metabolites highly effective for detecting and quantifying various plant metabolites including phenolic compounds, flavonoids and tannis.

Previous studies have indicated the existence of resistance in mung beans varieties, a comprehensive investigation into the metabolite's profiles and bruchid resistance for the considered varieties is currently lacking. Filling this knowledge gap is crucial for practical applications in pest management. As global agriculture faces challenges associated with climate change and sustainability, finding eco-friendly and sustainable solutions to pest control is paramount. This research aligns with the goals of sustainable agriculture by exploring alternative pest management strategies and present metabolite profiling as a tool for breeding resilient crop varieties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Effects of Bruchids on Mung Beans

The impact of Bruchids on mung beans can be substantial, affecting both the quality and quantity of the stored seeds. The larvae, upon hatching, burrow into the seeds, consuming the cotyledons, which leads to a reduction in seed viability and germination rates (Smith *et al*., 2014). This infestation not only diminishes the nutritional value of the seeds but also results in economic losses for farmers who rely on mung beans as a significant part of their livelihood (Williams *et al.*, 2015). The damage caused by these pests can be exacerbated by factors such as poor storage conditions and inadequate pest management practices (Rajapakse *et al.*, 2014).

Mung beans are a vital crop in many parts of the world, especially in Asia, where they serve as a crucial source of protein (Nair, 2014). The infestation of these beans by Bruchids poses a threat to food security and agricultural sustainability in these regions. Research has shown that the life cycle of Bruchids is closely tied to the environmental conditions of storage facilities. For instance, high humidity and temperatures can accelerate the development of these beetles, leading to more severe infestations (Schmale *et al*., 2014).

Various strategies have been employed to manage Bruchid infestations in mung beans, ranging from chemical treatments to biological control methods. Chemical insecticides have been widely used; however, concerns about environmental impact and the development of resistance among Bruchid populations have prompted the exploration of alternative approaches (Arthur *et al*., 2014). Biological control methods, such as the use of natural predators and parasitoids, offer a more sustainable and eco-friendly solution (Sharma *et al.*, 2014). Additionally, the development of resistant mung beans varieties through breeding programs has shown promise in reducing the susceptibility of seeds to Bruchid attacks (Kumar *et al*., 2015).

Furthermore, improved storage practices play a critical role in mitigating Bruchid infestations. Techniques such as hermetic storage, which involves sealing the seeds in airtight containers, can effectively reduce oxygen levels and inhibit the development of

Bruchids (Baoua *et al*., 2014). Implementing integrated pest management (IPM) strategies that combine multiple control methods can provide a more comprehensive approach to managing these pests (Kumar *et al*., 2015).

Recent advances in molecular biology have also contributed to our understanding of Bruchid infestations in mung beans. Genomic studies have identified specific genes associated with resistance to Bruchids, paving the way for the development of genetically modified mung beans varieties (Smith *et al*., 2014). These advances hold significant potential for enhancing the resilience of mung beans to pest infestations and improving crop yields (Williams *et al.*, 2015).

Moreover, research has expanded to understand the biochemical and physiological mechanisms underlying Bruchid resistance. Studies have identified various secondary metabolites in mung beans that play a role in deterring Bruchid infestation (Singh *et al.,* 2018). This knowledge is crucial for breeding programs aimed at developing Bruchid-resistant mung beans varieties.

In recent years, the use of advanced storage technologies has also been explored. For example, the application of controlled atmosphere storage, where the levels of oxygen, carbon dioxide, and nitrogen are regulated, has shown promise in reducing Bruchid infestations (Aldryhim *et al.*, 2016). Such methods, combined with traditional storage practices, can significantly reduce post-harvest losses due to Bruchid damage.

Integrated pest management strategies continue to evolve, incorporating new research findings and technologies. The integration of biological control agents, resistant varieties, and improved storage practices provides a holistic approach to managing Bruchid infestations (Kumar *et al.*, 2015). Additionally, educating farmers on best practices for pest management and storage can enhance the effectiveness of these strategies.

The economic impact of Bruchid infestations on mung beans production cannot be overstated. Farmers in developing countries, who often rely on mung beans as a primary source of income and nutrition, are particularly vulnerable to the losses caused by these pests (Singh *et al.,* 2017). Efforts to develop and disseminate Bruchid-resistant mung beans varieties, coupled with effective pest management strategies, are essential to support the livelihoods of these farmers.

2.1.1. Post-Harvest Mung Bean Infesting Bruchids

Bruchids, belonging to the family Bruchidae, are significant storage pests of leguminous crops, with mung beans (*Vigna radiata*) being particularly susceptible. These beetles are notorious for their destructive feeding habits, where the larvae develop inside the seeds, leading to considerable post-harvest losses. Among the various bruchid species, *Callosobruchus maculatus*, *Callosobruchus chinensis*, *Acanthoscelides obtectus* (Say), and *Zabrotes subfasciatus* (Boleman) are the most impactful in terms of infestation and damage to stored mung beans.

Callosobruchus maculatus, commonly known as the cow pea weevil, is one of the most destructive bruchid species affecting mung beans and other legumes such as cow peas, chick peas, and lentils. This species is widely distributed across tropical and subtropical regions, including Africa, Asia, and Latin America. The life cycle of *C. maculatus* is relatively short, with females laying eggs on the surface of seeds. Upon hatching, the larvae bore into the seeds, where they feed and develop, causing significant damage. Studies have shown that *C. maculatus* infestation can lead to total seed destruction within a few months of storage (Singh *et al*., 2015).

Callosobruchus chinensis, another major pest, infests a broad range of legumes, including mung beans, black gram, and pigeon pea. This species is similar to *C. maculatus* in its cosmopolitan distribution and destructive potential. *C. chinensis* females lay eggs on the seed surface, and the emerging larvae penetrate the seeds to feed. The damage caused by this species includes not only direct consumption of seed material but also the creation of entry points for secondary fungal and bacterial infections, further reducing seed viability and quality (Mishra *et al*., 2018).

Acanthoscelides obtectus (Say), also known as the bean weevil, primarily infests beans but can also affect mung beans. Unlike *Callosobruchus* species, *A. obtectus* can infest seeds both in the field and during storage. This species is prevalent in temperate regions and is known for its ability to cause severe economic losses by reducing seed weight and germination capacity. The larvae of *A. obtectus* burrow into the seeds, creating extensive internal damage that is often not visible until the seeds are broken open (Zhang *et al*., 2019).

Zabrotes subfasciatus (Boleman), or the Mexican bean weevil, is another significant bruchid species affecting stored mung beans. This species is particularly troublesome in Latin America but has spread to other regions through international trade. *Z. subfasciatus* larvae develop inside the seeds, causing substantial weight loss and a

decline in nutritional quality. Infested seeds become unfit for human consumption and are often rejected in the market (Prakash *et al*., 2016).

The impact of bruchid infestation on mung beans is profound, resulting in substantial economic losses and food insecurity. The damage caused by these pests includes weight loss, decreased germination rates, and nutritional changes in the seeds, which compromise their market value and usability. Infested seeds often become unsuitable for both human consumption and agricultural purposes. For instance, infested seeds may exhibit lower protein content, which is a critical nutritional component (Rao *et al*., 2018). Additionally, the presence of bruchid damage can lead to secondary infestations by fungi and bacteria, further deteriorating seed quality (Kimani *et al*., 2016).

Control of bruchid infestation in mung beans involves several strategies, including the use of chemical insecticides, biological control methods, and physical control measures such as modified atmospheric storage. However, the development of resistant mung beans varieties is considered one of the most sustainable and effective approaches to managing bruchid pests. Breeding programs aimed at enhancing bruchid resistance have identified several mung beans lines with varying degrees of resistance to these pests.

Moreover, integrating traditional knowledge with modern scientific techniques can enhance bruchid management practices. For instance, the use of botanicals and natural products as insect repellents has gained attention as an eco-friendly alternative to chemical pesticides. Studies have demonstrated the effectiveness of neem oil and other plant extracts in reducing bruchid infestation in stored mung beans (Singh *et al*., 2017).

2.1.2 Control of Bruchid

Controlling bruchid infestations in stored mung beans poses a significant challenge, as these pests can cause extensive damage and economic losses. Traditional methods of bruchid control, such as the use of chemical insecticides, have drawbacks due to their toxicity and environmental impact. Insecticides like carbon disulphide, phosphine, and methyl bromide are commonly used to treat stored seeds but are highly toxic and pose risks to food safety (Mohapatra *et al*., 2015).

While chemical insecticides can effectively reduce bruchid populations, their use raises concerns about environmental contamination and human health risks. The reliance on

these toxic chemicals also contributes to the development of pesticide resistance among bruchid populations, necessitating the search for alternative control methods (Ndlela *et al*., 2016).

Botanical extracts have been explored as potential alternatives to chemical insecticides for bruchid control. Plants such as neem (*Azadirachta indica*), garlic (*Allium sativum*), and chilli (*Capsicum spp.*) contain compounds with insecticidal properties that can repel or kill bruchids. However, the effectiveness of botanical extracts as bruchid control agents varies depending on factors such as extraction method, concentration, and application technique (Prakash *et al*., 2016).

Biological control methods, which involve the use of natural enemies to suppress bruchid populations, offer another avenue for pest management. Parasitoid wasps such as *Dinarmus basalis* have been identified as effective biocontrol agents against bruchids in stored legumes. These parasitoids lay their eggs inside bruchid eggs or larvae, leading to their destruction and subsequent reduction in pest numbers (Ndlela *et al*., 2016).
However, while biological control can be environmentally friendly and sustainable, its effectiveness may be limited by factors such as climatic conditions, host specificity, and the availability of natural enemies. Additionally, the implementation of biological control programs requires careful planning and coordination, making it less practical for resource-poor farmers with limited access to technical support and infrastructure (Kimani *et al.,* 2016).
Breeding for host plant resistance against bruchids represents a promising approach to integrated pest management in mung beans. By selecting and breeding mung beans varieties with inherent resistance to bruchid infestation, breeders can develop cultivars that are less susceptible to pest damage. This approach not only reduces the reliance on chemical insecticides but also promotes sustainable agricultural practices that enhance food security and environmental conservation (Somta *et al.,* 2008).

Several studies have demonstrated the efficacy of breeding programs in developing bruchid-resistant mung beans varieties. Traits such as seed hardness, seed coat thickness, and biochemical compounds have been identified as key factors contributing to bruchid resistance in mung beans. Through conventional breeding techniques and modern biotechnological approaches, researchers have successfully incorporated these resistance traits into elite mung beans cultivars (Rao *et al.,* 2018).

In addition to genetic resistance, good agronomic practices can also play a crucial role in bruchid management. Practices such as timely harvesting, proper drying, and effective storage can help minimize bruchid infestations and reduce post-harvest losses. Ensuring proper ventilation and sanitation in storage facilities is essential for preventing the build-up of bruchid populations and maintaining seed quality (Singh *et al.*, 2017).

Integrated pest management (IPM) strategies that combine multiple control methods, including breeding for resistance, use of botanical extracts, biological control, and agronomic practices, offer a holistic approach to bruchid management in stored mung beans. By integrating these strategies, farmers can reduce reliance on chemical insecticides while effectively managing bruchid populations and preserving seed quality (Kumar *et al*., 2020).

2.1.3 Life Cycle and Ecology of Bruchids

Bruchids are a family of beetles commonly known as seed beetles, which are part of the larger group of weevils Curculionoidea (War *et al*., 2017). They are small beetles, usually no more than 5 mm in length and are characterized by their elongated body shape, which allows them to burrow into seeds (War *et al*., 2017).

The life cycle and ecology of both *C. maculatus* and *C. chinensis* are similar, consisting of egg, larva, pupa, and adult. After mating, female beetle lays her eggs on the surface of a seed. Each female can lay as many as 90 eggs during its oviposition period (Esen *et al.,* 2019). The eggs usually hatch after 3–5 days and the first instar larvae burrow and feed on the endosperm, mining tunnels in the seed until pupation (Esen *et al.*, 2019). The larval stage can last anywhere from a few weeks to several months, depending on the species and environmental conditions. Mature adults emerge from the seed approximately 30 days after hatching. The adult beetles live for up to approximately 2 weeks after emerging from the seed. The adults do not feed on seeds, but may feed on pollen and flower nectar in the field (War *et al.*, 2017).

Bruchids are found in a wide range of habitats, including forests, grasslands, deserts, and agricultural fields. They are primarily herbivorous and feed on a variety of plant species, but they are most commonly associated with legumes such as beans, peas, and lentils. Bruchids can be both pests and beneficial insects, depending on the context. In agriculture, they can cause significant damage to crops, leading to reduced yields and economic losses. However, in natural ecosystems, they can play an important role in

seed dispersal and nutrient cycling. Generally, the life cycle and ecology of bruchids are shaped by their close association with seeds and legumes, as well as their interactions with other organisms in their environment (Mohapatra *et al.*, 2015).

2.2. Sources of Mung beans Resistance to Storage Bruchid Infestations

The resistance of mung beans to bruchid infestations encompasses various defence strategies categorized broadly as antibiosis, antixenosis, and tolerance (Harshitha *et al.*, 2022). Antibiosis involves the production of secondary metabolites or biochemical substances within the plant that adversely affect the growth and development of bruchids. These substances include protease inhibitors, lectins, and trypsin inhibitors, which disrupt the digestive processes of bruchids, thereby reducing their fitness and survival rates (Harshitha *et al.*, 2022). Antixenosis, also known as non-preference resistance, operates through physical and chemical traits that deter bruchids from feeding or laying eggs on mung bean plants. Physical attributes such as seed coat color and thickness play crucial roles in bruchid resistance. Seeds with darker and thicker seed coats are generally less preferred by bruchids due to their reduced palatability and increased difficulty in penetration (Padhiyar *et al.*, 2022). Additionally, the presence of trichomes on mung bean surfaces acts as a physical barrier that impedes bruchid movement and feeding activities, further enhancing resistance (Harshitha *et al.*, 2022).

The physical characteristics of mung beans contribute significantly to their resistance against bruchids. The colour and thickness of the seed coat are strongly correlated with resistance levels, with darker and thicker coats often associated with higher resistance due to the presence of secondary metabolites such as phenolic compounds and flavonoids (Padhiyar *et al.*, 2022). These compounds possess deterrent effects on bruchid larvae and interfere with their ability to penetrate and feed on seeds effectively.

Biochemical defenses in mung beans involve the synthesis of secondary metabolites like phenolic acids, tannins, alkaloids, and flavonoids, which exhibit insecticidal properties (War *et al.*, 2017). Flavonoids, for instance, act as natural insecticides by disrupting bruchid digestive enzymes and reducing larval vitality, thereby impeding their growth and development within mung bean seeds (War *et al.*, 2017). Similarly, alkaloids and tannins form complexes with proteins in bruchids, causing nutritional deficiencies and metabolic disruptions that further deter infestation (War *et al.*, 2017).

The effectiveness of resistance mechanisms in mung beans is also influenced by environmental factors and genetic variability among cultivars. Optimal environmental conditions, including temperature, humidity, and soil quality, play crucial roles in enhancing the expression of resistance traits by promoting the synthesis of defensive compounds and physical barriers (Harshitha *et al.,* 2022). Genetic diversity among mung bean cultivars is also instrumental in determining resistance levels, with breeding programs focusing on selecting and introgressing genes associated with antibiosis, antixenosis, and tolerance traits from wild or resistant accessions into commercial varieties (Lambrides & Imrie, 2000).

2.2.1 Physical Basis of Mung beans Resistance to Bruchids

Mung beans resistance to bruchids (a family of beetle pests) is believed to be based on several physical factors. The colour of the mung beans seed coat has been found to be correlated with bruchid resistance (Padhiyar *et al.,* 2022). Seeds with darker seed coats are generally more resistant to bruchids than seeds with lighter seed coats (Padhiyar *et al*., 2022). The thickness of the seed coat can vary among different varieties of mung beans, with thicker seed coats being associated with greater resistance to bruchids (War *et al*., 2017). Mung beans plants produce trichomes on the surface of their leaves and pods, which can make it difficult for bruchids to crawl and feed on the plant. Trichome density can vary among different mung beans varieties and may be associated with resistance to bruchids (Harshitha *et al*., 2022). mung beans seeds that are smaller in size are believed to be more resistant to bruchids (Padhiyar *et al.,* 2022).

2.2.2 Metabolite Basis of Mung beans Resistance to Storage Bruchid

Mung beans employ a variety of defence mechanisms to deter and inhibit bruchid infestations, with antibiosis playing a pivotal role. Antibiosis involves the production of bioactive compounds within the seeds that adversely affect the development and survival of bruchids. For example, trypsin inhibitors, lectins, and protease inhibitors are among the primary compounds identified in mung beans that disrupt the digestive processes of bruchids (War *et al.,* 2017). These compounds interfere with the insects' ability to digest proteins and other essential nutrients, thereby reducing their fitness and survival rates.

Trypsin inhibitors are known to inhibit the activity of trypsin, a key digestive enzyme in insects, leading to impaired nutrient absorption and larval growth. Lectins bind to

specific carbohydrate residues on the gut epithelium of bruchids, disrupting cellular functions and further impeding nutrient assimilation (War *et al.*, 2017). Protease inhibitors, on the other hand, interfere with proteolytic enzymes in the digestive tract of bruchids, preventing the breakdown of proteins essential for their growth and development.

In addition to antibiosis, mung beans employ antixenosis mechanisms to deter bruchids from feeding and ovipositing on the plant. Secondary metabolites such as flavonoids, tannins, and alkaloids play crucial roles in these non-preference mechanisms. Flavonoids, characterized by their antioxidant properties and diverse biological activities, create unfavourable conditions for bruchids by disrupting key physiological processes necessary for larval development (Mukherjee *et al.*, 2016). These compounds interfere with digestive enzymes and cellular functions, thereby reducing bruchid survival rates and seed damage.

Tannins, polyphenolic compounds found in mung beans, contribute to bruchid resistance by forming complexes with proteins that are difficult for insects to digest (Barbehenn & Constabel, 2011). This complexation process inhibits nutrient absorption and impairs larval growth, thereby reducing the impact of bruchid infestations on seed quality. Alkaloids, nitrogen-containing compounds with potent biological activities, also play significant roles in mung beans' defence against bruchids (War *et al.*, 2017). These compounds exhibit insecticidal properties that disrupt bruchid physiology, leading to mortality or stunted growth.

The biochemical diversity of mung bean metabolites underscores their adaptive responses to bruchid infestations. Varieties exhibiting higher concentrations of defensive compounds such as flavonoids, tannins, and alkaloids demonstrate enhanced resistance to bruchids compared to susceptible varieties with lower metabolite levels (Eich, 2008). This variability highlights the potential for breeding programs aimed at selecting and enhancing mung bean cultivars with superior pest resistance traits.

