DETERMINATION OF INSECTICIDAL AND SEED PROTECTIVE PROPERTIES OF PAWPAW EXTRACTS AGAINST BEAN WEEVILS (*Acanthoscelides obtectus*) IN STORAGE

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Determination of Insecticidal and Seed Protective Properties of Pawpaw Extracts against Bean weevils (*Acanthoscelides obtectus*) in storage

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DEDICATION

I dedicate this research to the Almighty God who gave me the energy and wisdom to persue this study. Special dedication to my father Mr. Kermundu Samson who was with me in prayers and encouraged me through the tough times I went through in the study.

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ACRONYMS

NaCRRI	National Crops Resources Research Institute
NABE 4	Namulonge Beans 4
NABE 6	Namulonge Beans 6
EOA	Ecological Organic Agriculture
SM	Secondary Metabolites
QE	Quercetin Equivalents
GAE	Galic Acid Equivalent
TAE	Tannic Acid Equivalent
US EPAP	United States Environment Protection Agency Pesticides

ABSTRACT

The study was an experiment carried out at the Nutrition and Bio-analytical laboratory at NaCRRI, Uganda from November 2016 to October 2017. The aim of the study was to investigate the efficacy of pawpaw leaf powder and liquid extracts in seed protection against bean weevils in storage over a time period. The specific objectives were; to determine the concentration of the secondary metabolites in pawpaw liquid extracts and pawpaw leaf powder; determine the effectiveness of pawpaw leaf powder, water and ethanol extracts in the control of bean weevils and to establish the effect of pawpaw leaf powder on seed germinability. Methods used in the study were; phyto-chemical screening of the secondary metabolites such as total tannins, total phenolics, total flavonoids, total alkaloids and total anti-oxidant activity. Pawpaw leaf extracts were prepared in water and ethanol at concentrations of 5%, 10%, 15% and 0% (control) where no treatment was applied. Pawpaw leaf powder was also applied at varying rates of 5%, 10% and 15% (w/w of sample) to bean seeds of NABE 6 cultivar. Pyto-chemical screening results revealed that the highest concentration of secondary metabolites in extracts was for total anti-oxidants at 5.5% in the pawpaw leaf accession M-5 which was significantly higher ($P<0.05$) than in other accessions. Powder treatment had the highest concentration of total anti-oxidants at 11.6% in M-2 followed by total tannins at 5.2%. Generally, pawpaw leaf powder had a higher concentration of secondary metabolites in the different pawpaw leaf accessions as compared to the liquid extracts. Pawpaw leaf powder provided the most effective control measure of bean weevils in bean storage. Pawpaw leaf powder significantly ($P<0.05$) reduced on the number of damaged seeds, the percentage weight loss of bean seeds, insect mortality, F1 progeny emergence. The same trends were observed from week one up to week 12 of the experimental set up. Pawpaw water extracts had the least effect in the control of weevils. Pawpaw leaf ethanol extract also had a significantly higher ($P<0.05$) mortality rate at 15% concentration as compared to other treatment levels. Pawpaw leaf powder did not significantly reduce on the germination percentage of the different bean varieties ($P<0.05$). It was discovered that the effect of pawpaw leaf powder water and ethanol extract in the control of bean weevil were doze dependent with 10% being the most ideal for powder extracts. The effectiveness of pawpaw leaf extracts in the control of bean weevils is attributed to the higher concentration of the different secondary metabolites in the leaves. The study demonstrated that pawpaw leaves could act as cost effective bio pesticides with potential to substitute synthetic insecticides for the control of bean weevils in stored beans.

CHAPTER ONE

INTRODUCTION

1.1 Botanical characteristics of pawpaw

Papaya, a tropical plant believed to have originated in Southern Mexico and Central-America, is now cultivated in tropical and sub-tropical regions worldwide (Merina, 2015). Papaya is a large, herbaceous like plant, with a single stem growing from 5 to 20 m (Anjum *et al*., 2013) and an extensive rooting system (Orwa *et al*., 2009). Papaya normally has a mono-axial stem without branching but it forms multi-stems when damaged. The tree is usually unbranched, unless lopped. Pawpaw leaves are spirally arranged, clustered near the apex of trunk; petiole up to 1 m long, hollow, greenish or purplish-green; lamina orbicular, glabrous, prominently veined; lobes deeply and broadly toothed (Orwa *et al*., 2009). The leaves are large, 50–70 cm in diameter, deeply palmately lobed, with seven lobes (Haslam, 2007). New leaves emerge from the apex and old leaves senescence and fall (Wang *et al*., 2014).