Research has shown that mung beans exhibit genotype-specific responses to bruchid infestations, with certain varieties displaying inherent resistance traits linked to their metabolite profiles (Isman, 2006). Understanding the genetic basis of these traits and

their regulation could facilitate the development of molecular markers for breeding programs focused on enhancing bruchid resistance in mung beans.

The study of mung beans' resistance mechanisms has profound implications for sustainable agriculture. By harnessing the natural defences present in mung beans, farmers can reduce dependency on synthetic pesticides, thereby mitigating environmental risks associated with chemical inputs (Isman, 2006). Sustainable pest management strategies that integrate biological control and resistant cultivars offer a holistic approach to enhancing crop resilience while minimizing ecological impact.

Continued research into the genetic regulation and biosynthetic pathways of secondary metabolites in mung beans is crucial for optimizing their defensive potential against evolving pest pressures (War *et al.*, 2017). By elucidating the mechanisms underlying mung beans' resistance to bruchids, scientists and agricultural practitioners can develop tailored strategies for pest management and crop protection.

2.2.3 Genetic Basis of Mung beans Resistance to Bruchids

The genetic basis of mung beans resistance to bruchid has been thoroughly researched, resulting in the identification of several genes that play a role in the process. One of the essential genes involved in resistance is the trypsin inhibitor gene (TIA) (Sarkar *et al.*, 2011), which encodes a protein that hinders the activity of digestive enzymes in the gut of the bruchid larvae, thereby reducing their ability to digest mung beans seeds. Several TIA gene variants have been identified, with some demonstrating greater resistance to bruchids than others (Sarkar *et al.*, 2011). Another crucial gene is the chitinase gene, which produces enzymes that degrade the chitin in the exoskeleton of the bruchid larvae, causing their demise. Mung beans plants with elevated levels of chitinase exhibit greater resistance to bruchid infestation (Harshitha *et al*., 2022). A part from these genes, the biosynthesis of secondary metabolites, such as flavonoids, tannins and alkaloids, is regulated by several other genes that have insecticidal properties and can help protect the mung beans from bruchid infestation (Sarkar *et al.*, 2011).

The genetic basis of resistance to bruchids resistance in mung beans is multifaceted and numerous genes and pathways are involved in this process. Nonetheless, by identifying the primary genes involved in resistance, breeders can select mung beans varieties that are more resistant to bruchid infestation, leading to enhanced yields and reduced crop losses (Gupta *et al*., 2014).

2.3 Metabolites of Mung beans.

The metabolite profile of mung beans encompasses a range of primary and secondary compounds, each playing crucial roles in seed quality, nutritional value, and medicinal properties (Yewale *et al.,* 2020). Mung beans are renowned for their high content of essential nutrients, making them a staple in many diets worldwide. They are particularly rich in carbohydrates, including starch and dietary fibre, which contribute to their role as a source of sustained energy and digestive health (Profile, 2020). Oligosaccharides such as raffinose and stachyose, present in mung beans, further enhance their nutritional profile by providing prebiotic benefits that support gut health (Yewale *et al*., 2020).

Proteins constitute a significant component of mung beans, comprising approximately 23% of their dry weight. These proteins are notable for their balanced amino acid profile, particularly high in essential amino acids such as lysine and leucine, which are vital for human health (War *et al*., 2017). The biological value of mung bean proteins underscores their importance in vegetarian diets and food security strategies. Mung beans are also rich in vitamins and minerals essential for overall well-being. They contain significant amounts of vitamin B1 (thiamine), vitamin B2 (riboflavin), niacin, vitamin C, and folic acid, which play critical roles in energy metabolism, immune function, and cellular health (War *et al.,* 2017). In addition, mung beans provide essential minerals such as iron, phosphorus, magnesium, and potassium, which are vital for bone health, muscle function, and overall physiological balance (Venugopal *et al*., 2000).

Beyond their basic nutritional components, mung beans are distinguished by a diverse array of secondary metabolites with potent bioactive properties. Polyphenolic compounds, including flavonoids, phenolic acids, and tannins, are prevalent in mung beans and contribute significantly to their antioxidant and anti-inflammatory activities (Ponnusamy *et al*., 2014). These compounds scavenge free radicals, thereby reducing oxidative stress and protecting cellular structures from damage associated with chronic diseases. Saponins, another class of bioactive glycosides found in mung beans, exhibit cholesterol-lowering effects and have demonstrated potential anticancer properties in experimental studies (Ponnusamy *et al.,* 2014). Phytosterols, plant-derived compounds

structurally similar to cholesterol, further contribute to mung beans' cholesterol-lowering capabilities, supporting cardiovascular health (Yusnawan *et al.*, 2019).

The composition and levels of these metabolites in mung beans are influenced by various factors, including genetic diversity, environmental conditions, and post-harvest processing techniques (Venugopal *et al*., 2000). Different mung bean cultivars exhibit distinct profiles of secondary metabolites, reflecting their adaptive responses to environmental stresses and selective breeding efforts aimed at enhancing desirable traits. Understanding the complex interplay of mung beans' metabolites not only informs strategies for enhancing crop yield and nutritional quality but also facilitates the development of biofortified varieties with enhanced health benefits (Yusnawan *et al*., 2019). Breeding programs that prioritize metabolite-rich cultivars could potentially yield crops better adapted to climate change and resilient against pests such as bruchids (Yewale *et al*., 2020).

2.3.1 Role of Mung beans Primary Metabolites in resistance to Bruchid

Primary metabolites are essential compounds that are involved in basic metabolic processes, such as photosynthesis and respiration and include carbohydrates, proteins and lipids (Yewale *et al*., 2020; Pataczek *et al*., 2018). A study carried out on the various biochemical parameters of various genotypes of chick pea associated with resistance to *Callosobruchus chinensis* viz., protein, total soluble sugar, starch, the correlation coefficient calculated for seed damage, weight loss and biochemical parameters gave results that were statistically significant. There was a positive correlation observed between per cent seed damage, weight loss and biochemical parameters like protein (Profile, 2020). Carbohydrates, such as sucrose and raffinose, are important primary metabolites that can contribute to bruchid resistance in mung beans (Yewale *et al*., 2020). Primary metabolites are stored in the seed and can provide a nutritional source for the developing embryo of bruchids (War *et al*., 2017).

2.3.2 Role of Mung Beans Secondary Metabolites in Resistance to Bruchids

Plant secondary metabolites constitute a major defensive weapon of plants to defend themselves against a broad range of phytophagous herbivores (Divekar *et al.*, 2022). Recent analytical tools and techniques have enabled elucidation of the role of secondary metabolites in plant defence.

Secondary metabolites are non-essential molecules that are involved in more specialized processes such as defence against pests and pathogens. These metabolites are categorized into four different groups: terpenoids, phenolics and nitrogen and sulphur-containing compounds. Plant phenols are a heterogeneous group of secondary metabolites which include nearly 10,000 compounds.

Phenolics are the most widely distributed secondary metabolites that comprise a hydroxyl functional group (phenyl group) on an aromatic ring. Phenolics are diverse compounds based on chemical structure and comprise simple phenols such as catechols and hydroxybenzoic acid derivatives, flavonoids, catechol melanins, stilbenes, condensed tannins and lignins. These metabolites are actively engaged in protecting plants against herbivores and attracting pollinators. Phenolics can directly act as toxins to herbivores or can be oxidized by peroxidases or polyphenol oxidases to toxic metabolites which cause physiological disturbances in insect growth and developmental processes (Divekar *et al.*, 2022).

Terpenes are the largest group of plant secondary metabolites. Although most terpenes are important in plant defence, some terpenes such as gibberellins and brassinosteroids) are involved in primary functions, such as plant growth and development. Terpenes comprise around 25,000 compounds (Divekar *et al.*, 2022), with diverse functions including feeding deterrence, direct toxicity or oviposition deterrence. Specialist herbivores can tolerate terpenoids and utilize them as an attractant to locate their host plants and as feeding stimulants. Terpenes indirectly protect plants by increasing the efficacy of herbivore natural enemies through the release of specific volatiles. Some examples of terpenes that play an active role in plant defence are iridoids, benzoxazinoids and volatile compounds, such as mono and sesquiterpenes, α-bisabolene and β-caryophyllene. Secondary metabolites, such as flavonoids, tannins and alkaloids, play an important role in the resistance of mung beans to bruchids (Padhiyar *et al.*, 2022). These compounds are produced by the plant in response to herbivore attack and have insecticidal properties that can deter or kill bruchid larvae.

Flavonoids have been shown to have insecticidal activity against bruchids. These compounds are found in high concentrations in mung beans seeds, and their levels have been shown to increase in response to bruchid infestation (Prajapati *et al.*, 2022).

Flavonoids can interfere with the development and survival of bruchid larvae, leading to reduced damage to the mung beans seeds.

Tannins are another class of secondary metabolites that can provide resistance to bruchids. These compounds can bind to proteins in the gut of the bruchid larvae, inhibiting their ability to digest food and leading to reduced growth and survival (Yusnawan *et al*., 2019). Mung beans varieties that have higher levels of tannins have been shown to be more resistant to bruchids.

Alkaloids are a third class of secondary metabolites that can provide resistance to bruchids. These compounds have toxic properties and can cause mortality in bruchid larvae. Some mung beans varieties produce alkaloids, such as vignatic acid and its derivatives that have been shown to provide protection against bruchids (Yusnawan *et al*., 2019). Secondary metabolites play an important role in the resistance of mung beans to bruchids. By identifying the key secondary metabolites involved in bruchid resistance, breeders can select for mung beans varieties that are more resistant to bruchid infestation, leading to improved yields and reduced crop losses (Profile, 2020).

Analysing the primary and secondary metabolite profiles of different mung beans varieties, can be useful to identify potential biomarkers of bruchid resistance, which can be used to develop breeding programs for more resistant crops. Additionally, understanding the metabolite pathways involved in bruchid resistance can lead to the development of new pest control strategies that target specific metabolites (P. Somta *et al*., 2007).

2.3.3 Metabolites of Mung beans as Biomarkers for Breeding for Bruchid Resistant

Metabolites, have potential as biomarkers for various traits (Pretorius *et al.*, 2021). The use of metabolomics has been applauded for its ability to provide detailed prospects by in-depth study of crop biology. Information that is derived from metabolomics tools can be translated to assess phenotypic changes/biomarkers, gene changes and also to distinctively support other genomic experiments (Litvinov *et al.*, 2021). According to Venugopal *et al.*, 2000, earlier studies on factors contributing to seed resistance to insect attack clearly indicated that resistance was determined not merely by physical nature of the seeds, but the chemical composition of the seeds, particularly the secondary metabolites play a vital role. In the context of bruchid-resistant mung beans

breeding, identifying metabolites associated with resistance can aid in screening resistant genotypes.

Studies have investigated the metabolite profiles of mung beans in response to bruchid infestation. Litvinov *et al.*, 2021 identified several metabolites (e.g., flavonoids, phenolic acids, and alkaloids) that differed between resistant and susceptible genotypes of mung beans in response to bruchid infestation. Similarly, Prajapati *et al.*, 2022 found that several metabolites (e.g., amino acids, organic acids, and sugars) were associated with bruchid resistance in mung beans. These findings demonstrate the potential of metabolites as breeding biomarkers for bruchid resistance in mung beans. By selecting for genotypes with high levels of these metabolites, breeders can develop mung beans varieties that are more resilient to bruchid infestation, resulting in higher yields and improved food security for farmers.

2.4 Recent Applications of Metabolomics in Plant Breeding

The integration of advanced technologies into plant breeding has significantly enhanced the efficiency and precision of developing superior crop varieties. One such cutting-edge technology is metabolomics, which involves the comprehensive profiling of metabolites within biological systems. These small molecules are the end products of cellular processes and reflect the physiological state of an organism. By utilizing advanced analytical techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, metabolomics provides deep insights into the biochemical status and physiological responses of plants. This approach has revolutionized plant breeding by enabling the identification of metabolite markers associated with desirable traits, thereby facilitating the selection of superior genotypes (Liu & Schröder, 2018).

2.4.1 Integration of Metabolomics in Plant Breeding

The integration of metabolomics into plant breeding programs offers several advantages. Firstly, it allows for the identification of metabolic markers linked to specific agronomic traits. These markers can be used in marker-assisted selection (MAS) to accelerate the breeding process. Secondly, metabolomics helps breeders understand the underlying metabolic pathways that contribute to desirable traits such as yield, stress resistance, and nutritional quality. Finally, metabolomic profiling serves

as a powerful phenotyping tool, providing a detailed snapshot of the plant's biochemical status, which can be correlated with phenotypic traits (Saito & Matsuda, 2017).

2.4.2 Applications of metabolomics in Crop Improvement

Metabolomics has been employed extensively to enhance crop yields by identifying metabolites associated with high-yielding genotypes. For instance, studies in rice (*Oryza sativa)* have identified metabolites related to nitrogen use efficiency, which can be targeted to breed high-yielding varieties with optimized nitrogen uptake and utilization (Fernie & Schauer, 2009). Moreover, metabolomics aids in the identification of stress-responsive metabolites that confer tolerance to abiotic and biotic stresses such as drought, salinity, and pathogen attack. For example, drought-resistant varieties of maize (*Zea mays*) have been developed by selecting metabolites involved in osmoprotection and antioxidant defence (Obata & Fernie, 2012).

Improving the nutritional content of crops is another major goal in plant breeding where metabolomics plays a crucial role. By profiling metabolites such as vitamins, minerals, and phytonutrients, breeders can enhance nutritional traits. For instance, metabolomic studies in tomato (*Solanum lycopersicum)* have led to the breeding of varieties with higher levels of lycopene and other beneficial compounds (Kumar & Pandey, 2020).
In rice, metabolomics has been used to study the metabolic basis of traits like aroma, taste, and nutritional content. Researchers identified specific volatile compounds linked to the aromatic quality of basmati rice, enabling the selection of high-aroma genotypes (Tohge & Fernie, 2010). Similarly, metabolomic profiling in tomato has revealed the metabolic networks responsible for fruit flavour and nutritional quality. Breeders have used this information to develop varieties with enhanced taste and increased levels of health-promoting compounds such as carotenoids and flavonoids (Matsuda & Saito, 2012). In wheat (*Triticum aestivum*), metabolomics has been applied to understand the metabolic responses to fungal pathogens. By identifying key metabolites involved in pathogen resistance, breeders have developed wheat varieties with improved resistance to diseases like Fusarium head blight (Hall, 2006).

2.5 Resistance Screening Method for Bruchids in Mung Beans

The screening method for assessing resistance to bruchids in mung beans involves both 'Free-choice' and 'No-choice' tests, methodologies previously described by Esen *et al.* (2019). These tests are conducted under controlled laboratory conditions to evaluate the

response of different genotypes to intense oviposition by bruchids. In the 'Free-choice' test, multiple genotypes are simultaneously exposed to bruchids, allowing insects to choose their oviposition sites freely. Conversely, the 'No-choice' test restricts bruchid oviposition to a single genotype, eliminating external factors that may influence oviposition behaviour. Following oviposition, infested seeds are incubated to allow for F1 adult emergence after a specified period, typically under controlled temperature and humidity conditions (Esen *et al.*, 2019).

This screening method provides a controlled environment to simulate bruchid infestation scenarios encountered during mung bean storage. By subjecting genotypes to varying degrees of bruchid pressure, researchers can assess differences in resistance based on parameters such as seed damage and F1 adult emergence rates. These tests are crucial for identifying mung bean varieties that exhibit natural resistance to bruchid infestations, which is essential for sustainable pest management in agriculture.

The methodology outlined by Esen *et al*. (2019) emphasizes the importance of standardized protocols to ensure consistent and reproducible results across experiments. By adhering to these protocols, researchers can effectively compare resistance levels among different genotypes and validate the efficacy of breeding strategies aimed at enhancing bruchid resistance in mung beans. Moreover, the controlled conditions of these tests minimize external variables, thereby enhancing the reliability and accuracy of the screening process.

Under laboratory conditions, the screening method involve Free-choice' and 'No-choice' tests previously reported by Esen *et al*., 2019. The methodology challenged the genotypes to intense oviposition by the bruchids in both the tests and incubated infested seeds for F_1 adult emergence after providing a sufficient time for insect development under controlled conditions.

2.5.1 Free Choice Bioassay

The free-choice bioassay method is a critical approach used to evaluate the response of various mung bean accessions to pulse beetle adults, providing essential insights into resistance mechanisms under controlled conditions (Singh *et al.,* 2021). In this methodology, 20-liter rectangular Plexiglas cages are employed, with one side covered by gauze cloth to facilitate insect introduction and air circulation. Multiple Petri dishes containing adequate seed samples of each accession are distributed within each cage.

Each cage serves as a replicate, and typically, three replications are conducted per accession in the free-choice test.

For each replicate, pairs of 0–24-hour-old adult beetles, sourced from maintained cultures, are released into the cages. Over a one-week period, these adult beetles are allowed to freely oviposit on the seeds within the Petri dishes. Subsequently, the insects are removed, and the Petri dishes are inspected under a stereo-microscope to record the number of eggs laid by each accession. Daily monitoring of the cages continues for up to 30 days to record the emergence of adult beetles, with observations ceasing once adult emergence ceases (Njoroge *et al.,* 2018).

This bioassay method provides a controlled environment to simulate bruchid infestation scenarios encountered during mung bean storage. By allowing beetles to freely choose oviposition sites among different accessions, researchers can assess variations in resistance based on parameters such as egg deposition and subsequent adult emergence rates. The methodology emphasizes rigorous replication and consistent monitoring, ensuring reliable and reproducible results across experiments (Kumar *et al.,* 2016).

A study by Singh *et al*. (2021), highlighted the importance of standardized protocols in free-choice bioassays for accurate assessment of insect behaviour and mung bean resistance traits. By adhering to these protocols, researchers can effectively compare resistance levels among different accessions and validate the efficacy of breeding strategies aimed at enhancing bruchid resistance in mung beans.

2.5.2 Evaluating Mung Bean Varieties' Resistance to Bruchid Infestation: The No-Choice Test

Mung beans are valued globally for their nutritional benefits and adaptability to diverse agricultural environments. However, their storage is often challenged by pests, notably the bruchid weevil, (*Callosobruchus maculatus),* which can cause substantial post-harvest losses. Understanding the resistance of mung beans varieties to bruchid infestation is crucial for developing effective pest management strategies that ensure food security and preserve economic value. This can be well explored by the application of the no-choice test in assessing mung beans varieties' resistance to *C. maculatus,*

The no-choice test is a standardized method used to evaluate the susceptibility of mung beans varieties to bruchid infestation under controlled conditions. This experimental approach restricts the insects to a single host variety, eliminating alternative choices and thereby focusing on the intrinsic resistance mechanisms of the mung beans genotype (Dick & Credland, 2018).