The flowers appear on the axils of the leaves, maturing into large fruits. Fruit is large, oblong and weighs from 3-5 kg (Silva *et al*., 2007). Pawpaw plant has a complicated means of reproduction (Silva *et al*., 2007). The plants are either male or female and sometimes hermaphrodite and are self-pollinated. Papaya flowers are born in inflorescences which appear in the axils of leaves. Female flowers are held close against the stem as single flowers or in cluster of 2-3 flowers with large functional pistils, no stamens and ovoid-shaped ovary. Male flowers are smaller and more numerous (Karunamoorthi *et al.*, 2014).

Fruits are large, cylindrical and are ripe when they feel soft and the skin has attained orange-red, yellow-green and yellow-amber, with rich orange pulp (Silva *et al*., 2007). In some varieties, the fruits are usually picked in a mature green state and allowed to ripen (Wolters, 2009). When ripe, the flesh is sweet and juicy (Singh and Rao, 2011). The fruit superficially resembles a melon puriform, with its oval and elongated shape (Cavalcante *et al*., 2012).The fruits range in size from 7-30 cm, with numerous seeds which are small, grey-black (about 5 mm), round, covered with gelatinous (Otsuki *et al*., 2010). Small latex vessels extend throughout the tree and are particularly abundant in fruits that have reached full size but have not yet begun to ripen (Ravi, 2008).

1.2 Socio-economic importance of pawpaw

Every part of pawpaw is of economic value and its uses range from nutritional to medicinal (Ezugwu, 2008; Iroka *et al*., 2016). Pawpaw is cultivated for its edible ripe fruit; its juice is a popular beverage, and its young leaves, shoots, and fruits are cooked as a vegetable (Ikram *et al.,* 2015:Iroka *et al*., 2016).The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like; papain and chymopapain which have anti-viral, anti-fungal and anti-bacterial properties (Bhadane *et al*., 2014). The fruit is not just delicious and healthy, but whole plant parts, fruits, roots, bark, peel, seeds and pulp are also known to have medicinal and insecticidal properties (Iwu, 2014).

The fruits are a source of flavouring used in candies, jellies, preservatives, and ice cream (Johari and Kawatra, 2016).They contain papain, a proteolytic enzyme, which has a wealth of industrial uses. It has milk-clotting (rennet) and protein-digesting properties used in meat tenderizing, pharmaceuticals, beauty products, and cosmetics (Nitsawang *et al.,* 2006). Besides, it has been used in brewing and wine making, and in the textile and tanning industries. Nearly, 80% of American beer is treated with papain, which digests the precipitable protein fragments, and causes the beer to remain clear upon cooling (Mamboya, 2012). Cosmetically, papain is used in some dentifrices, shampoos, and facial creams (Anibijuwon and Udeze, 2009; Edi, 2012).

The cultivation of papaya is important in the tropics because it is a source of income to the farmer within a relatively short time (Chaves-Bedoya and Nuñez, 2007, Chan, 2009). Pawpaw can be used for treatment of numerous diseases like; warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expels worms and stimulate reproductive organs (Aravind *et al*., 2013).The leaves are also used as traditional medicines for treatment of asthma, colic, fever, beriberi, malaria, dengue fever, cancer and it has a high antioxidant and anti-inflammatory activity (Patil *et al.*, 2014). It is relatively cheap to prepare bio-insecticides from pawpaw leaves because the materials are within the homesteads reach, hence economically reducing the cost of purchasing insecticides (Chaves-Bedoya and Nuñez, 2007).