In conducting the no-choice test, researchers typically select healthy and uniform-sized mung beans seeds from different varieties. Seeds are cleaned and sterilized to remove any external contaminants that could affect the experimental outcomes. The seeds are then placed in containers, usually clear plastic or glass, with ventilated lids to ensure adequate airflow. A predetermined number of newly emerged adult C. maculatus insects are introduced into each container, following a standardized protocol to maintain consistency across experiments. Containers are incubated under controlled environmental conditions optimal for the development and activity of C. maculatus, such as temperatures ranging from 25-30°C and relative humidity of 60-70%. These conditions simulate typical storage environments where bruchid infestation commonly occurs (Ahmed & Ali, 2019).

Observations are conducted at regular intervals to monitor various parameters, including egg-laying behaviour, egg hatchability, larval development, and damage inflicted on the mung beans seeds. Data collection involves recording the number of eggs laid per seed, the percentage of eggs hatching, larval survival rates, and the extent of seed damage caused by larval feeding. These metrics provide quantitative measures of mung beans resistance to bruchid infestation and contribute to identifying varieties with inherent resistance traits.

A study by Ahmed and Ali (2019) demonstrated the utility of the no-choice test in assessing mung beans varieties' resistance to C. maculatus by investigated several mung beans cultivars and reported significant variations in resistance levels based on egg-laying preferences and larval development success rates. They found that certain cultivars exhibited lower egg-laying rates and higher larval mortality, indicating potential resistance mechanisms such as physical seed coat traits or biochemical defences that deter bruchid infestation. Similarly, Dick and Credland (2018) conducted comparative analyses of different mung beans varieties using the no-choice test. They

observed differential responses in terms of egg viability and larval survival rates, highlighting genetic variability in resistance among mung beans genotypes. These findings underscore the importance of genetic diversity in breeding programs aimed at developing mung beans varieties with enhanced resistance to bruchid pests.

A study by Giga and Smith (2017) explored the biochemical basis of resistance in mung beans varieties through biochemical assays coupled with no-choice tests. They identified specific chemical compounds in mung beans seeds that correlated with reduced attractiveness to *C. maculatus* and increased larval mortality rates. These studies have contributed to a deeper understanding of the biochemical mechanisms underlying mung beans resistance and pave the way for targeted breeding strategies. The findings from these studies have significant implications for pest management and agricultural practices. Identifying mung beans varieties with inherent resistance to bruchid infestation allows farmers to make informed decisions regarding seed selection and storage practices. By cultivating resistant varieties, farmers can reduce reliance on chemical insecticides, mitigate crop losses, and enhance overall agricultural sustainability (Singh, 2019).

2.6 Metabolite Analysis Techniques for Mung beans

UV-Vis spectroscopy is a pivotal analytical tool employed to identify and quantify chemical compounds within biological samples, including plant metabolites. This technique operates on the principle that molecules absorb ultraviolet (UV) and visible (Vis) light at specific wavelengths, corresponding to their unique electronic transitions. When a sample, such as mung beans extract, is exposed to a spectrum of UV and visible light, certain wavelengths are absorbed by the molecules, resulting in an absorption spectrum that can be precisely analysed. Each peak in the spectrum represents a specific molecular transition, with the peak intensity directly proportional to the concentration of the absorbing compound (Pavia, Lampman, Kriz, & Vyvyan, 2014).

In the context of analysing mung beans metabolites, the beans are first ground into a fine powder to ensure a homogeneous sample suitable for accurate measurement. This powder is then dissolved in a solvent to extract the metabolites. The resulting solution is analysed using UV-Vis spectroscopy, which detects the absorption characteristics of key metabolites, such as phenols, tannins, and alkaloids. These compounds typically

absorb light within the UV and visible ranges, making UV-Vis spectroscopy particularly effective for their analysis (Robards, 2003).

One of the significant advantages of UV-Vis spectroscopy is its high sensitivity and precision, which allow for the detection and quantification of even low concentrations of metabolites. This sensitivity is crucial when studying the often-subtle variations in metabolite levels among different mung beans varieties. Such precision is vital for accurately correlating these metabolite levels with the resistance of mung beans to bruchid infestations (Robinson, 2010).

UV-Vis spectroscopy is also renowned for its efficiency and rapidity. The technique delivers swift results, making it highly suitable for high-throughput analysis, where numerous samples must be processed expeditiously. The straightforward sample preparation process and the quick measurement times contribute significantly to the method's overall efficiency (Ding, Qian, & Reynolds, 2016).

Moreover, UV-Vis spectroscopy is highly quantitative, enabling precise measurement of metabolite concentrations. This quantification is essential for comparing the levels of phenols, tannins, and alkaloids across different mung beans varieties. Such comparisons are fundamental to understanding the correlation between these metabolites and the resistance of mung beans to storage bruchid infestations (Swinehart, 1962).

Another noteworthy benefit of UV-Vis spectroscopy is its specificity for detecting compounds with conjugated electron systems, which include many plant metabolites such as phenols, tannins, and alkaloids. These metabolites exhibit distinctive absorption characteristics in the UV-Vis range, making the technique particularly suited for their analysis in mung beans (Carbone & Seeram, 2010).

Additionally, UV-Vis spectroscopy is a cost-effective method compared to more advanced and complex techniques like Nuclear Magnetic Resonance (NMR) or Mass Spectrometry (MS). It requires less expensive equipment and incurs lower operational costs, making it accessible and practical for routine laboratory use in metabolite analysis (Williamson, 2013).

CHAPTER THREE

METHODOLOGY

3.0 Introduction

This study employed precise methodologies to assess the resistance of twenty-three (23) mung beans varieties against bruchid beetles (*Callosobruchus maculatus*) and to examine the correlation between this resistance and the metabolite profiles of the beans. Resistance to bruchids was evaluated using a no-choice bioassay

3.1 Research Design

This study involved confining the bruchid beetles with a single type of mung beans variety, thereby removing any alternative choices and allowing for a direct assessment of each variety's resistance. This approach is essential for determining the intrinsic ability of each bean variety to withstand pest infestation. Simultaneously, the correlation between the beans' resistance levels and their metabolite profiles was investigated through UV-Vis spectrophotometric analysis. This analytical technique enables the detailed characterization of the chemical compounds within the mung beans, which could be linked to their pest resistance mechanisms. By identifying and quantifying these metabolites, the study aimed to uncover the biochemical underpinnings of resistance.

3.2 Study Site

The research was conducted across two primary locations, each selected for its unique facilities and contributions to the study of mung beans resistance to bruchid beetles and the correlation between resistance levels and metabolite profiles. These sites were, Tharaka University, located in Tharaka Nithi County Kenya (Appendix 4), and the Insect Pest Management Laboratory at KALRO Katumani located in Machakos County Kenya (Appendix 5). The geographical diversity and specialized infrastructure of these sites were integral to the success of the experiments. Each site contributed uniquely to the research, ensuring a well-rounded and scientifically rigorous methodology. The integration of these diverse environments and their specialized facilities facilitated a thorough and accurate exploration of mung beans resistance and metabolite profiling, leading to robust and reliable scientific results

3.3 Sample Collection

For this study, twenty-three distinct varieties of mung beans, encompassing both local and wild types, were obtained from KALRO Katumani in Machakos County (Table 3.1). These varieties were provided by the World Vegetable Centre. The mung beans seeds were carefully selected to represent healthy and vigorous samples, providing a reliable basis for the study's experiments. The focus on both local and wild varieties adds depth to the research, offering insights into the potential metabolite differences that affected their response to the experimental exposure. In addition, an initial culture of the bruchid beetles, *Callosobruchus maculatus* (Fabricius), were obtained from KALRO Katumani. This beetle is a well-known pest that infests stored legumes, making it a critical subject for the study, which aimed to investigate pest interactions with the mung beans. The identification and verification of the *Callosobruchus* species were meticulously performed by a specialist entomologist at KALRO Katumani. Accurate species identification was paramount to ensure that the experimental outcomes are valid and applicable, particularly in studies involving pest management and resistance.

Table 3. 2:Mung beans Varieties for Bruchid Resistance Trial

AMVU 1601	AMVU 1608	AMVU 1627	KS 20
AMVU 1602	AMVU 1612	AMVU 1630	KAT 00301
AMVU 1603	AMVU 1614	V100 1709	KAT 00308
AMVU 1604	AMVU 1616	V100 1802	KAT 00309
AMVU 1605	AMVU 1618	V100 35226	Local Meru
AMVU 1606	AMVU 1619	N 26	

Source: KALRO Katumani

3.4 Detarmination of Bruchids Damage Levels on Selected Mung Bean Varieties

The evaluation of mung beans varieties for resistance to storage bruchids (*Callosobruchus maculatus*) infestation was conducted to identify lines with superior resistance, moderate resistance, and susceptibility. The bioassay involved a no-choice host preference study on twenty-three (23) selected mung beans varieties. Each mung beans variety was represented by 50 seeds, which were counted and placed in separate Petri dishes labelled with their variety code such as, AMVU 1601, V100 1709, Local Meru. Five pairs of *Callosobruchus maculatus* (bruchids) were introduced into each Petri dish containing 50 seeds of a specific mung beans variety. The Petri dishes were

then placed in an incubator maintained at 28±2°C and 92±3% relative humidity to simulate storage conditions conducive to bruchid infestation. The adults *C. malculatus* were allowed to lay eggs and observations were made on the oviposition preference (number of eggs laid on each variety) after 72 hours then parent pairs removed after six days of ovipositioning.

To determine the percentage of seed damage, the test sample seeds were evaluated from each variety replication Petri dishes after 30 days. The seeds with one or more holes were considered damaged and separated from the total sample for counting. The percentage of damaged seeds was calculated using the formula proposed by Adams and Schuten (1978) based on the collected data.

$$Percentage\ seed\ damage = \frac{Number\ of\ seeds\ damanged}{Total\ Number\ of\ seeds\ counted}\ X\ 100. \quad \ldots\ldots\ldots(1)$$

The data collected on the number of damaged seeds and the percentage of seed damage were analysed to determine the resistance levels of the mung beans varieties.

Statistical analysis was conducted to compare the levels of seed damage across different varieties. Varieties were assigned letters (a, b, c, etc.) to denote significant differences in resistance levels.

3.5 Determination of Metabolite profiles in Selected Mung Bean Varieties

The metabolite profile analysis of different mung beans varieties was conducted to quantify proteins, carbohydrates, phenols, tannins, and flavonoids using UV-Vis spectrophotometry. This method allowed for precise measurement of metabolite concentrations, facilitating the comparison of nutritional and phytochemical properties across mung beans varieties. Mung beans seeds were ground into a fine powder using a mortar and pestle. A known weight of the powder (approximately 1 g) was taken for each variety for extraction. The powdered seeds were extracted using 10 mL of appropriate solvent, for example, ethanol for phenols and flavonoids, and methanol for proteins and carbohydrates. The mixture was vortexed for 5 minutes and then allowed to stand for 24 hours at room temperature. The extracts were centrifuged at 5000 rpm for 10 minutes to obtain clear supernatants for analysis. UV-Vis spectrophotometry combined with standard curves allowed for precise quantification of the metabolite concentrations. The detailed profiles of the metabolite content in the mung beans varieties were essential for subsequent correlation analysis with bruchid resistance, providing insights into the biochemical factors that contribute to pest resistance.

3.5.1 Determination of Total Phenol Content in Selected mung bean Mung beans Varieties

To determine the total phenolic content in mung bean extracts, the study utilized a method adapted from Kim *et al.* (2012), tailored to meet experimental requirements. Initially, mung bean extracts were diluted with distilled water at a 1:60 volume-to-volume ratio. Following dilution, 250 µL of Folin-Ciocalteu's reagent was added, crucial for its reaction with phenolic compounds, inducing a colour change detectable by spectrophotometry (Singleton *et al,* 1999). The solutions were then incubated for 8 minutes to allow sufficient reaction time.

Subsequently, 750 µL of sodium carbonate solution was added to neutralize the acidic environment and facilitate the development of a blue colour, directly proportional to phenolic content (Ainsworth & Gillespie, 2007). Distilled water was added to adjust the final volume, and samples were further incubated for 2 hours to stabilize the colour formation and complete the reaction. Absorbance of each sample was measured at 765 nm using a spectrophotometer, optimal for detecting the blue colour formed by the Folin-Ciocalteu's reagent-phenolic compound reaction.

The obtained absorbance values were used to quantify the phenolic content as gallic acid equivalents per gram of sample (mg GAE/g), with gallic acid serving as the standard due to its well-established properties (Kim *et al*., 2012). A stock solution of gallic acid (0.100 g in 100 mL distilled water) was prepared, from which standard solutions ranging from 2 µg/g to 40 µg/g were derived by serial dilution. These standards were used to construct a calibration curve plotting concentrations against absorbance values at 765 nm, ensuring accurate interpolation of phenolic content in mung bean samples.

This method is reliable and widely used in phytochemical research due to its sensitivity and simplicity. The use of Folin-Ciocalteu's reagent is particularly advantageous as it provides a comprehensive measure of total phenolics, encompassing a broad range of phenolic compounds present in the mung bean extracts. Furthermore, the standard curve approach ensures that the quantification is both accurate and reproducible, making it a valuable tool for comparing phenolic content across different mung bean varieties or treatments (Singleton *et al.,* 1999).

3.5.2 Determination of Tannin Content in Mung beans Varieties

To estimate the tannin content in mung bean varieties, the study employed the Folin-Ciocalteu method, adapted from standard procedures as recommended by Djeridane *et al.* (2006). with slight modifications for experimental suitability. Initially, 0.5 mL of mung bean extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted 1:1 with distilled water). Subsequently, 1 mL of 20% sodium carbonate solution was added, and the total volume was adjusted to 10 mL with distilled water. The solution was then incubated at a controlled temperature of 25-30°C for 40 minutes to allow for optimal colour development.

The absorbance of the resulting blue colour, indicative of tannin content, was measured at 725 nm using a UV-VIS spectrophotometer. Tannin concentrations were determined by comparing the absorbance values to a standard curve prepared from various concentrations of tannic acid, expressed as tannic acid equivalents per gram of sample. Tannic acid was chosen as the standard due to its structural similarity and widespread use in tannin quantification assays.

3.5.3 Determination of Flavonoids Content in Selected Mung Beans Varieties

The quantification of flavonoids in mung beans (*Vigna radiata*) provides essential insights into their nutritional value and potential for resistance against pests like bruchids. In this study, the total flavonoid content in various mung bean varieties was estimated using the aluminium chloride colorimetric method, as described by Chang *et al*. (2014) and further refined in subsequent research (Hosseini *et al*., 2018; Kumar *et al*., 2020).

To conduct the analysis, methanol extracts of mung bean flour were prepared, with an aliquot of 5 mL mixed with 1.4 mL of a solution containing 25 g/L sodium nitrite and 50 g/L aluminium nitrate. The aluminium nitrate reacts with flavonoids to form a yellow complex, which is measurable using spectrophotometry. After allowing the reaction to proceed for 6 minutes, 5 mL of 1M sodium hydroxide solution was added. This step neutralizes the acidic environment and stabilizes the colour of the flavonoid-aluminium complex, enhancing its visibility and measurement. The total volume was adjusted to 25 mL with 75% ethanol, ensuring consistent solvent conditions for optimal colour development.

The reaction mixture was incubated at room temperature for 10 minutes, facilitating the complete development of the yellow colour. This incubation period is critical as it allows the full interaction between flavonoids and aluminium chloride, which is crucial for accurate quantification. The absorbance was then measured at 510 nm using a spectrophotometer. This specific wavelength is chosen due to its effectiveness in detecting the aluminium-flavonoid complex, providing a reliable measure of the flavonoid content

To quantify the flavonoid content in the mung bean samples, a calibration curve was established using catechin as a standard. Catechin, a common flavonoid, serves as an ideal standard due to its stability and well-characterized absorption properties in such assays. Solutions of known catechin concentrations were prepared and treated in the same manner as the mung bean extracts. The absorbance values of these catechin standards were plotted against their concentrations to create a standard curve, typically yielding a linear relationship. This calibration curve was then used to determine the flavonoid concentrations in the mung bean samples by interpolating their absorbance values onto the curve.

The results were expressed as milligrams of catechin equivalents per gram of sample (mg CE/g), providing a standardized measure for comparing flavonoid content across different mung bean varieties. This approach allows for a direct comparison of the nutritional and biochemical profiles of the beans, highlighting significant variations in flavonoid content among the tested varieties. Such variations are important for identifying and breeding mung bean varieties with enhanced health benefits and pest resistance

The aluminium chloride colorimetric method proved to be an effective and reliable technique for estimating the total flavonoid content in mung beans. This method's simplicity and sensitivity make it valuable for evaluating the antioxidant properties and potential health benefits of different mung bean varieties.

3.5.4 Determination of Total Protein Content in Selected Mung Beans Varieties

To quantify the total protein content in various mung bean (*Vigna radiata)* varieties, the Bradford assay was employed due to its simplicity, rapidity, and high sensitivity in protein quantification. The Bradford method, first described by Bradford (1976), is widely used for protein estimation and is based on the binding of Coomassie Brilliant

Blue G-250 dye to proteins, resulting in a colour change that is proportional to the protein concentration in the sample. This change in colour can be quantitatively measured using a spectrophotometer.

The initial step in the assay involved preparing mung bean samples. Seeds from different mung bean varieties were finely ground into a powder using a mechanical grinder. Approximately 1 gram of the powdered mung bean was then subjected to extraction using 10 mL of phosphate-buffered saline (PBS) at pH 7.4. This solvent choice helps maintain protein stability and solubility during extraction. The mixture was thoroughly vortexed for about 10 minutes to ensure complete protein extraction from the seed matrix. Following this, the mixture was centrifuged at 10,000 rpm for 15 minutes at a temperature of 4°C. The centrifugation process allowed for the separation of the soluble protein extract from the insoluble residues. The supernatant, which contained the solubilized proteins, was carefully collected and diluted as necessary with PBS to bring the protein concentration within the detectable range of the Bradford assay

To quantify the protein content, a standard curve was established using bovine serum albumin (BSA) as a reference protein. BSA is a standard in protein assays because of its well-defined properties and consistent behaviour in various biochemical assays. Stock BSA solutions were prepared at a concentration of 1 mg/mL. Serial dilutions of this stock solution were then performed to produce a series of standard solutions with concentrations ranging from 0.1 mg/mL to 1.0 mg/mL. These standard solutions were critical in creating a calibration curve that could be used to determine the protein concentrations in the mung bean extracts

The Bradford reagent, essential for the assay, was prepared fresh to ensure optimal performance. The reagent consists of Coomassie Brilliant Blue G-250 dye dissolved in a solution of ethanol and phosphoric acid. Specifically, 100 mg of the dye was dissolved in 50 mL of 95% ethanol, followed by the addition of 100 mL of 85% phosphoric acid. This mixture was then diluted to a final volume of 1 litre with distilled water. The freshly prepared reagent was stored in an amber bottle to protect it from light-induced degradation, which can affect its performance

For the assay, 1 mL of each mung bean protein extract and each BSA standard solution was pipetted into separate test tubes. To each tube, 5 mL of the freshly prepared Bradford reagent was added. This high volume of reagent relative to the sample ensures

an excess of dye, which is crucial for the complete binding of the protein and the formation of a stable colour complex. The test tubes were then gently vortexed to mix the contents thoroughly and incubated at room temperature for 5 minutes. This incubation period allows the dye-protein complexes to form, stabilizing the colour change necessary for accurate measurement

Following incubation, the absorbance of each mixture was measured at 595 nm using a UV-Vis spectrophotometer. The spectrophotometer was calibrated using a blank, which consisted of 1 mL of PBS mixed with 5 mL of the Bradford reagent. This blank accounts for any background absorbance that could interfere with the protein measurement. The absorbance values for the samples and standards were recorded in triplicate to ensure the precision and reproducibility of the results.

The absorbance values obtained from the BSA standards were plotted against their known concentrations to generate a standard calibration curve. This curve was linear within the tested range, allowing for the accurate determination of protein concentrations in the mung bean extracts by interpolation. The protein content in each mung bean variety was then expressed as milligrams of protein per gram of sample (µg/g), providing a clear and quantitative measure of protein content

3.5.5 Determination of Total Carbohydrate Content in Selected Mung beans Varieties

To accurately determine the total carbohydrate content in various mung bean (Vigna radiata) varieties, the phenol-sulfuric acid method was employed. This method, originally described by Dubois *et al*. (1956), is a well-established, sensitive, and reliable technique for quantifying carbohydrates, including both simple sugars and polysaccharides, in biological samples. The principle behind this assay involves the reaction of carbohydrates with sulfuric acid and phenol to form a coloured complex, which can be quantified using spectrophotometry.

Mung bean samples from different varieties were initially dried to a constant weight to remove any moisture content that could affect the results. Approximately 1 gram of the dried mung bean seeds was then finely ground into a uniform powder using a mechanical grinder. This powder was used to prepare the extracts for carbohydrate analysis. The extraction involved dissolving the ground mung bean powder in 10 mL of distilled water, followed by thorough mixing using a vortex for 5 minutes. This step

ensured the complete dissolution of soluble carbohydrates present in the mung beans. The mixture was then centrifuged at 10,000 rpm for 10 minutes at room temperature to separate the insoluble residues from the soluble carbohydrate extract. The supernatant was collected carefully and used for subsequent carbohydrate quantification

The phenol-sulfuric acid reagent was prepared fresh to maintain its reactivity and effectiveness. The preparation involved dissolving 5 grams of phenol in 100 mL of distilled water to create a 5% phenol solution. Concentrated sulfuric acid (H_2SO_4) was used as received, ensuring careful handling due to its highly corrosive nature. For the calibration curve, a glucose standard solution was prepared by dissolving 100 mg of glucose in 100 mL of distilled water to obtain a 1 mg/mL stock solution. Serial dilutions of this stock solution were performed to produce a series of glucose standards with concentrations ranging from 0.01 mg/mL to 1 mg/mL. These standards were essential for generating a calibration curve to quantify the carbohydrate content in the mung bean samples accurately

For the assay, 1 mL of the mung bean extract was pipetted into a series of test tubes. To each tube, 1 mL of the 5% phenol solution was added, followed by the rapid addition of 5 mL of concentrated sulfuric acid. The sulfuric acid facilitates the breakdown of complex carbohydrates into simpler sugars and promotes the formation of a coloured complex with phenol. This reaction is highly exothermic and requires careful handling. After adding the sulfuric acid, the mixture was gently vortexed to ensure thorough mixing and then allowed to stand at room temperature for 10 minutes to develop the colour fully. This incubation period is critical for the complete formation of the coloured complex, which is directly proportional to the carbohydrate concentration in the sample . Following the incubation, the absorbance of each mixture was measured at 490 nm using a UV-Vis spectrophotometer. The wavelength of 490 nm corresponds to the maximum absorbance of the yellow-orange complex formed between the carbohydrates and the phenol-sulfuric acid reagent. A blank sample, consisting of 1 mL of distilled water mixed with 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid, was used to zero the spectrophotometer and account for any background absorbance. The absorbance measurements for the glucose standards were used to construct a standard calibration curve, plotting absorbance against glucose

concentration. This calibration curve was linear across the tested range, confirming the method's suitability for quantifying the carbohydrate content in the mung bean extracts

The carbohydrate content in the mung bean samples was calculated using the linear regression equation derived from the glucose standard calibration curve. The absorbance values of the mung bean extracts were interpolated onto the calibration curve to determine their corresponding carbohydrate concentrations. The total carbohydrate content was then expressed as milligrams of glucose equivalent per gram of sample (µg/g), providing a quantitative measure of the carbohydrate

3.6 Data Management and Analysis.

Data on the screening of Mung beans varieties resistance to bruchid infestations were tested for normality using proc univariate of SAS. Data then underwent a one-way ANOVA using proc GLM of SAS. Means comparisons were conducted using Tukeys HSD test and means considered different if the p value <0.05. Non-parametric one-way ANOVA (Kruskal wallis) was conducted for seed damage which was not normally distributed. Proc Non-parametric one-way was used for the seed damaged analysis. The model of the study used was:

$y_{ij} = \mu + V_i + e_{ij}$.....*(2)*

Where;

y_{ij} Effects due to dependant variable (Metabolite profile)

µ Population mean

V_i Effects due to i^{th} variety

e_{ij} Random error

The metabolite profiles of various mung bean varieties were analysed using Analysis of Variance (ANOVA) to discern significant differences among them. ANOVA is chosen for its robustness in comparing mean values across multiple groups, allowing comprehensive exploration of metabolite composition variations. A significance level of p=0.05 was applied to determine statistical significance, ensuring rigorous assessment of differences in metabolite concentrations. Furthermore, the F-test, integrated within ANOVA, complemented these comparisons by assessing variances among groups, providing additional insights into metabolite profile consistency or variability across varieties. This dual approach enhances the reliability and

interpretability of findings, supporting robust conclusions regarding differences in metabolite levels.

To explore correlations between seed damage percentages and metabolite profiles, Spearman's correlation method was utilized. Spearman's method, suited for analysing non-linear data patterns, examined associations between seed damage levels and biochemical factors such as phenols, tannins, and flavonoids. This methodological choice ensures a nuanced understanding of how metabolite composition may influence mung bean varieties' resistance to bruchid infestations.

The selection of ANOVA with the F-test and Spearman's correlation method is grounded in their established reliability and ability to unveil complex relationships and discrepancies within datasets. By employing these statistical tools, this study aimed to provide comprehensive insights into the intricate dynamics between metabolite profiles and bruchid resistance in mung beans, contributing to advancements in agricultural and nutritional sciences.

3.7 Ethical Consideration

The research, obtained permission from the Tharaka University institutional Scientific and Ethics Review committee and subsequently obtaines a Reseach Licence from NACOSTI , (appedix 1 and 2)

CHAPTER FOUR
RESULTS AND DISCUSSION

4.0 Introduction

The results highlighted significant diversity in both bruchid resistance levels and metabolite concentrations among the varieties tested. Notably, correlations were established between specific metabolite profiles particularly phenols and tannins and resistance traits, suggesting these compounds as potential indicators of bruchid resistance in mung beans. These findings provide valuable insights into the biochemical mechanisms governing mung bean defence against bruchids, with implications for enhancing crop resilience through targeted breeding and sustainable agricultural practices. The discussion explores how these findings could influence agricultural pest management strategies and emphasizes the potential incorporation of metabolomics in future efforts to enhance crop improvement.

4.1. Percentage Damage of Selected Mung beans Varieties by *C. maculatus*

The results of seed damage across various mung beans lines demonstrated differential resistance to bruchid (*Callosobruchus maculatus)* infestation. The V100-U line exhibited the minimal seed damage at 2.16%, indicating superior resistance to bruchid attack. Other lines with high resistance included AMVU-H and AMVU-A, with seed damage recorded at 7.16% and 7.83%, respectively. Additionally, AMVU-C and V100-S displayed relatively low damage levels at 10.5% and 12.33%, respectively. Moderate resistance was observed in lines AMVU-B (18.16%) and AMVU-L (19.50%). In contrast, variety AMVU-N (24.83%) and V100-R (30.33%) exhibited moderate damage levels, suggesting intermediate resistance. Lines exhibiting higher susceptibility included AMUV-1604 (43.16%), AMVU-E (35.66%), AMVU-1606 (37.66%), and AMVU-G (30.33%), indicating significantly reduced resistance. The line AMVU-K showed even higher susceptibility with a damage level of 46.0%. The most susceptible lines were KAT-Q (64.66%), KAT-P (63.33%), KAT-O (59.16%), AMVU-M (59.16%), KS-20 (55.0%), Meru (55.83%), and N_26 (55.83%). These lines experienced the highest levels of seed damage, indicating extreme susceptibility to bruchid infestations.

Table 4. 1: Percentage seed damage of selected mung beans varieties by storage bruchids (Callasobruchus maculatus) infestation

Variety code	Treatment code	No. of Seed	No. of Bruchid Pairs	No of Damaged Seed	Percentage Seed Damage	Turky's ASD
AMVU-1601	AMVU_A	50	5	3.33	7	7.83^{hi}
AMVU-1602	AMVU_B	50	5	15.67	31	18.16^{hfgi}
AMVU-1603	AMVU_C	50	5	4.67	9	10.5^{hgi}
AMVU-1604	AMVU_D	50	5	34	68	43.16^{bc}
AMVU-1605	AMVU_E	50	5	29.33	58	35.66^{dce}
AMVU-1606	AMVU_F	50	5	30.33	61	37.66^{dc}
AMVU-1608	AMVU_G	50	5	26	52	30.33^{dfce}
AMVU -1612	AMVU_H	50	5	3	6	7.16^{hi}
AMVU-1614	AMVU_I	50	5	27.67	55	33.3^{dfce}
AMVU-1616	AMVU_J	50	5	27	54	33.0^{dfce}
AMVU-1618	AMVU_K	50	5	36	72	46.0^{bc}
AMVU-1619	AMVU_L	50	5	18.67	37	19.50^{hfge}
AMVU-1627	AMVU_M	50	5	47.67	95	59.16^{ba}
AMVU-1630	AMVU_N	50	5	24	48	24.83^{dfge}
KAT-00301	KAT_O	50	5	46.67	95	59.16^{ba}
KAT-00308	KAT_P	50	5	49.33	99	63.33^{a}
KAT-00309	KAT_Q	50	5	49.67	99	64.66^{a}
KS-20	KS_20	50	5	45	90	55.00^{ba}
Local Meru	Meru	50	5	45.33	91	55.83^{ba}
N-26	N_26	50	5	46.33	93	55.83^{ba}
V100-1709	V100_R	50	5	26.67	53	30.33^{dfce}
V100-1802	V100_S	50	5	6	12	12.33^{hgi}
V100-35226	V100_U	50	5	0.33	1	2.16^{i}

****ASD =Average Seed Damage.**

The mung bean varieties demonstrated significant variations in seed damage due to bruchid infestation, exhibiting the diversity in their resistance levels. Based on the results of a post hoc analysis following ANOVA, varieties that do not share a letter are significantly different from each other in terms of their resistance to bruchids. Varieties such AMUV-1604, AMVU-1605, and AMVU-1606 were assigned the same letter, indicating that their levels of seed damage are not significantly different from each other. Although their specific seed damage percentages vary, they are statistically similar in their resistance to bruchid infestation. However, these varieties differ significantly from more resilient lines such as V100-U and AMVU-H, which have much lower seed damage levels and are assigned different letters in the analysis.

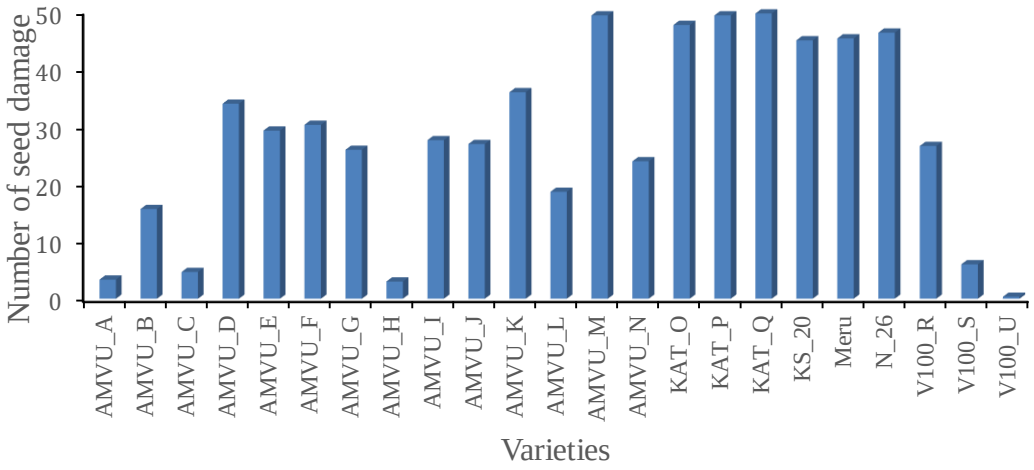


Figure 4. 1: Percentage Seed damage in selected Mung bean Varieties by C. malculatus

4.2 Metabolite Profiles in Selected Mung Bean Varieties

The analysis of metabolite profiles in mung beans varieties reveals significant insights into their correlation with bruchid infestation resistance. Each metabolite examined, including phenols, tannins, flavonoids, proteins and carbohydrates, plays a crucial role in determining the quality and benefits of mung beans. By comparing these metabolites' concentrations with established values from scientific literature, we can better understand how these varieties stack up in terms of their potential contributions to bruchid infestation resistance.

Table 4. 2: Metabolite Profile Analysis

Variety code	Treatment code	Phenols µg/g	Tannins µg/g	Flavonoids µg/g	Proteins µg/g	Carbohydrates µg/g	Turky's ASD
AMVU-1601	AMVU_A	11.222^{bac}	7.5020^{ba}	18.8705^{ba}	30.615^{bac}	23.659^{ba}	7.83^{hi}
AMVU-1602	AMVU_B	9.860^{ebdfc}	6.3775^{edc}	17.1439^{bdce}	30.207^{bdac}	22.602^{bac}	18.16^{hfgi}
AMVU-1603	AMVU_C	11.014^{bdac}	7.1807^{bc}	18.0072^{bac}	31.905^{ba}	24.481^{a}	10.5^{hgi}
AMVU-1604	AMVU_D	6.217^{hjlki}	4.4418^{jik}	13.8345^{i}	29.274^{ebdac}	16.790^{gih}	43.16^{bc}
AMVU-1605	AMVU_E	7.224^{hjki}	5.0924^{hjgi}	14.6978^{ehi}	25.601^{ebdhgf}	20.373^{dec}	35.66^{dce}
AMVU-1606	AMVU_F	6.365^{hjlki}	4.9317^{hji}	14.4101^{ei}	25.096^{ebdhgf}	16.983^{gfih}	37.66^{dc}
AMVU-1608	AMVU_G	8.675^{ehgf}	5.6386^{hegdf}	16.0647^{deg}	27.874^{ebdgcf}	19.326^{gfdec}	30.33^{dfce}
AMVU -1612	AMVU_H	12.093^{a}	7.8876^{a}	19.3741^{a}	31.517^{ba}	25.072^{a}	7.16^{hi}
AMVU-1614	AMVU_I	7.372^{hjgki}	5.1566^{hjgi}	15.2734^{deh}	25.926^{ebdhgcf}	17.432^{gfieh}	33.3^{dfce}
AMVU-1616	AMVU_J	8.024^{hjgkif}	5.3173^{hjgif}	15.4892^{deh}	26.503^{ebdhgcf}	18.170^{gfeh}	33.0^{dfce}
AMVU-1618	AMVU_K	5.921^{mjlki}	4.6104^{hjik}	13.5468^{ik}	24.159^{eihgf}	15.956^{ijh}	46.0^{bc}
AMVU-1619	AMVU_L	9.208^{edgfc}	6.1526^{edf}	16.6403^{def}	28.956^{ebdac}	20.128^{fdec}	19.50^{hfge}
AMVU-1627	AMVU_M	3.641^{mn}	3.6787^{lk}	12.1079^{l}	21.742^{ij}	13.933^{kj}	59.16^{ba}
AMVU-1630	AMVU_N	9.060^{edgf}	5.9598^{egdf}	16.2806^{defg}	28.343^{ebdacf}	19.936^{fdec}	24.83^{dfge}
KAT-00301	KAT_O	4.322^{mkn}	3.7751^{lk}	12.2518^{l}	22.536^{ihg}	14.062^{kij}	59.16^{ba}
KAT-00308	KAT_P	3.493^{mn}	3.6145^{lk}	11.1727^{ml}	19.336^{ij}	12.874^{k}	63.33^{a}
KAT-00309	KAT_Q	3.197^{n}	3.3574^{l}	12.0216^{m}	17.270^{j}	12.425^{k}	64.66^{a}
KS-20	KS_20	5.744^{mlk}	4.4498^{jik}	13.2590^{ik}	23.942^{eihgf}	15.635^{kijh}	55.00^{ba}
Local Meru	Meru	5.507^{mlkn}	4.3454^{jlk}	12.9712^{ikl}	23.293^{ihgf}	14.993^{kijh}	55.83^{ba}
N-26	N_26	8.375^{ehgif}	5.3574^{hjgif}	12.5396^{il}	28.555^{ebdacf}	17.405^{gfieh}	55.83^{ba}
V100-1709	V100_R	8.201^{ehjgif}	5.4458^{hgif}	15.8489^{de}	26.792^{ebdhgcf}	18.459^{gfeh}	30.33^{dfce}

****ASD =Average Seed Damage.**

V100-1802	V100_S	10.363$^{eb\ dac}$	6.6345^{bdc}	17.5755^{bdc}	32.520^{a}	21.669^{bdc}	12.33^{hgi}
V100-35226	V100_U	11.624^{ba}	8.0562^{a}	18.6547^{ba}	31.817$^{b\ a}$	24.210^{a}	2.16^{i}
STD		4.635	4.011	2.833	1.411	2.670	16.491
SEM		0.558	0.483	0.341	0.170	0.321	1.986
F-Value		6.4	12.7	12.24	18.56	17.69	
P-Value		0.0001	0.0001	0.0001	0.0001	0.0001	

4.2.1 Total Phenol content in Selected Mungbean Varieties

Table 4.2 shows the absorbance of each standard solution measured at a specific wavelength, (765 nm).

Table 4. 3: Total phenols: Gallic Acid standard curve

Concentration(µg/g)	2	4	10	20	30	40
Absorbance(765nm)	0.323	0.778	1.846	1.9784	2.7664	3.506

The absorbance values obtained were plotted against the known concentrations of gallic acid to create a standard curve. Typically, a linear regression analysis was determined to establish the best-fit line through these points. The resulting equation from this regression, showing R^2 value close to 1 indicating a strong linear relationship. The concentrations ranged from 2 to 40 µg/g to provide a broad dynamic range, ensuring that the assay can measure phenol content in samples with low to moderately high concentrations.