1.3 Bio-pesticides as a pest control strategy

Bio-pesticides are naturally occurring substances derived from animals, plants and micro-organisms like *Bacillus thuringenesis* and *Trichoderma* and are applied in similar manner like chemical pesticides. They are effective in the control of pests and they are ecologically friendly in nature (Gupta and Dikshit, 2010; Singh *et al*., 2017). Based on the natural resources from which they are isolated, bio-pesticides are classified as microbial pesticides, botanical pesticides and zooid pesticides (Leng *et al.,* 2011; Glare *et al*., 2012; Zhong *et al.,* 2016). The use of natural products derived from metabolic activity of plants may constitute a new avenue of pest control (Flint and Van den Bosch, 2012; Mining *et al.,* 2014). Protection of stored products generally involves mixing grains with protectants made up of plant materials with or without minerals (Obeng-Ofori, 2010). The use of oils in stored products as pest control is also an ancient practice and it can provide seed protection against weevils for a period of up to six months (Pangnakorn *et al*., 2011; Lashari *et al*., 2013).

The precise strategy used by different communities varies from region to region and appears to depend partly on the type and efficiency of suitable flora available in different locations (Obeng-Ofori, 2010; Oerke *et al.,* 2012). In ancient cultures and in different parts of the world, there exist empirical knowledge of plants for the control of storage pests for thousands of years approximately (1500BC). Pesticidal plants have been used for more than 150 years (El-Wakeil, 2013); the Egyptian and Indian farmers used to mix the stored grains with fire ashes (Chandaliya, 2014).The ancient Romans used false hellebore (*Veratrum album*) as a rodenticide while pyrethrum was used as an insecticide in Persia and China (Costa, 1987; Talukder, 2006a).Indian farmers used neem leaves and seeds for the control of stored grain pests (Ahmed and Grainge, 1986; Chomchalow, 2003). In some south Asian countries, food grains such as rice or wheat are traditionally stored by mixing with 2% turmeric powder (Jilani and Su, 1983; Ahuja *et al.,* 2015).

In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Tripathi *et al*., 2009). In northern Cameroon, cowpeas are traditionally mixed with sieved ash after threshing and the mixture put into mud granaries or clay jars (Wolfson *et al.,* 1991). In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants (Powel, 1989). In Rwanda, farmers store edible beans in a traditional closed structure (imboho) and whole leaves of basil *(Ocimum canum)* are usually added to the stored foodstuff to prevent insect damage within these structures (Weaver *et al.,* 1991). In addition

to the pesticidal plants, farmers in Tanzania have been using other products such as cow's urine, cow dung, and ashes (Mihale *et al*., 2009).

In Uganda, most farmers have opted for traditional preservation and storage means to curb down pest infestation which include; use of inert materials like ash, sand, lime and use of insecticidal plants (Fields *et al.*, 2001:Al-Fuhaid, 2018). Around the Lake Victoria basin in Uganda, small holder farmers have used marigold (*Mexican marigold*), tobacco, cypress, tephrosia , neem, banana leaves, moringa, tree marigold, tick berry (*Lantana camara)*, African soapberry, bitter leaf, eucalyptus among others to control insect pests (Mugisha-Kamatenesi *et al*., 2008)

1.4 Benefits of bio-pesticides in the control of storage pests

Botanical control is among the best storage pest control methods of grain pests since it is bio-degradable, environmentally friendly and does not leave toxic residues in the produce (Onoja, 2015). They can mitigate environmental pollution caused by chemical pesticide residues and promote sustainable development of agriculture (Mazid *et al.*, 2011). Most of these plant products are cheap, readily available, edible and ecologically safer means of controlling insect pest infestations of stored cereal and grains especially in the tropics (Phillips and Throne, 2010; Ileke *et al.*, 2012). Bio-pesticides can make more contribution for humans to fight against diseases, insects and other agricultural pests and can be the focus of the pesticide industry in future (Rodgers, 1993; Abhilash and Singh, 2009).