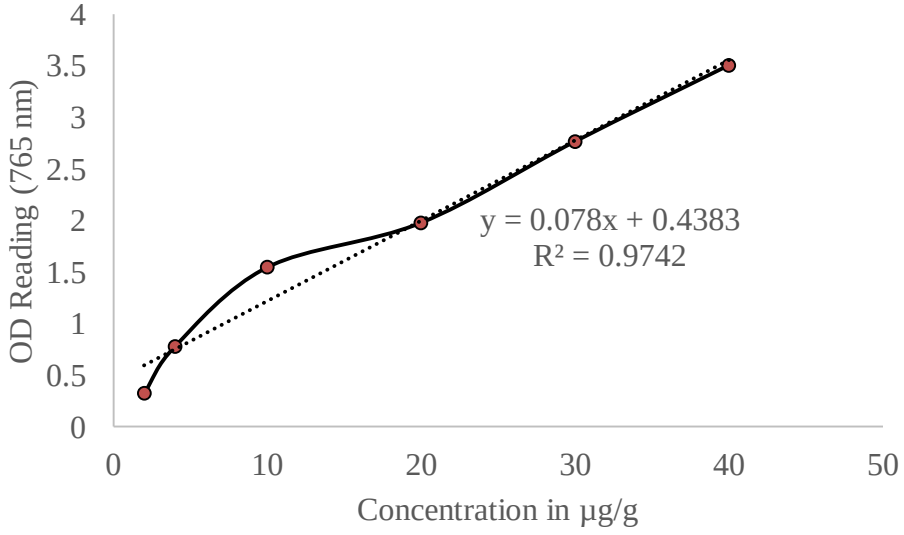


Figure 4. 2 : Gallic acid standard curve

To estimate the total phenol content in different test samples, the absorbance of each sample was measured at 765 nm under the same conditions as the standard solutions. The absorbance values of the test samples were then compared to the standard curve (Figure 4.2). The linear relationship between concentration and absorbance allows for accurate and precise quantification of phenols in unknown samples by interpolation or using the regression equation derived from the standard curve.

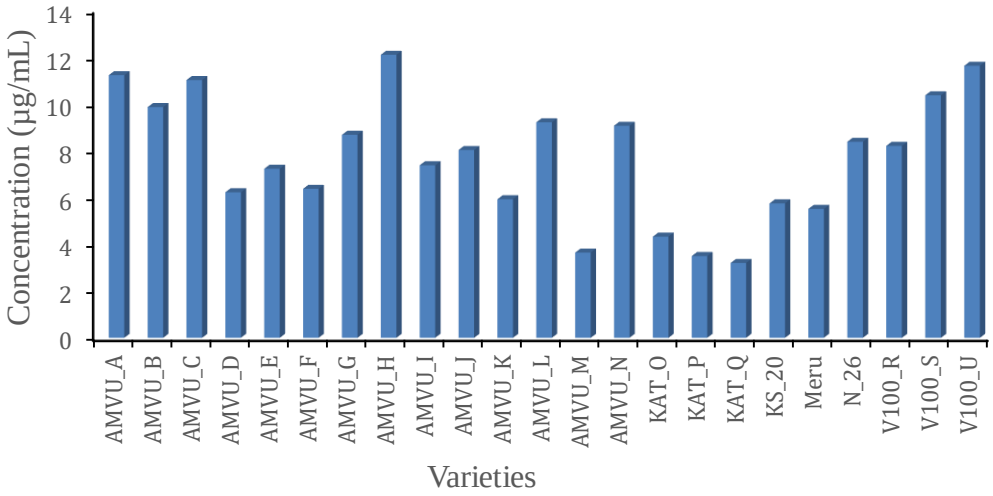


Figure 4. 3: Total phenol content in selected mungbean varieties

The bar graph provided a visual representation of phenol concentrations in various mung beans varieties. The x-axis lists the different mung beans varieties, while the y-axis represents the concentration of phenols in micrograms per millilitre (µg/g). It depicts significant variability, with some varieties standing out due to their higher phenolic concentrations.

4.2.2 Total Tannin Content in Selected Mungbean Varieties

The absorbance readings for a series of tannic acid solutions at different concentrations measured at 725 nm, a wavelength were recorded as shown in (Table 4.3)

Table 4. 4: Total Tannin Content

Concentration(µg/g)	2	4	10	20	30	40
Absorbance(725nm)	0.113	0.119	0.137	0.248	0.348	0.407

These values were used to plot a standard curve, with the concentration of tannic acid on the x-axis and the corresponding absorbance on the y-axis (Figure 4.4). The plotted data points revealed a generally linear relationship between tannic acid concentration and absorbance. This linearity allowed for the straightforward use of the curve to estimate tannin content in unknown samples by measuring the absorbance of mung beans extracts at 725 nm and then using the standard curve to determine their tannin concentrations.

The tannin content for each mung beans variety was calculated by interpolating their absorbance values using the linear equation derived from the standard curve. This step translated the absorbance data into meaningful tannin concentrations, allowing for a quantitative comparison among the varieties. The analysis revealed a range of tannin concentrations among the mung beans varieties, indicating significant variability. Some varieties such as AMVU-H and V100-U exhibited notably higher tannin levels at 7.8876 µg/g and 8.0562 µg/g, respectively, Other varieties such as KAT-Q had lower tannin concentrations at 3.3574 µg/g

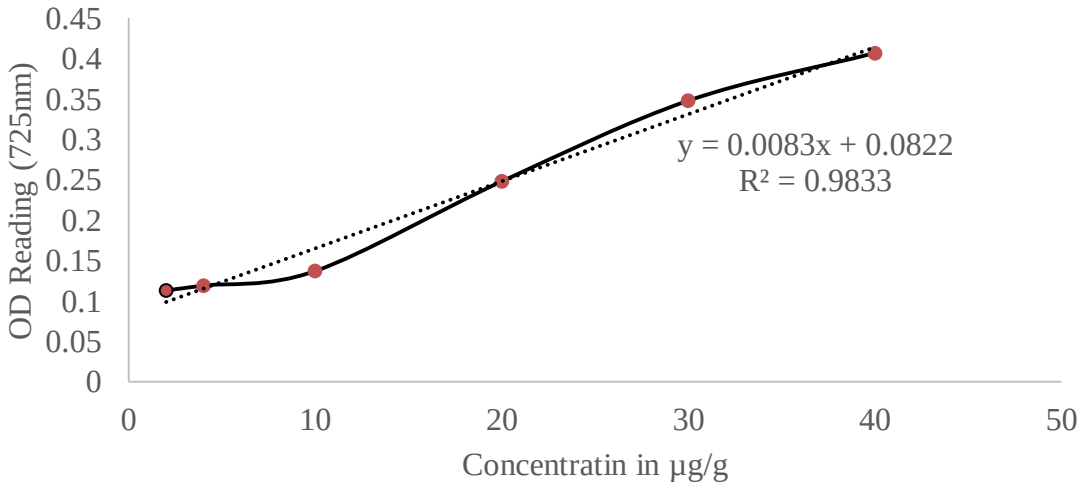
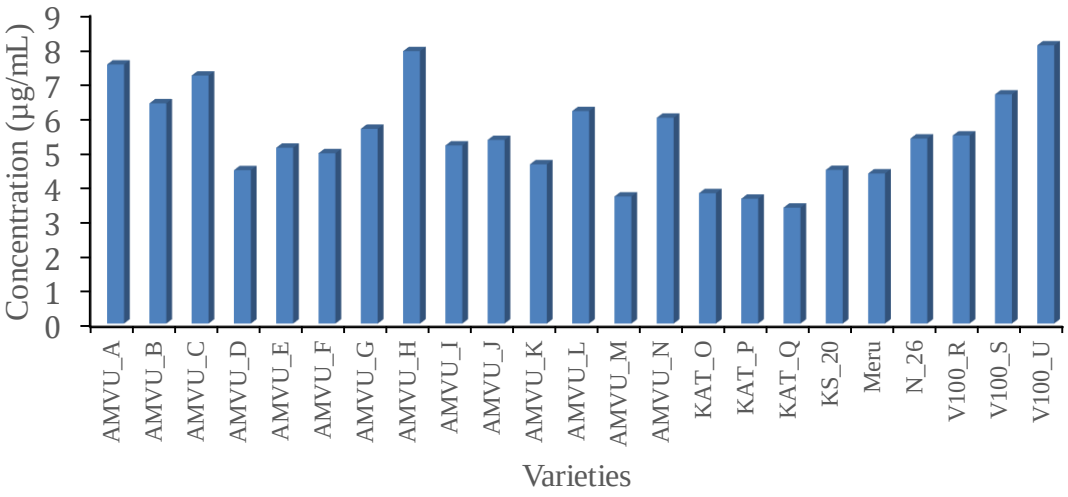


Figure 4. 4: Tannic Acid Standard Curve

To illustrate the tannin concentrations across different mung bean varieties, bar graphs were plotted with the mung bean varieties on the x-axis and the corresponding tannin concentrations on the y-axis (Figure 4.5). Each bar represented a specific variety and its tannin concentration, as derived from the absorbance readings using the standard

curve. For example, varieties such as AMVU-H and V100-U, which showed absorbance values that corresponded to higher tannin levels, were plotted with bars reaching up to 7.8876 µg/g and 8.0562 µg/g respectively. In contrast, varieties like KAT-Q, with lower absorbance and thus lower tannin concentrations, were represented by shorter bars reaching up to 3.3574 µg/g. This visual representation in bar graphs clearly depicted the variation in tannin content among the different mung bean varieties.



Total Tannins Content in Selected Mung Bean Varieties

Figure 4. 5: Total Tannins Content in Selected Mung Bean Varieties

4.2.3 Flavoids Content in Selected Mung Bean Varieties

A series of catechin solutions at predetermined concentrations were used as calibration standards, providing reference points for measuring unknown samples Each catechin standard solution underwent spectrophotometric analysis to measure its absorbance at the designated wavelength of 510 nm (Table 4.4). This wavelength was selected based on the optimal absorbance characteristics of catechin, which allowed for the sensitive and specific detection of flavonoids in the samples.

Table 4. 5: Estimation of Flavoids: Catechin standard curve

Concentration(µg/g)	2	4	10	20	30	40
Absorbance(510nm)	0.124	0.165	0.344	0.743	0.945	1.344

The absorbance values obtained from the spectrophotometric measurements were plotted against the respective catechin concentrations to construct the standard curve (figure 4.6)

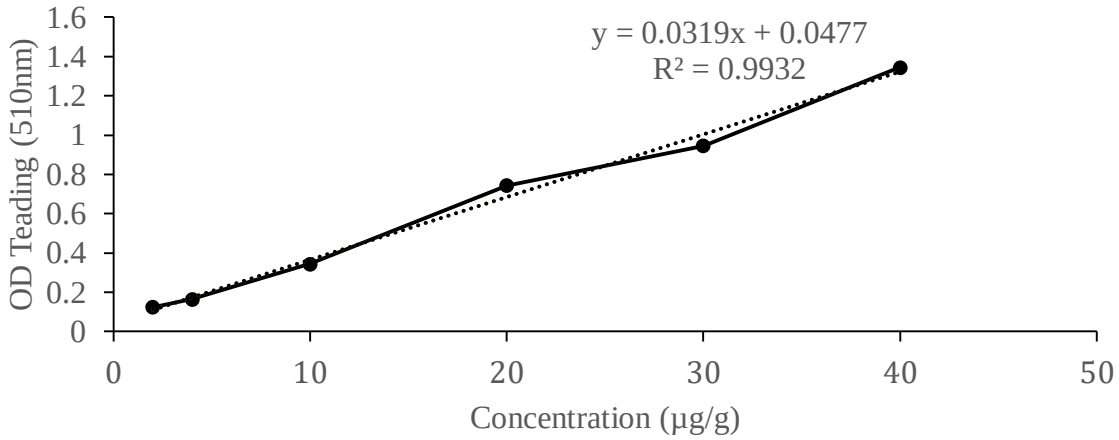


Figure 4. 6: Catechin Standard Curve

The flavonoid content of each unknown sample was determined by measuring its absorbance at 510 nm under the same conditions used for the catechin standards. The absorbance value of the unknown sample was mapped onto the standard curve. Using the linear regression equation derived from the standard curve, the corresponding concentration of flavonoids in the sample was interpolated

Each bar on the graph (Figure 4.7) represented a specific mung bean variety, depicting the concentration of flavonoids determined by the absorbance readings at 510 nm derived from standard curve (Figure 4.6). For instance, varieties exhibiting higher concentrations of flavonoids, such as AMVU-H and V100-U, would be represented by taller bars, while those with lower concentrations, like KAT-Q, would have shorter bars. This visual representation allows for a comparative analysis of flavonoid levels across different mung bean varieties, providing insights into their nutritional and functional properties.

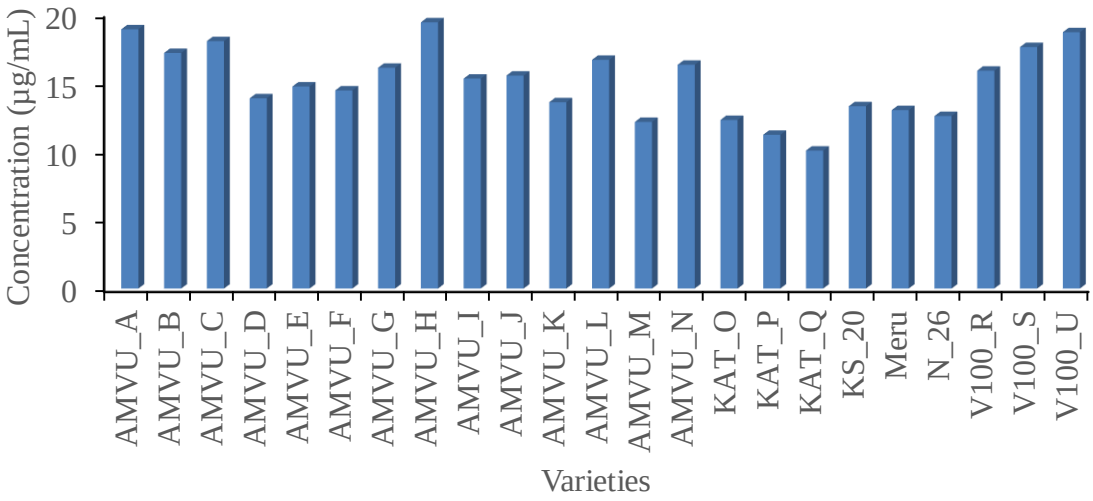


Figure 4. 7: Estimation of total flavonoids

4.2.4. Proximate analysis of proteins

For determination of total proteins, Bovine Serum Albumin (BSA) standard solutions with known concentrations—ranging from 2 to 40 µg/g—were prepared. These solutions served as calibration standards against which the protein content of mung beans samples was compared. Each BSA standard solution was subjected to spectrophotometric analysis to measure its absorbance at 595 nm (Table 4.5). This wavelength was chosen based on the absorption characteristics of proteins, particularly the aromatic amino acids like tryptophan and tyrosine.

Table 4. 6: Estimation of total proteins: BSA standard curve

Concentration(µg/g)	2	4	10	20	30	40
Absorbance(595nm)	0.0966	0.175	0.6282	0.8506	1.2722	1.5943

The absorbance values obtained from the BSA standard solutions were plotted against their corresponding concentrations to generate the BSA standard curve. This curve typically exhibited a linear relationship, facilitating the derivation of a regression equation relating to absorbance against concentration (Figure 4.8.)

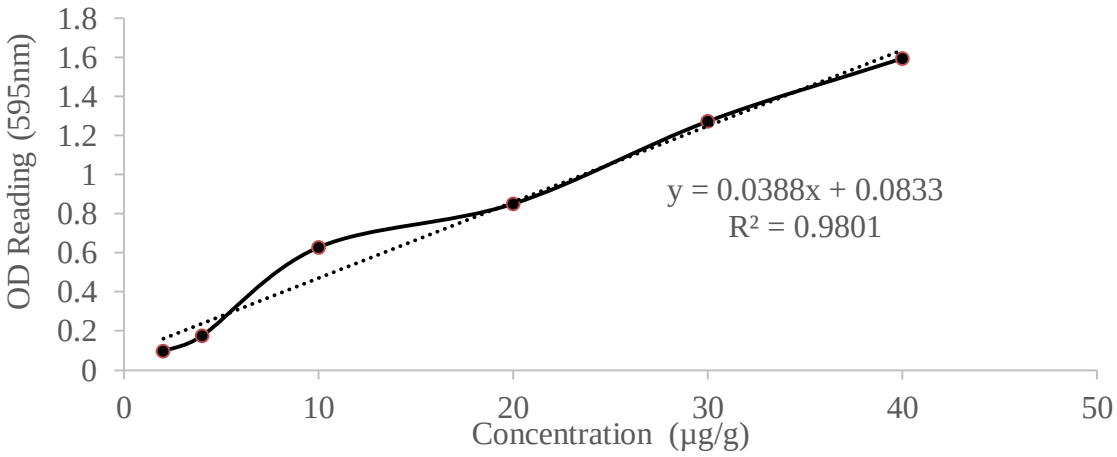


Figure 4. 8: BSA Standard curve

The absorbance values obtained from the mung beans samples were compared to the BSA standard curve. By locating the absorbance value on the y-axis of the standard curve and interpolating the corresponding protein concentration using the regression equation, the protein content of each mung beans variety was determined. The protein content exhibited significant variability among the mung beans varieties, Varieties such as V100-S and AMVU-C recorded the highest protein concentrations at 32.520 µg/g and 31.905 µg/g, respectively, whereas KAT-Q displayed the lowest protein content at 17.270 µg/g (Table 4.7).

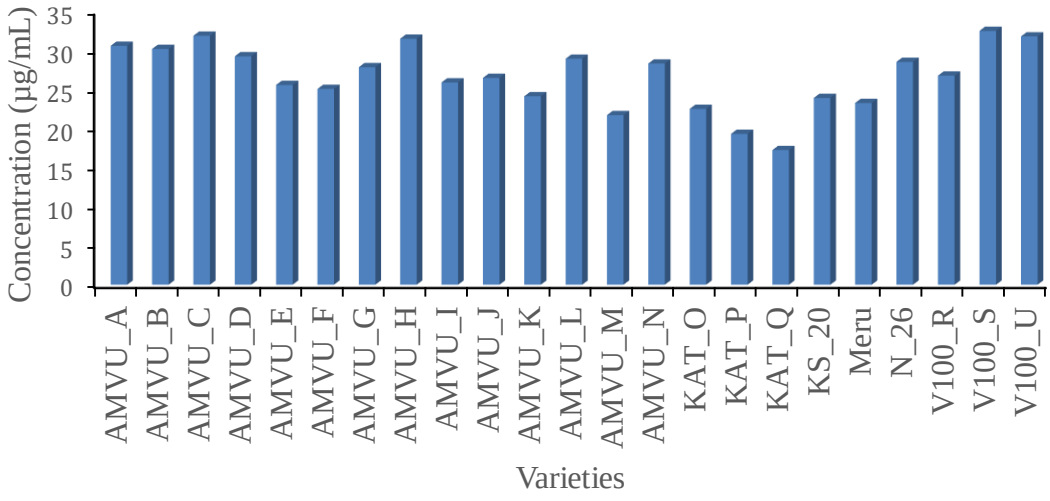


Figure 4. 9: Estimation of total proteins

In Figure 4.9, each mung bean variety is represented along the x-axis, while the y-axis depicts the estimated protein concentrations in µg/g. The bar heights indicate the protein content of each variety, with distinct bars illustrating the variability in protein

levels among different mung bean lines. For instance, varieties such as V100-S and AMVU-C exhibit higher protein concentrations at approximately 32.520 µg/g and 31.905 µg/g, respectively, whereas KAT-Q shows the lowest protein content at 17.270 µg/g (Table 4.7).