Bio-pesticides are used to control rather than to eradicate pests, often incorporating a delay factor. They are more selective than chemical pesticides (Marrone, 2014). Most bio-pesticides have an advantage of higher selectivity and are relatively safe, inexpensive, and are readily available in many areas of the world (Leng *et al.*, 2011). Botanical extracts kill and repel pests, affect insect growth and development, have anti-feedant, arrestant, anti-fungal, anti-viral, and anti-bacterial properties against pathogens (Isman, 2008; Senthil-Nathan, 2015). Botanicals may not kill an insect for hours or days, but they act very quickly to stop its feeding. The botanical insecticide can work by ingestion, through contact, as a deterrent and by disrupting developmental processes (Achakzai *et al*., 2009). Contact poisoning is through the solution or powder killing the larvae through their skin and other tissues; a deterrent through the insecticide preventing the larvae and insects from feeding in order to starve them (Sarwar, 2015b).

Although bio-pesticides are slow to act, they supply environment protection and do not harm the natural environment (Leng *et al.*, 2011). Many plant products break down naturally outdoors and do not cause any long term toxic effects. Botanicals are generally short-lived in the environment, as these are broken down rapidly in the presence of light and air, thus they do not provide pest control for very long time or perhaps several days (Ahmad *et al*., 2011; Sarwar, 2012). On the other hand, the fast breakdown of botanical insecticides into less toxic or nontoxic compounds poses less risk to non-target organisms, hence environmentally compatible (Sarwar, 2015). Synthesis of bio-pesticides by using plants and their extracts is a very cost effective process than microbial bio-pesticides (Sarwar, 2015). Phyto-chemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safer and inexpensive since they are readily got within the natural environment (Mohankumar *et al*., 2016).

1.5 The bean weevils (*Acanthoscelides obtectus*)

The bean bruchid, *Acanthoscelides obtectus* is among the most important and widespread storage pests in all major dry common bean (*Phaseolus vulgaris* L.) growing regions worldwide (Alvarez *et al*., 2005; Govindarajan *et al*., 2013). The adult body is 2-5 mm in length, light to dark brown to black in color. The elytra, which do not completely cover the abdomen, are with longitudinal spots, a golden pubescence, and a red posterior border. The head is bent forward. The larvae are white with a brown head, setae and three pairs of reduced legs (Minney, 1990; Leroi, 2013). Adults of *A. obtectus* do not feed, are weak fliers, and feign death when disturbed; only the larvae cause damage (Baier and Webster, 1992; Abate and Ampofo, 1996). Freshly emerged adults copulate at any time within 24 hours after their emergence. During copulation, males normally raise their fore and middle legs to hold the females; copulation lasts for 4-5 minutes. The bean weevil starts to infest beans in the field and continues to develop during storage; its life-cycle is thus holometabolous (Agah and Leroi, 2013; Thakur *et al.*, 2014).

Oviposition starts within few hours after mating. The freshly emerged female lays the maximum number of eggs on the first and second day of oviposition and then oviposition decreases subsequently. The adult female of ***A. obtectus*** lays an average of 60 eggs in her lifetime (Tabu *et al*., 2012; Leroi, 2013). The female glues only a small number of eggs to the seeds, while the majority of the eggs are released freely among the seeds and the first instar larva burrows into the bean (Paul, 2009). Freshly laid eggs are milky white and ellipsoidal in shape. Oviposition lasts for 7-10 days. Larval instars are completed inside the

host seeds and all the larval instars are voracious feeders. The last larval instar prepares an emergence window before moulting to pupal stage. Larval development takes 14-20 days. The pupal stage also completes development inside the host seeds and pupal development takes 14-17 days. The total life cycle takes 44-54 days (Ramírez, 2015; Ebinu *et al*., 2016).

These beetles cause serious economic loss of legume commodities both in fields and stores every year (Thakur and Sharma, 2014) .The bruchids cause extensive grain weight and quality losses through their feeding (Okwute *et al*., 2009) and product alterations such as reduction of nutritional and aesthetic value; alteration of cooking characteristics (Mutungi *et al.,* 2007). They lead to reduction in viability of bruchid-damaged seeds (Jones *et al*., 2011). The adult beetles do not cause damage to the pulse grains by feeding but they mate and oviposit on grains and contaminate with their excreta. The larva is solely responsible for the grain damage. The larvae destroy seeds by feeding inside partially or completely and make them unfit for human consumption (Hossain *et al*., 2014).