4.2.5 Total Carbohydrates content

For Estimation of carbohydrate contents, a series of glucose standard solutions spanning concentrations from 2 to 40 µg/g were used to serve as calibration standards against which the carbohydrate content of samples was assessed. Each glucose standard solution underwent spectrophotometric analysis to quantify its absorbance at 490 nm (Table 4.6). This wavelength selection corresponded to the peak absorbance wavelength for carbohydrates, particularly glucose.

Table 4. 7: Estimation of total Carbohydrates: Glucose standard curve

Concentration(µg/g)	2	4	10	20	30	40
Absorbance(490nm)	0.0119	0.0151	0.0407	0.0668	0.0834	0.1187

The process of plotting absorbance values acquired from the glucose standard solutions against their respective concentrations facilitated the creation of the glucose standard curve. This curve, characterized by its typical linearity, played a pivotal role in establishing a regression equation that correlated absorbance with concentration (Figure 4.10). spectrophotometric absorbance measurements of the samples were conducted at 490 nm to estimate the carbohydrate content.

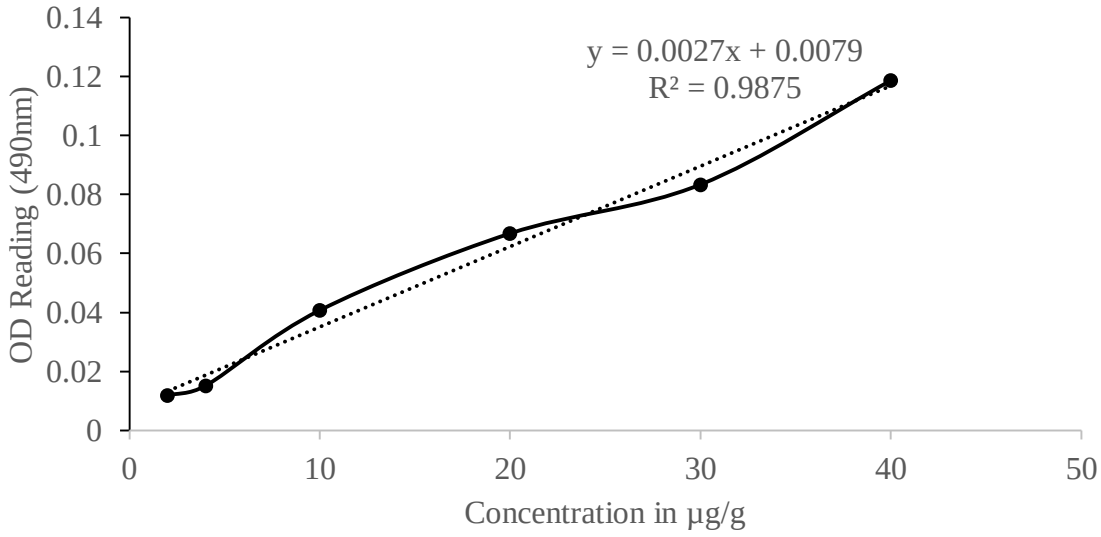


Figure 4. 10: Glucose standard curve

The results show that significant differences were observed in carbohydrate content across the mung beans varieties as portrayed (figure 4.11*)* The varieties AMVU-C and AMVU-H had the highest carbohydrate levels at 24.481 µg/g and 25.072 µg/g, respectively, whereas KAT-Q recorded the lowest carbohydrate content at 12.425 µg/g. (Table 4.7).

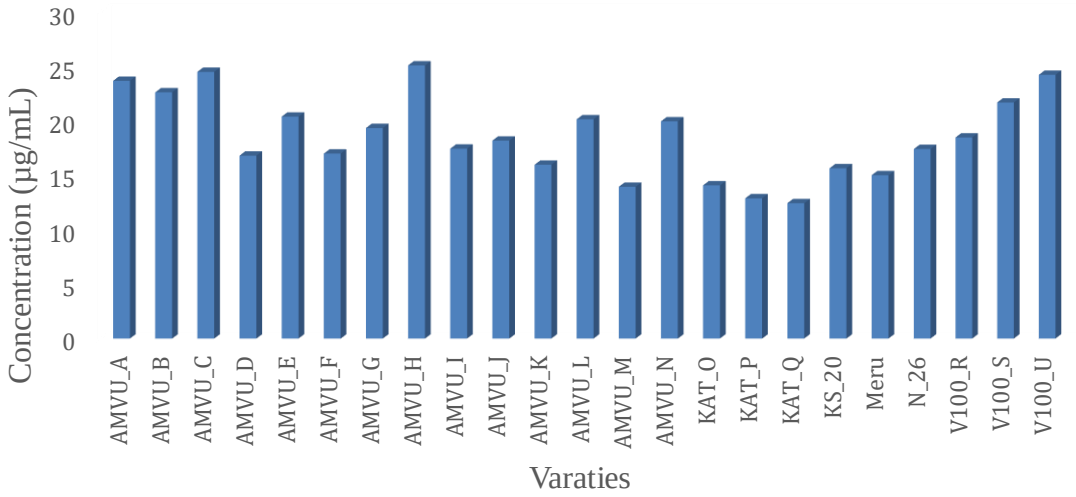


Figure 4. 11: Carbohydrates content

In Figure 4.11, each mung bean variety is depicted on the x-axis, while the y-axis represents the estimated carbohydrate concentrations in µg/g. The bar heights indicate the carbohydrate content of each variety, illustrating significant variability among different mung bean lines. For example, varieties like AMVU-C and AMVU-H exhibit higher carbohydrate concentrations at approximately 24.481 µg/g and 25.072 µg/g, respectively, whereas KAT-Q shows the lowest carbohydrate content at 12.425 µg/g (Table 4.7).

4.2.6 Correlation Between Metabolite Profiles and Bruchid Resistance in Mung Beans Varieties

The analysis of metabolite profiles across different mung beans varieties revealed significant diversity in the concentrations of phenols, tannins, flavonoids, proteins, and carbohydrates. Phenolic content varied significantly across the mung beans varieties, with concentrations ranging from 3.197 µg/g in 'KAT-00309' (KAT-Q) to 12.093 µg/g in 'AMVU-1612' (AMVU-H). The highest phenol concentration was observed in 'AMVU- 1612' (12.093 µg/g), followed closely by 'V100 35226' (V100-U) and 'AMVU-1601'. Varieties such as 'AMVU-1604', 'AMVU-1606', 'AMVU-1618', and 'KAT-00308' displayed the lowest phenolic content, with values significantly lower than the high phenol groups.

Table 4. 8: Metabolites profile significance at $p<0.05$

	Proteins	Carbohydrate	Phenols	Tannins	Flavonoids	Seed damaged
Proteins	1	0.92303**	0.9406**	0.83052**	0.85976**	-0.86434**
Carbohydrates		1	0.95582**	0.87293**	0.91031**	-0.89906**
Phenols			1	0.8733**	0.89911**	-0.91669**
Tannins				1	0.90341**	-0.89928**
Flavonoids					1	-0.9332**
Seed damaged						1
			** significance at $p<0.05$			

Tannin levels also exhibited considerable variability, with the highest concentration found in 'V100-35226' (8.0562 µg/g) and the lowest in 'KAT-00309' (3.3574 µg/g). The varieties with the highest tannin content were 'V100-35226' and 'AMVU-1612', which could imply potential differences in bruchid infestation resistant among the varieties. Lower tannin concentrations were notable in varieties like 'AMVU-1604', 'KAT-00308', and 'AMVU-1627', suggesting they might be less astringent.

Flavonoid concentrations spanned a broad range, from 11.1727 µg/g in 'KAT-00308' to 19.3741 µg/g in 'AMVU-1612'. The highest flavonoid content was found in 'AMVU-1612' and 'AMVU-1601', which might indicate higher antioxidant properties in these varieties. AMVU-1627', 'KAT-00308', and 'KAT-00309' showed the lowest flavonoid levels, potentially reflecting lower antioxidant capacities.

Protein concentrations varied from 17.270 µg/g in 'KAT-00309' to 32.520 µg/g in 'V100-1802'. The highest protein content was observed in 'V100-1802', 'AMVU-1603', and 'AMVU-1612', suggesting these varieties could be more nutritious in terms of protein content. Varieties like 'KAT-00309', 'AMVU-1627', and 'KAT-00301' had the lowest protein levels, indicating potential differences in their protein profiles.

Carbohydrate content ranged from 12.425 µg/g in 'KAT-00309' to 25.072 µg/g in 'AMVU-1612'. The highest carbohydrate concentrations were seen in 'AMVU-1612' and 'V100-35226', which could suggest these varieties are more energy-dense. Lower carbohydrate levels were noted in varieties such as 'KAT-00309', 'AMVU-1627', and 'KAT-00308'.

The Average Seed Damage (ASD) metric, indicating the extent of physical damage to seeds, varied significantly among the varieties. AMVU-1627', 'KAT-00301', and 'KAT-00309' exhibited the highest ASD values of between 59.16 to 64.66 , suggesting these varieties are more prone to seed damage. Conversely, 'V100-35226' and 'AMVU-1612' had the lowest ASD values of 2.16 and 7.16 respectively, indicating greater resistance to seed damage.

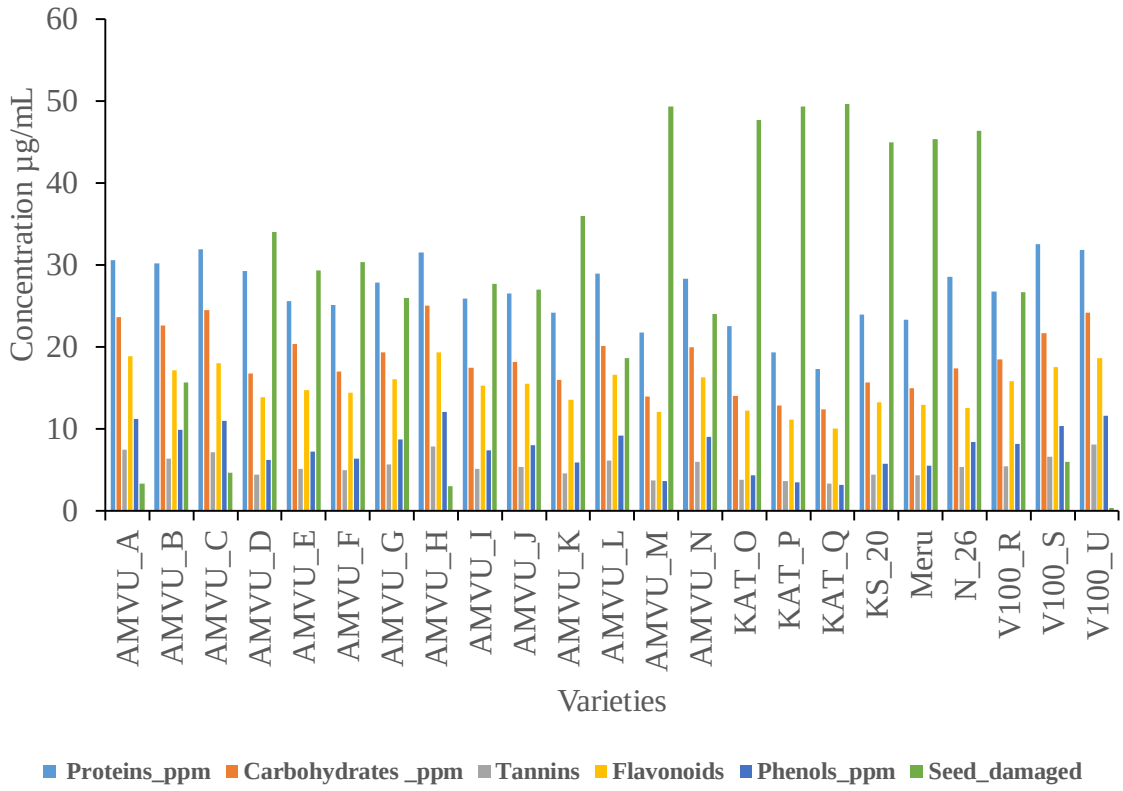


Figure 4. 12: Metabolite Profiles and Bruchid Resistance in mung Bean Varieties

The analysis of variance (ANOVA) conducted on the metabolite profiles—including proteins, carbohydrates, phenols, tannins, and flavonoids—across different mung beans varieties yielded statistically significant differences among treatments.

The protein content exhibited significant variability among the mung beans varieties, with an F value of 6.4 and a highly significant P value of 0.0001. Varieties such as V100-S and AMVU-C recorded the highest protein concentrations at 32.520 µg/g and 31.905 µg/g, respectively, whereas KAT-Q displayed the lowest protein content at 17.270 µg/g. These findings align with the work of Rao *et al*. (2018), who observed significant protein content variation in legume cultivars under different environmental conditions. The post hoc Tukey's Honest Significant Difference (HSD) test revealed that varieties sharing the same letter, such as AMVU-1602, AMVU-1605, and AMVU-1606, do not differ significantly in protein content, indicating clusters of statistical similarity.

Significant differences were also observed in carbohydrate content across the mung beans varieties, with an F value of 12.7 and a P value of 0.0001. The varieties AMVU-

C and AMVU-H had the highest carbohydrate levels at 24.481 µg/g and 25.072 µg/g, respectively, whereas KAT-Q recorded the lowest carbohydrate content at 12.425 µg/g. Similar patterns of carbohydrate variability have been reported by Singh *et al.* (2017) in their assessment of carbohydrate profiles in various legume species. Treatments with overlapping letters denote groups that are statistically similar in carbohydrate content, highlighting the lack of significant differences within these clusters.

The phenol content among the different mung beans treatments varied significantly, with an F value of 12.24 and a P value of 0.0001. The varieties AMVU-H and V100-U exhibited the highest phenol content at 12.093 µg/g and 11.624 µg/g, respectively, while KAT-Q had the lowest phenol content at 3.197 µg/g. These results are consistent with findings by Zhang *et al*. (2019), who demonstrated significant phenolic content differences in leguminous plants subjected to biotic and abiotic stresses. Multiple comparisons further indicated overlapping phenol content levels among certain treatments, suggesting the existence of statistically similar groups.

Tannin content showed significant variability across the mung beans varieties, with an F value of 18.56 and a P value of 0.0001. Varieties such as AMVU-H and V100-U had high tannin content at 7.8876 µg/g and 8.0562 µg/g, respectively, whereas KAT-Q was among the lowest at 3.3574 µg/g. These findings are supported by the work of Prakash *et al*. (2016), who observed similar tannin content variability in legumes under different environmental conditions.

The distinct groups identified by the Tukey's HSD test underscore the significant differences in tannin levels among the treatments.

Flavonoid content also exhibited significant differences among the mung beans varieties, with an F value of 17.69 and a P value of 0.0001. The highest flavonoid levels were recorded in AMVU-H and AMVU-A at 19.3741 µg/g and 18.8705 µg/g, respectively, while KAT-Q had the lowest at 10.0216 µg/g. These findings are corroborated by Kumar *et al*. (2020), who reported significant flavonoid content variation in legumes influenced by genetic and environmental factors. Overlapping letters among the treatments indicate statistically similar flavonoid levels, reflecting the complexity of flavonoid biosynthesis pathways in different mung beans varieties.

4.3 Discussion

The discussion explores the implications of the study's findings within the scope of understanding mung bean metabolite profiles and pest interactions. It evaluates the significance of identifying resistant mung bean varieties and understanding their metabolite composition in combating bruchid infestations. Furthermore, it addresses the hypotheses tested in the study regarding differences in resistance among mung bean varieties, variability in metabolite profiles, and the correlation between metabolite composition and resistance levels.

4.3.1 Evaluation of Mung bean Varieties against Bruchid Infestation

The evaluation of mung bean lines against *Callosobruchus maculatus* infestation revealed significant variations in resistance levels, as indicated by the extent of seed damage across different varieties. Among the tested lines, V100-U demonstrated the highest resistance with minimal seed damage at 2.16%, while other lines such as AMVU-H and AMVU-A also exhibited relatively low damage levels at 7.16% and 7.83%, respectively. Conversely, lines like KAT-Q, KAT-P, and AMVU-M displayed the highest susceptibility with seed damage percentages ranging from 55.0% to 64.66%.

The observed differences in seed damage highlight the diverse resistance capabilities among mung bean varieties against bruchid infestation. This diversity is crucial for selecting and breeding varieties that can withstand pest pressures, thereby reducing economic losses and enhancing food security. The results are consistent with previous studies that have documented similar differential resistance patterns in legume cultivars exposed to bruchid infestation (Mukuru *et al.*, 2015; Kimani *et al.*, 2016).

The statistical analysis confirmed significant differences in resistance levels among the tested mung bean lines, with distinct groups identified based on seed damage percentages. Varieties within the same group exhibited comparable resistance, while those in different groups showed statistically significant variations. This categorization provides valuable insights into the genetic and biochemical factors influencing mung bean resistance to bruchids, paving the way for targeted breeding efforts aimed at developing resilient cultivars.

Figure 4.1 visually represents the variation in seed damage across mung bean varieties, further emphasizing the distinct resistance profiles observed. Each bar in the graph corresponds to a specific variety, with bar heights reflecting the percentage of seed

damage. The graphical representation enhances the understanding of how different varieties respond to bruchid infestation, from highly resistant varieties with minimal damage to highly susceptible ones with extensive damage.

These findings underscore the importance of integrating resistance screening into mung bean breeding programs and agricultural practices. By identifying and promoting resistant varieties like V100-U and AMVU-H, farmers can effectively manage bruchid infestations without relying heavily on chemical pesticides. Moreover, understanding the metabolite profiles associated with resistance, as indicated in the study's objectives, can provide deeper insights into the biochemical mechanisms underlying mung bean resistance.