1.6 Control of bean weevils

Beans are preserved against bean weevils through a number of methods. The hermetic storage is used to preserve beans against damage by beans weevils and it has been used since ancient times in an attempt to preserve grains (Freitas *et al*., 2016). The technology is based on the creation of storage environments that are unfavourable to pests by means of one of the following methods: vacuum hermetic fumigation, gas hermetic fumigation, or bio-generated modified atmosphere (Navarro, 2012). Once the product is wrapped and the bag is closed, the level of oxygen within the bag drastically falls as a result of grain, insect, and fungus respiration. On the other hand, the level of carbon-dioxide increases (Hell *et al.,* 2014; Njoroge *et al.,* 2014). Consequently, insects stop feeding, become inactive and eventually die of asphyxiation or desiccation (Murdock *et al.,* 2012). Bruchid control is also done through the use of plastic bags that suppress air circulation from them (Baoua *et al*., 2014).

Synthetic pesticides are also used to control storage pests and these include; pyrethroids, organo-phosphates, and aluminum phosphide fumigant (Corrêa *et al*., 2011; Pimentel *et al.,* 2012). This is suitable for seeds for planting only. They cannot be re-infested once treated and the most used insecticides include; Malathion which kills 85%-99 % of adult weevils in the first 24 hours after application. Lindane dust gives longer protection but is toxic to humans and must only be used for seeds for planting (Pavela, 2010). Another possible approach to sustainable bruchid management is the exploitation of host-plant resistance; an

effective, economical, and environmentally friendly method of pest control (Sharma *et al*., 2007; Shankar and Abrol, 2012). Weevils are controlled mechanically by mixing the stored beans with ashes to fill the spaces between seeds making it hard for bruchids to infest at the optimal rate of 20%ash to the weight of bean seeds. This method only works before infestation. Sand, lime, or other fillers can be substituted for ash (Prakash *et al*., 2016).

Control of bean weevils may also be done by coating the bean seeds with edible vegetable oils like soybean, sesame oil which is relatively effective permitting storage for at least 6 months without insect damage. It also makes the seeds look more attractive. The oil penetrates bruchid eggs and destroys them and also reduces on the oviposition and increases adult mortality (Mushobozy *et al*., 2009; Omulo *et al*., 2017). Shelling and cleaning of beans immediately after harvest eliminates eggs and insects of common bean weevils coming from the field to the pods (Reddy, 2009; Sarwar, 2015).Use of wood vinegars can also be effective in controlling bean weevils in storage (Pangnakorn *et al*., 2011).

1.7 Statement of the problem

Post-harvest pests are known to cause severe losses of beans in particular the bean weevil, *Acanthoscelides obtectus*, necessitating immediate and long term control measures (Mining *et al*., 2014; Mutungi *et al.,* 2014). Common bean weevils are important pests of common beans (*Phaseolus vulgaris)* and are responsible for the heavy losses of bean cultivars both in fields and in stores (Thakur, 2012). Losses in dry weight of bean seeds due to bruchid damage have been estimated at about 70%, especially where post-harvest management is poor (Mulungu *et al*., 2007).

Conventional pesticides have been used for decades to protect stored pulses from pests. However, their harmful environmental impacts and emergence of pest resistance due to the increased pesticide use, their deleterious effect to non-target organisms and toxic residues in food grains have triggered a search for eco-friendly, bio-degradable and potential bio-pesticides (Onoja, 2015). Therefore, there is an utmost need to implement safe, eco-friendly and agro-ecological alternatives to protect stored grain products and to restrict the use of toxic chemicals globally. The aim of this study was to determine the insecticidal and seed protective properties of pawpaw leaf liquid and powder extracts in the control of weevils during storage.

1.8 Objectives of the study

1.8.1 Main objectives

The overall objective of this study was to establish the potential of pawpaw leaf powder and liquid extracts as bio-pesticides against the bean weevils in storage.

1.8.2 Specific objectives

I. To determine the concentration of selected secondary metabolites in pawpaw leaf powder and liquid extracts with bio-pesticide properties.

II. To determine the effectiveness of pawpaw leaf powder and liquid extracts as bio-pesticides against bean weevils over specific time period.

III. To establish the effect of pawpaw leaf powders on the seed germination of beans after storage.

1.8.3 Research hypotheses

i. Pawpaw leaf powder and liquid extracts contain varying concentration of secondary metabolites that have insecticidal properties.

ii. Pawpaw leaf powder and liquid extracts are effective in the control of bean weevils over specific time period.

iii. Pawpaw leaf powder has no effect on bean seed germinability in storage.

1.9 Significance of the study

This study provides solutions which are environmentally sound to post harvest storage losses of pulses that have been rampant for a long time in Uganda and other developing countries. The information on the concentration of secondary metabolites, the most effective application rates and timing of applications of pawpaw leaf powder and liquid extracts is very crucial for commercial agriculture companies with interests in the industrial production of bio-pesticides. The knowledge of the most effective concentration of secondary metabolites in pawpaw leaf powders and liquid extracts is important to the farmers as it will help to minimize the use of inorganic pesticides that have adverse effects to humans as they consume the stored beans and to the environment during planting time.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Secondary metabolites are substances which are produced by plants as defense chemicals. They are organic molecules that are not involved in the normal growth and development of an organism. Metabolites are considered as end products of primary metabolism and not involved in metabolic activity (Ruby, 2015; Kumar *et al*., 2015). Secondary metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (Anurag *et al*., 2014; Kumar *et al*., 2015). The secondary metabolites are naturally produced from plants and posses biological properties like anti-microbial, anti-fungal and pesticidal properties (Sher, 2009; Bodaiah, 2015). Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivorous predators and other environmental factors (Sarwar, 2015a).

Tissues of higher plants contain arrays of bio-chemicals known as "secondary plant chemicals" (allelo-chemicals), which are defensive in function. Plants contain several groups of phyto-chemicals such as; alkaloids, steroids, terpenoids, essential oils, phenolics among others which have been reported previously for their insecticidal activities (Merina, 2015). Plants also contain; saponins, resins, various organic acids and other compounds (Teoh, 2016; Singh and Rao, 2011). Many plant allelo-chemicals including; azadirachtin, nicotine, pyrethrins and rotenoids have been developed as commercial insecticides (Regnault, 2012). Plant secondary metabolites are effective against pest and disease agents because they are either analogues of certain vital components of the cellular signalling system, or can interfere with vital enzymes and block metabolic pathways (Makkar *et al*., 2007). The mode of action for plant derivatives used for insect pest management is as; contact poisons, ingestion or stomach poisons, feeding deterrence, repellents and confusants, which paralyze nerve activity, respiratory arrest, and act on the central and peripheral nervous system leading to convulsions and finally death of the insect victims (Rahuman *et al*., 2008; Silva, 2013).

2.2 The secondary metabolites in plant extracts

Plant derived extracts and phyto-chemicals have long been a subject of research in an effort to develop alternatives to conventional pesticides which have reduced health and environmental impact (Akhtar *et al*., 2008). It is well known that secondary plant metabolites act as; kairomones, allomones, stimulants or deterents of feeding and oviposition, and as anti-feedants, help in reduction of fecundity, insecticides and insect hormone mimic (Talukder, 2006b; Adeyemi and Mohammed, 2014). The production and accumulation of a wide variety of organic chemicals is one of the major mechanisms by which plants defend themselves against herbivory, and attack by microbial pathogens and invertebrate pests (Rahuman *et al*., 2008).