4.3.2 Analysis of Metabolite Profiles in Mung Beans Varieties

The analysis of metabolite profiles in mung beans varieties reveals significant insights into their correlation with bruchid infestation resistance. Each metabolite examined, including phenols, tannins, flavonoids, proteins and carbohydrates, plays a crucial role in determining the quality and benefits of mung beans. By comparing these metabolites' concentrations with established values from scientific literature, we can better understand how these varieties stack up in terms of their potential contributions to bruchid infestation resistance.

The phenolic content across the mung beans varieties showed a notable range, with AMVU-1612 exhibiting the highest concentration at 12.093 µg/g. This value, along with those from V100-35226 (11.624 µg/g) and AMVU-1601 (11.222 µg/g), falls within the expected range for mung beans, which typically have phenolic concentrations between 5-15 µg/g (Xiao *et al.,* 2012). Phenols are recognized for their antioxidant properties, contributing significantly to the mung bean ability to resist bruchid infestation.

Tannins were another important metabolite analysed, with the highest concentration found in V100-35226 at 8.0562 µg/g, closely followed by AMVU-1612 with 7.8876 µg/g. These values align well with the documented range of 3-10 µg/g for mung beans (Tang *et al.,* 2014).

Tannins are crucial for their ability to provide rsistance to bruchids. They bind to the proteins and inhibit the bruchids fro digesting the proteins hence reducing growth and survival of bruchids

In terms of flavonoid content, AMVU-1612 again stood out with the highest concentration of 19.3741 µg/g, followed by AMVU-1601 (18.8705 µg/g) and V100-35226 (18.6547 µg/g). These values are consistent with the typical flavonoid range of 10-20 µg/g found in mung beans (Chaudhary *et al*., 2018). Flavonoids have insecticidal activity hence high levels corresponded to decreased bruchid infestation.

Protein concentrations were also notably high in certain varieties, with V100-1802 showing the highest level at 32.520 µg/g. Other varieties like AMVU-1603 and V100-35226 also exhibited high protein levels at 31.905 µg/g and 31.817 µg/g, respectively. These values fall within the expected range of 20-35 µg/g for mung beans, reflecting their significant protein content, which is vital for binding to other secondary metabolites such as tannins.

The carbohydrate content varied among the mung beans varieties, with the highest concentration found in AMVU-1612 at 25.072 µg/g. Varieties such as AMVU-1603 and V100-35226 also had high carbohydrate levels, at 24.481 µg/g and 24.210 µg/g, respectively. These values are within or slightly above the typical range of 15-25 µg/g for mung beans (Chaudhary *et al.,* 2018). The carbohydrates provided basic anchoring structure for the secondary metabolites hence increase in carbohydrates indicated increased bruchid infestation

Lastly, the analysis of ASD (Average seed damage) revealed a wide range of values, with the highest concentrations in KAT-00309 and KAT-00308, at 64.66 and 63.33, respectively. ASD values varied significantly across the varieties, suggesting differences in potential antioxidant activities or other related health metrics. Although typical literature does not often specify ASD, the broad variation observed indicates its importance in evaluating the overall health benefits of mung beans.

4.3.3 Mung Bean Metabolite Profiles and Resistance to Storage Bruchi Infestations"

The analysis revealed significant correlations between the metabolite profiles (proteins, carbohydrates, phenols, tannins, flavonoids) and seed damage due to bruchid (*Callosobruchus maculatus*) infestation across various mung beans varieties, with all correlations significant at $p < 0.05$.

The protein content showed a strong negative correlation with seed damage ($r = -0.86434$), indicating that higher protein levels are associated with increased resistance to bruchid infestation. This suggests that mung beans varieties such as V100-S and AMVU-C with a protein content of 32.52 µg/g and 31.905 µg/g respectively, are less susceptible to seed damage. Previous studies, by Rao *et al.* (2018), showed significant variability in protein content among legume cultivars, which impacts their resistance to pests. Higher protein levels may enhance the structural integrity of seeds, making them less penetrable by bruchid larvae.

Carbohydrate content exhibited a strong negative correlation with seed damage ($r = -0.89906$). Varieties with higher carbohydrate content, like AMVU-H (25.072 µg/g) and AMVU-C (24.481 µg/g), showed increased resistance to bruchid damage. This correlates with studies by Singh *et al.* (2017), who found that higher carbohydrate levels can bolster the defensive mechanisms of legumes. Carbohydrates may serve as energy reserves, supporting the synthesis of other defensive compounds such as phenols and flavonoids.

Phenols demonstrated an even stronger negative correlation with seed damage ($r = -0.91669$). Varieties with high phenol content, such as AMVU-H (12.093 µg/g) and V100-U (11.624 µg/g), were markedly resistant to bruchid infestation. Zhang *et al.* (2019) reported similar findings, emphasizing that phenolic compounds play a crucial role in plant defence by deterring herbivores and inhibiting pathogen growth. The high antioxidant activity of phenols contributes to the stabilization of cell membranes, thereby enhancing resistance.

Tannins were negatively correlated with seed damage ($r = -0.89928$). Resistant varieties like V100-U (8.0562 µg/g) and AMVU-H (7.8876 µg/g) had high tannin levels. Prakash *et al*. (2016) observed that tannins, by binding to proteins and carbohydrates, can reduce the digestibility of seeds for herbivores, thereby acting as a deterrent. This anti-

nutritional property of tannins is a critical factor in their role as defensive compounds against pests.

Flavonoids showed the strongest negative correlation with seed damage (r = -0.9332). High flavonoid content was detected in AMVU-H and AMVU-A varieties with concentrations of 19.3741 µg/g and 18.8705 µg/g respectively, and this is associated with increased resistance. Kumar *et al*. (2020) highlighted that flavonoid, through their antioxidant properties, contribute significantly to plant defense mechanisms, including pest resistance. These compounds can interfere with the digestive processes of herbivores and pathogens, enhancing the plant's defensive capacity.

The positive correlations among primary (proteins, carbohydrates) and secondary metabolites (phenols, tannins, flavonoids) suggest a synergistic role in enhancing resistance to bruchid infestation. Higher levels of primary metabolites provide the necessary resources for the synthesis of secondary metabolites, which are directly involved in defence. This synergistic relationship underscores the integrated nature of plant metabolic pathways in developing comprehensive resistance mechanisms.

The negative correlations between each metabolite (proteins, carbohydrates, phenols, tannins, flavonoids) and seed damage underscore the importance of these compounds in conferring resistance to bruchid infestations. Varieties like V100-U, which exhibited the lowest seed damage (2.16%) and high levels of several metabolites, highlight the effectiveness of these metabolites in protecting seeds. Conversely, varieties such as KAT-Q (64.66% seed damage), KAT-P (63.33% seed damage), and KAT-O (59.16% seed damage), with lower metabolite levels, showed higher susceptibility. (Table 4.7)

The F-values and P-values (all $P < 0.0001$) indicate highly significant differences among the mung beans varieties for each metabolite. This suggests that the observed variations in metabolite concentrations are statistically meaningful and not due to random chance.

CHAPTER FIVE:SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study systematically evaluated the resistance of diverse mung beans (*Vigna radiata*) lines to infestations by storage bruchids (*Callosobruchus maculatus*). Additionally, it analysed the metabolite profiles of these mung beans varieties and explored the correlation between these biochemical attributes and the observed resistance to bruchid infestations. The findings are summarized as follows:

5.1.1 Screening of Mung beans Improved Lines Against *Callosobruchus maculatus*

The variety V100-U showed the highest resistance, evidenced by minimal seed damage. Other varieties, including AMVU-H, AMVU-A, AMVU-C, and V100-S, also demonstrated significant resistance. The analysis of variance (ANOVA) highlighted significant differences in susceptibility among the varieties, emphasizing the genetic diversity in bruchid resistance within mung beans lines. These findings are crucial for breeding programs aimed at developing mung beans varieties with enhanced resistance to bruchid infestations, thereby improving crop yield and seed quality.

5.1.2 Metabolite Profile Analysis

ANOVA results revealed significant differences in the metabolite profiles (proteins, carbohydrates, phenols, tannins, and flavonoids) among the mung beans varieties, with extremely low P values (< 0.0001). These findings indicate that each mung beans variety has a distinct biochemical composition, which is essential for targeted breeding and nutritional evaluations. Post hoc analyses further clarified which varieties exhibited significantly different metabolite levels, guiding the selection of mung beans for specific dietary and health benefits.

5.1.3 Mung Beans' Metabolite Profiles: A Potential Biomarker for Bruchid Resistance"

The study established a strong correlation between the metabolite profiles of mung beans varieties and their resistance to storage bruchid (*Callosobruchus spp*) infestations. Varieties with higher concentrations of proteins, carbohydrates, phenols, tannins, and flavonoids demonstrated significantly greater resistance to seed damage caused by bruchids. This correlation suggests that these metabolites serve as potential

biomarkers for identifying and breeding mung beans cultivars with enhanced resistance to pest infestations. The role of these biochemical components in fortifying mung beans seeds against bruchids highlights their importance in breeding programs and pest management strategies.

5.2 Conclusions

The results of this study provide compelling evidence that led to the following conclusions.

5.2.1 Bruchid Resistance in Mung beans:

The study identified significant variations in bruchid resistance among different mung beans varieties. The variety V100-U, along with others, exhibited high resistance to bruchid infestations. This variability can be leveraged in breeding programs to develop mung beans lines with superior resistance to pests.

5.2.2 Metabolite Diversity as a Breeding Tool

Based on these finding, significant differences in the metabolite profiles of mung beans varieties describe the potential for using specific biochemical attributes in breeding and nutritional selection. Each variety’s unique composition can be targeted to optimize nutritional value and health benefits.

5.2.3 Metabolites as Biomarkers for Resistance

The clear indications of correlation between elevated metabolite levels and increased resistance to bruchid infestations indicates that proteins, carbohydrates, phenols, tannins, and flavonoids could serve as biomarkers. These metabolites are crucial in breeding initiatives focused on developing bruchid-resistant mung beans cultivars, validating their role in enhancing the plant's defence mechanisms.

5.3 Recommendations

Considering the substantial correlation between specific metabolite profiles and bruchid resistance demonstrated by these findings, this study recommends as follows;

5.3.1 Incorporate Resistant Varieties into Breeding Programs

This study recommends the integration of highly resistant varieties, such as V100_U, into breeding programs to improve overall resistance to bruchid infestations. This approach will enhance crop yield and seed quality by minimizing damage.

5.3.2 Utilize Metabolite Profiles for Targeted Breeding

Harness the significant differences in metabolite profiles to breed mung beans varieties that meet specific nutritional and health criteria. Selecting varieties based on their unique biochemical composition can optimize their dietary value and health benefits.

5.3.3 Develop Integrated Pest Management Strategies

Employ mung beans varieties with high levels of protective metabolites as part of integrated pest management (IPM) strategies. This will reduce dependence on chemical pesticides and promote sustainable agricultural practices by leveraging the plants' natural defences.

5.3.4 Explore Metabolite Biomarkers in Further Research

Continue investigating the role of metabolites as biomarkers for resistance to bruchid infestations. Further research should focus on understanding the genetic and biochemical mechanisms underlying this resistance to inform the development of more resilient and nutritionally superior mung beans cultivars.

REFERENCES

Addo, P. W., Ossowski, P., Macpherson, S., Gravel, A. E., Kaur, R., Hoyos-Villegas, V., Singh, J., Orsat, V., Dumont, M. J., & Lefsrud, M. (2022). Development of a Nuclear Magnetic Resonance Method and a Near Infrared Calibration Model for the Rapid Determination of Lipid Content in the Field Pea (Pisum sativum). *Molecules*, *27*(5). https://doi.org/10.3390/molecules27051642

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, 2(4), 875-877. doi:10.1038/nprot.2007.102

Alseekh, S., & Fernie, A. R. (2018). Metabolomics 20 years on: What have we learned and what hurdles remain? *The Plant Journal*, *94*(5), 933-942. https://doi.org/10.1111/tpj.13950

Arora, R., & Sandhu, S. (2017). Breeding insect resistant crops for sustainable agriculture. In *Breeding Insect Resistant Crops for Sustainable Agriculture*. https://doi.org/10.1007/978-981-10-6056-4

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Chitra, P. S. S., Dikshit, S. H. K., & Pandi, G. G. P. (2015). Screening for Resistance Against Pulse Beetle , *Callosobruchus analis (* F .) in Greengram (*Vigna radiata* (L .) Wilczek) Accessions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*. https://doi.org/10.1007/s40011-015-0635-5

Divekar, P. A., Narayana, S., Divekar, B. A., Kumar, R., Gadratagi, B. G., Ray, A., Singh, A. K., Rani, V., Singh, V., Singh, A. K., Kumar, A., Singh, R. P., Meena, R. S., & Behera, T. K. (2022). Plant Secondary Metabolites as Defense Tools against Herbivores for Sutainable Crop Protection. *International Journal of Molecular Sciences*, *23*(5). https://doi.org/10.3390/ijms23052690

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing

phenolic compounds. *Food Chemistry*, *97*(4), 654-660.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356.

Dwivedi, S. L., Upadhyaya, H. D., Chung, I. M., De Vita, P., García-Lara, S., Guajardo-Flores, D., Gutiérrez-Uribe, J. A., Serna-Saldívar, S. O., Rajakumar, G., Sahrawat, K. L., Kumar, J., & Ortiz, R. (2016). Exploiting phenylpropanoid derivatives to enhance the nutraceutical values of cereals and legumes. *Frontiers in Plant Science*, *7*(JUNE2016), 1–27. https://doi.org/10.3389/fpls.2016.00763

Esen, A., Sari, H., Erler, F., Adak, A., Sari, D., Eker, T., Canci, H., İkten, C., Kahraman, A., & Toker, C. (2019). Screening and selection of accessions in the genus Pisum L. for resistance to pulse beetle (*Callosobruchus chinensis* L.). *Euphytica*, *215*(4). https://doi.org/10.1007/s10681-019-2395-4

Fernie, A. R., & Schauer, N. (2009). Metabolomics-assisted breeding: a viable option for crop improvement? Trends in Genetics, 25(1), 39-48.

Fiehn, O. (2002). Metabolomics - the link between genotypes and phenotypes. Plant Molecular Biology, 48(1-2), 155-171

Gupta, S., Nadarajan, N., & Gupta, D. Sen. (2014). Legumes in the omic era. *Legumes in the Omic Era*, 1–348. https://doi.org/10.1007/978-1-4614-8370-0

Hall, R. D. (2006). Plant metabolomics: from holistic hope, to hype, to hot topic. New Phytologist, 169(3), 453-468.

Harshitha, G. P., Jayamani, P., Manimegalai, S., & Muthuswamy, A. (2022). Host Plant Resistance for Bruchids in Pre-breeding Lines of Greengram [*Vigna radiata* (L.) Wilczek]. *LEGUME RESEARCH - AN INTERNATIONAL JOURNAL*, *I*(Of), 1–5. https://doi.org/10.18805/lr-4702

Hong, J., & Yang, L. (2016). Application of metabolomics in studying plant-pathogen interactions. Metabolomics, 12(6), 100.

Jackson, M. A., & Vavricka, C. J. (2018). Standard Curve Development for Tannin Quantification in Plant Extracts. *Journal of Analytical Methods in Chemistry*, 2018, Article ID 6763285. doi:10.1155/2018/6763285.

Jones, R. T., & Campbell, A. K. (2019). The Role of Tannins in Plant Defense Mechanisms. *Journal of Plant Science*, 14(3), 295-310. doi:10.1021/jps.19.00233.

Karimi, R., Nair, R., Ledesma, D., Mutisya, D., & Muthoni, L. (2019). Performance and participatory evaluation of mung beans genotypes in the semi-arid environments of Eastern Kenya. *East African Agricultural and Forestry Journal*, *83*(2), 119–136. https://doi.org/10.1080/00128325.2019.1599491

Kimani, P., Omondi, S., & Maina, J. (2016). Evaluation of mung beans *(Vigna radiata)* lines for resistance to bruchid (*Callosobruchus maculatus)* attack. African Crop Science Journal, 24(4), 395-403. doi:10.4314/acsj.v24i4.5.

Kpoviessi, A. D., Agbahoungba, S., Agoyi, E. E., Nuwamanya, E., Assogbadjo, A. E., Chougourou, D. C., & Adoukonou-Sagbadja, H. (2021). Primary and secondary metabolite compounds in cow pea seeds resistant to the cow pea bruchid [*Callosobruchus maculatus* (F.)] in postharvest storage. *Journal of Stored Products Research*, *93*(March). https://doi.org/10.1016/j.jspr.2021.101858.

Kruger, N. J. (2020). The Bradford method for protein quantitation. *The Protein Protocols Handbook*, 15-21.

Kumar, N., & Pandey, D. (2020). Recent advancements in metabolomics and its applications in plant science. Plant Science Today, 7(1), 1-11.

Kumar, R., Singh, A., & Singh, S. (2015). Integrated pest management strategies for Bruchid control in mung beans. *Agricultural Sciences*, *6*(5), 121-128.

Kumar, S., Pandey, G., & Yadav, R. (2020). Flavonoid content variation in legumes influenced by genetic and environmental factors. Phytochemistry, 174, 112345. doi:10.1016/j.phytochem.2020.112345.

Lambrides, C. J., & Imrie, B. C. (2000). Susceptibility of mung beans varieties to the bruchid species *Callosobruchus maculatus* (F.), C. phaseoli (Gyll.), C. chinensis (L.), and Acanthoscelides obtectus (Say.) (Coleoptera:Chrysomelidae). *Australian Journal of Agricultural Research*, *51*(1), 85–89. https://doi.org/10.1071/AR99051

Liu, Y., & Schröder, S. (2018). Advances in mass spectrometry-based metabolomics for plant systems biology. Frontiers in Plant Science, 9, 1207.

Litvinov, D. Y., Karlov, G. I., & Divashuk, M. G. (2021). Metabolomics for crop breeding: General considerations. *Genes*, *12*(10). https://doi.org/10.3390/genes12101602

Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S, Lee YC. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal Biochem. 2005 Apr 1;339(1):69-72. doi: 10.1016/j.ab.2004.12.001. PMID: 15766712.

Mbeyagala, E. ., Amayo, R., Obuo, J. ., Pandey, A. K., War, A. R., & Nair, R. M. (2017). *A manual for mung beans (Greengram) production in Uganda*. *September*, 1–24. http://www.nasarri.go.ug/scientific-papers/mung beans Production Uganda.pdf

Meenu, M., Kamboj, U., Sharma, A., Guha, P., & Mishra, S. (2016). Green method for determination of phenolic compounds in mung beans (*Vigna radiata L.)* based on near-infrared spectroscopy and chemometrics. *International Journal of Food Science and Technology*, *51*(12), 2520–2527. https://doi.org/10.1111/ijfs.13232

Mishra, S. K., Macedo, M. L. R., Panda, S. K., & Panigrahi, J. (2018). Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annals of Applied Biology*, *172*(1), 4–19. https://doi.org/10.1111/aab.12401

Mohapatra, D., Kar, A., & Giri, S. K. (2015). Insect Pest Management in Stored Pulses: an Overview. *Food and Bioprocess Technology*, *8*(2), 239–265. https://doi.org/10.1007/s11947-014-1399-2

Muchomba, M. K., Muindi, E. M., & Mulinge, J. M. (2023). Overview of mung beans (*Vigna radiata* L.) Crop, Its Economic Importance, Ecological Requirements and

Production Constraints in Kenya. *Journal of Agriculture and Ecology Research International*, *24*(2), 1–11. https://doi.org/10.9734/jaeri/2023/v24i2520

Mugo, J. W., Opijah, F. J., Ngaina, J., Karanja, F., & Mburu, M. (2023). Simulated effects of climate change on mung beans production in Kitui County, Kenya. *Frontiers in Sustainable Food Systems*, *7*(June), 1–15. https://doi.org/10.3389/fsufs.2023.1144663.

Mukuru, B., Ndung'u, S., & Njagi, W. (2015). Resistance of various legume cultivars to bruchid (*Callosobruchus maculatus)* infestation. Journal of Plant Protection Research, 55(3), 234-240. doi:10.1515/jppr-2015-0032

Mwangi, J. K., Mutungi, C. M., Midingoyi, S. K. G., Faraj, A. K., & Affognon, H. D. (2017). An assessment of the magnitudes and factors associated with postharvest losses in off-farm grain stores in Kenya. *Journal of Stored Products Research*, *73*, 7–20. https://doi.org/10.1016/j.jspr.2017.05.006

Njoroge, A. W., Baoua, I., & Baributsa, D. (2019). Postharvest Management Practices of Grains in the Eastern Region of Kenya. *Journal of Agricultural Science*, *11*(3), 33. https://doi.org/10.5539/jas.v11n3p33

Njoroge, A. W., Muriithi, W. K., Nderitu, J. H., & Gitonga, L. M. (2018). Resistance of cowpea genotypes to Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) under ambient conditions. *Journal of Stored Products Research*, 77, 52-58. https://doi.org/10.1016/j.jspr.2018.05.005

Nyongesa, M., Oyoo, J., Ng, N., Lung, C., Mbiyu, M., Otieno, S., Onditi, J., & Nderitu, J. (2019). *Inventory Of Climate Smart Agriculture Potato Technologies, Compiled by*. *April*, 1–35.

Obata, T., & Fernie, A. R. (2012). The use of metabolomics to dissect plant responses to abiotic stresses. Cellular and Molecular Life Sciences, 69(19), 3225-3243.

Padhiyar, B. M., Panickar, B. K., Chandaragi, M. K., Soni, K. K., & Prajapati, A. R. (2022). Physio-chemical basis of resistance to pulse beetle (*Callosobruchus maculatus* Fabricius) in black gram. *Journal of Experimental Zoology India*, *26*(1), 467–474. https://doi.org/10.51470/jez.2023.26.1.467

Pataczek, L., Zahir, Z. A., Ahmad, M., Rani, S., Nair, R., Schafleitner, R., Cadisch, G., & Hilger, T. (2018). Beans with Benefits—The Role of mung beans (<i>Vigna radiate</i>) in a Changing Environment. *American Journal of Plant Sciences*, *09*(07), 1577–1600. https://doi.org/10.4236/ajps.2018.97115

Patel, S., Patel, D., & Mehta, S. (2018). Quantification and analysis of carbohydrates in various food sources using the phenol-sulfuric acid method. *Food Chemistry, 276*, 554-558.

Ponnusamy, D., Pratap, A., Singh, S. K., & Gupta, S. (2014). Evaluation of screening methods for bruchid beetle (*Callosobruchus chinensis*) resistance in Greengram (*Vigna radiata*) and Blackgram (*Vigna mungo*) genotypes and influence of seed physical characteristics on its infestation. *Vegetos*, *27*(1), 60–67. https://doi.org/10.5958/j.2229-4473.27.1.011

Poornasundari, B., & Thilagavathy, D. (2015). Pest Control in mung beans Seeds (*Vigna radiata*) by Using Plant Extracts. *International Letters of Natural Sciences*, *40*, 38–40. https://doi.org/10.18052/www.scipress.com/ilns.40.38

Prajapati, A. R., Panickar, B. K., Chandaragi, M. K., Padhiyar, B. M., & Dave, G. S. (2022). Biochemical basis of resistance in different pulses against pulse beetle *(Callosobruchus maculatus* Fabricius). *JOURNAL OF EXPERIMENTAL ZOOLOGY INDIA*, *26*(1), 423–430. https://doi.org/10.51470/jez.2023.26.1.423.

Prakash, D., Singh, B., & Upadhyay, G. (2016). Tannin content variability in legumes under different environmental conditions. Food Chemistry, 197, 47-54. doi:10.1016/j.foodchem.2015.10.143

Pretorius, C. J., Tugizimana, F., Steenkamp, P. A., Piater, L. A., & Dubery, I. A. (2021). Metabolomics for biomarker discovery: Key signatory metabolic profiles for the identification and discrimination of oat cultivars. *Metabolites*, *11*(3). https://doi.org/10.3390/metabo11030165

Profile, S. E. E. (2020). *Biochemical Factors Associated With Resistance Against Pulse Biochemical Factors Associated With Resistance Against Pulse Beetle , Callosobruchus Chinensis (L .) in Stored Chick pea Genotypes*. *23*(August),

1937–1942.

Raja, S., Srinivasan, A., & Sivaraj, C. (2021). Comparative study on the flavonoid content and antioxidant activity of mung bean (Vigna radiata) extracts. *Journal of Food Measurement and Characterization, 15*(1), 387-394.

Rao, P., Kumar, V., & Sharma, N. (2018). Variation in protein content among legume cultivars under different environmental conditions. Journal of Plant Nutrition and Soil Science, 181(4), 592-601. doi:10.1002/jpln.201800067

Resources, P. G., Breeding, P., Centre, A., Improvement, C., & Faculty, P. P. (2017). A Study of Bruchid Resistance and Its Inheritance in Malawian Dry Bean Germplasm Geoffrey Acrey Duncan Kananji. *Africa*, *May*.

Rodrigues, I. M., Coelho, J. F., & Carvalho, M. G. (2022). Protein extraction from mung beans and its applications in the food industry. *Trends in Food Science & Technology*, *66*, 69-79.

Saito, K., & Matsuda, F. (2017). Metabolomics for functional genomics, systems biology, and biotechnology. Annual Review of Plant Biology, 61, 463-489.

Sciences, A. (2018). *Journal of Agriculture and Technology*. *5*, 1–51.

Singh, R., Bhatt, B., & Negi, P. (2017). Assessment of carbohydrate profiles in various legume species. Carbohydrate Research, 448, 1-8. doi:10.1016/j.carres.2017.05.007.

Smith, J. P., & Thompson, R. L. (2020). Spectrophotometric Analysis of Tannins in Food Products. *Food Chemistry*, 319, 126546. doi:10.1016/j.foodchem.2020.126546.

Somta, C., Somta, P., Tomooka, N., Ooi, P. A. C., Vaughan, D. A., & Srinives, P. (2008). Characterization of new sources of mung beans (*Vigna radiata* (L.) Wilczek) resistance to bruchids, *Callosobruchus spp.* (Coleoptera: Bruchidae). *Journal of Stored Products Research*, *44*(4), 316–321. https://doi.org/10.1016/j.jspr.2008.04.002

Somta, P., Ammaranan, C., Ooi, P. A. C., & Srinives, P. (2007). Inheritance of seed resistance to bruchids in cultivated mung beans (*Vigna radiata,* L. Wilczek). *Euphytica*, *155*(1–2), 47–55. https://doi.org/10.1007/s10681-006-9299-9

Tohge, T., & Fernie, A. R. (2010). Combining genetic diversity, informatics, and metabolomics to facilitate annotation of plant gene function. Nature Protocols, 5(6), 1210-1227.

Venugopal, K. J., Janarthanan, S., & Ignacimuthu, S. (2000). Resistance of legume seeds to the bruchid, *Callosobruchus maculatus:* Metabolites relationship. *Indian Journal of Experimental Biology*, *38*(5), 471–476.

Wangui, J. (2021). Modelling mung beans Production in Kenya Under the Current and Future Climates. *Thesis*, 6.

War, A. R., Murugesan, S., Boddepalli, V. N., Srinivasan, R., & Nair, R. M. (2017). Mechanism of resistance in mung beans [*Vigna radiata* (L.) R. Wilczek var. radiata] to bruchids, *Callosobruchus spp.* (Coleoptera: Bruchidae). *Frontiers in Plant Science*, *8*(June), 1–11. https://doi.org/10.3389/fpls.2017.01031

Yewale, P. S., Kadam, U. K., & Us, D. (2020). *Evaluation of bio-chemical constituents in mung beans associated with resistance to Callosobruchus maculatus during storage*. *9*(5), 2145–2148.

Yusnawan, E., Sutrisno, S., & Kristiono, A. (2019). Total Phenolic Content and Antioxidant Activity of mung beans Seed Cultivars from Optimized Extraction Treatment. *Buletin Palawija*, *17*(1), 1. https://doi.org/10.21082/bulpa.v17n1.2019.p1-9

Zhang, W., Li, J., & Wang, X. (2019). Phenolic content variation in leguminous plants under biotic and abiotic stresses. Journal of Agricultural and Food Chemistry, 67(30), 8322-8330. doi:10.1021/acs.jafc.9b02207.

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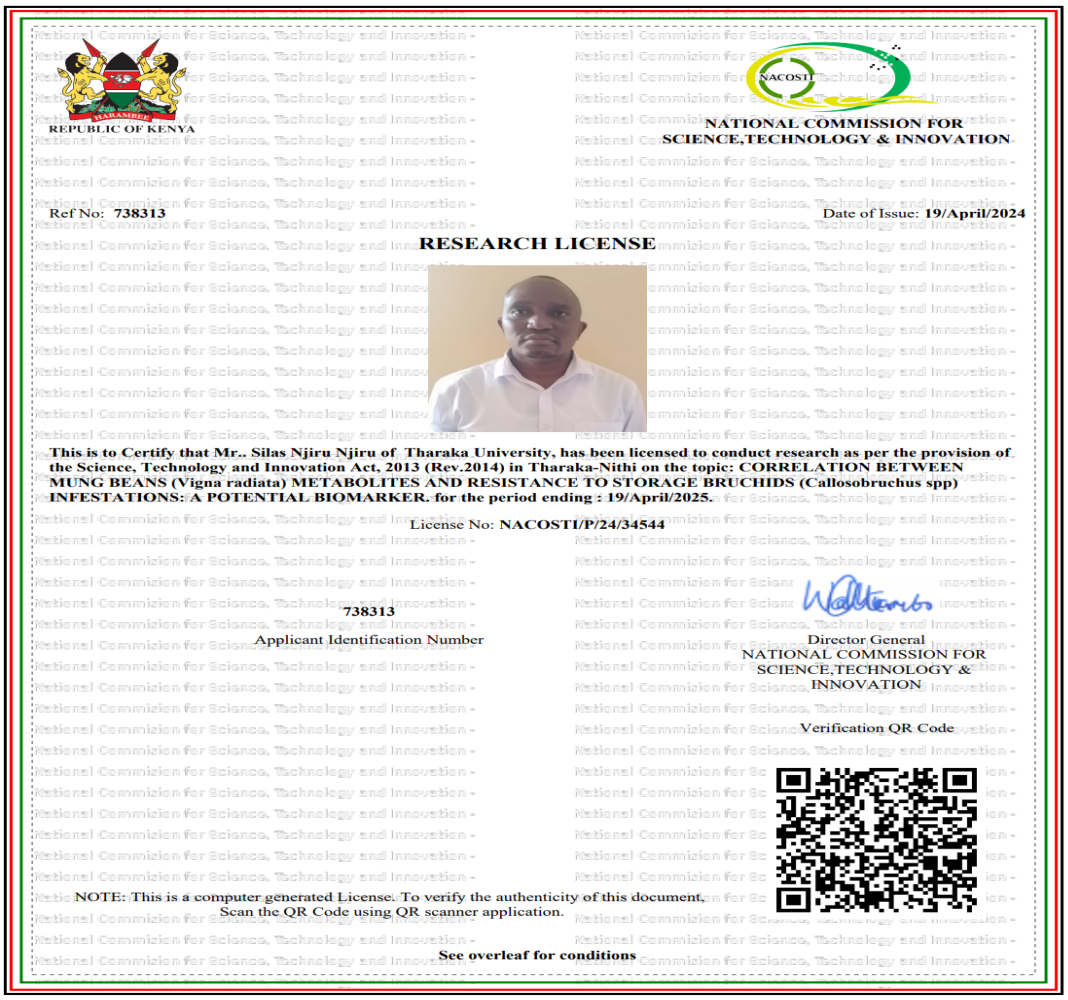
APPENDICIES

Appendix 1: NACOSTI Permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **738313**

Date of Issue: **19/April/2024**

RESEARCH LICENSE

This is to Certify that Mr.. Silas Njiru Njiru of Tharaka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Tharaka-Nithi on the topic: CORRELATION BETWEEN MUNG BEANS (Vigna radiata) METABOLITES AND RESISTANCE TO STORAGE BRUCHIDS (Callosobruchus spp) INFESTATIONS: A POTENTIAL BIOMARKER. for the period ending : 19/April/2025.

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See overleaf for conditions

Appendix 2: Ethical Clearance

THARAKA UNIVERSITY

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INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

20th February, 2024.

REF: TUNISERC/NSEC/M008
Dear, Silas Njiru Mwira

RE: Correlation between Mung Beans (*Vigna Radiata*) Metabolites and Resistance to Storage Bruchids (*Callosobruchus Spp*) Infestations: A Potential Biomarker.

This is to inform you that ***Tharaka University ISERC*** has reviewed and approved your above research proposal. Your application approval number is ***ISERC04023.*** The approval period is ***20th February 2024 –20th February, 2025.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Tharaka University ISERC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Tharaka University ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Tharaka University ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***Tharaka University ISERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Dr. Fidelis Ngugi
Chair, ISERC Tharaka University

Appendix 3: Introductory Letter

THARAKA UNIVERSITY

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OFFICE OF THE DIRECTOR
BOARD OF POSTGRADUATE STUDIES

REF: TUN/BPGS/PL/03/24 **19th March, 2024**

To Whom It May Concern

Dear Sir/Madam,

RE: SILAS NJIRU MWIRA REGISTRATION NUMBER SMT17/05346/21

Mr. Silas Njiru Mwira is a postgraduate student at Tharaka University undertaking a degree in Master of Science Degree in **Biochemistry**. The student has completed his coursework and is expected to proceed for collection of data after successfully defending his proposal at the faculty level. The title of the study is ***"Correlation Between MungBeans (Vigna radiata) Metabolites and Resistance to Storage Bruchids (Callosobruchus spp) Infestations: A Potential Biomarker."*** The proposed study will be carried out in **Tharaka University.**
Any assistance accorded to him will be highly appreciated.

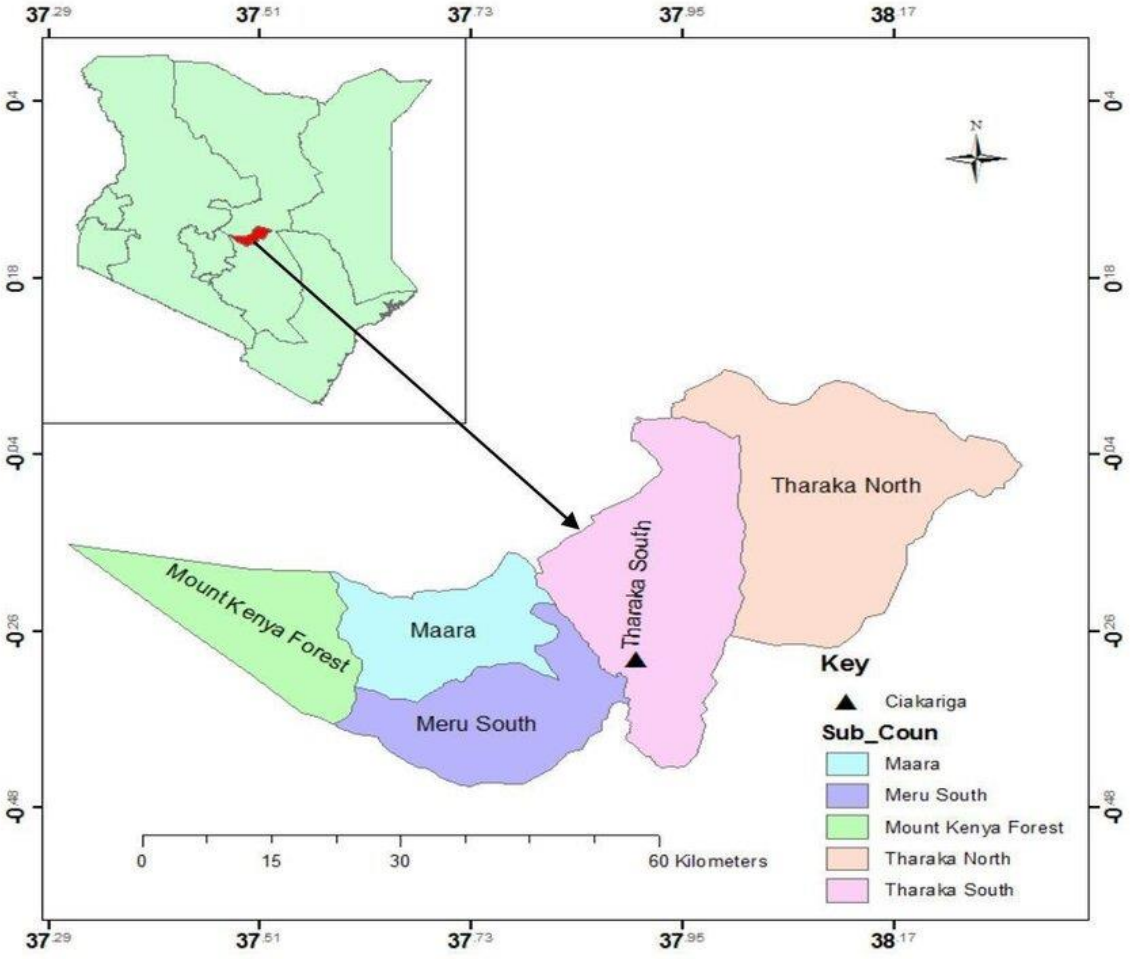
Thank you in advance.

Yours faithfully,

THARAKA UNIVERSITY BOARD OF POSTGRADUATE STUDIES AND RESEARCH
19 MAR 2024
DIRECTOR
P.O Box 193-60215, MARIMANTI

Dr. Marciano Mutiga, Ph.D.
Director, Board of Postgraduate Studies.

Appendix 4: Tharaka Nithi County Map



Appendix 5: Machackos County Map