Phenolics are organic compounds that are characterised by the presence of a hydroxyl group, attached to a benzene ring or to other complex aromatic ring structures. Phenols with more than one hydroxyl group per aromatic ring are known as polyhydric phenols (Golawska *et al*., 2008; Oleg, 2014). Plant phenols represent a structurally diverse and widely distributed class of allelo-chemicals. Phenols are found in the bark and more abundant in the old leaves than older stems and older leaves of plants. Plant phenolics, in particular phenolic acids occur in vegetables, fruits, nuts, seeds, roots, leaves and barks (Kamath *et al.*, 2015).The protection afforded by phenols against plant pathogens and herbivores is a primary factor in selection during plant evolution (Akhtar *et al*., 2008). The ecological roles of phenolic compounds include constitutive and induced roles in toxicity and feeding deterrence in insects (Napal *et al.,* 2010). Phenolic compounds have also been reported to exert a synergistic effect on aphids' behaviour, physiology and metabolism and as a result reduce the aphid population on the resistant plants (Golawska *et al*., 2008).

Flavonoids are polyphenolic compounds that are essentially a class of water-soluble pigments from plants. Flavonoids are a group of natural compounds with variable phenolic structures and are found in plants. Chemically, flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyrane ring (Pandey, 2013). They can be divided into a variety of classes such as flavones for example; flavone, apigenin, and luteolin ; flavonols such as; quercetin, kaempferol, myricetin, and fisetin;, flavanones such as; flavanone, hesperetin, and naringenin (Pandey, 2013; Panche, 2016).

Flavonoids are naturally occurring substances in plants and they commonly occur in foliage, bark, sapwood and heartwood in trees and are abundant in fruits and vegetables (Momina Hayat, 2017). The main properties reported for flavonoids include; anti-oxidant, anti-inflammatory, anti-histaminic, and antiviral (Anand, 2016). Flavonoids can act as repellents or deterrents against insects (Nuessly, 2007). Flavonoids can modulate the feeding behaviour of insects (Mierziak, 2014). They also have an adverse effect on the feeding, development and survival rates of beetles and can cause a high mortality rate in adult insects (Deepak *et al.*, 2012).

Alkaloids contain Nitrogen heterocycles, and are mainly present in plants as salts of carboxylic acids (such as citric, lactic, oxalic, acetic, malic and tartaric, fumaric and benzoic acids). Alkaloids are distinguished on the basis of a structural similarity like; indole alkaloids or a common precursor like benzy-lisoquinoline, tropane, pyrrolizidine, or purine alkaloids (Sayeed, 2013). Alkaloids have a wide variety of chemical structures like monocyclic, dicyclic, tricyclic, tetracyclic, and more complex cage structures (Rasmana *et al.,* 2015).

Alkaloids are generally present in higher concentrations in barks, seeds, roots, stems of plants and leaves than in wood of plants (Achakzai, 2009). Generally young plant parts contained greater level of alkaloids as compared to old parts. Alkaloids and amines often affect neuro-receptors as agonists or antagonists and modulate other steps in the signal transduction like ion channels and enzymes. This is because alkaloids are derived from the same amino acid precursors as neuro-transmitters, and their structures often mimic those of neuro-transmitters. Furthermore, alkaloids may affect the function of ion channels by inhibiting neurotransmitter-degrading enzymes (such as acetyl-cholinesterase, monoamine oxidase) or by modulating enzymes involved in signal transduction (such as adenyl-cyclase, phosphodiesterase, protein kinase, phospholipase) (Shields, 2008; Hayatie *et al*., 2015).

Alkaloids are responsible for larval mortality of many pests and this may be due to the effect of alkaloids that can affect protein kinase that is involved in signal transduction and development process of most cells and tissues (Lvovich, 2014; Qadir, 2014). Alkaloid extracts from plants have high mortality on the adult insects (Habimana and Hakizayezu, 2014). As an insect neurotoxin, exposure to these toxins generally causes insects to experience spasms and paralysis before death. This toxic effect is attributed by various authors to the presence of some chemical compounds of the alkaloid groups (Mulungu *et al.*, 2007; Taylor, 2011)

Tannins are secondary plant metabolites defined by their ability to precipitate proteins, a property usually inherent to tannins with a molecular weight from 500–3000 g/mol (Mona *et al*., 2008, Salminen *et al.,* 2011). Their binding affinity and ability to precipitate proteins depends, in addition to the tanniń s molecular weight, also on protein size and structure, as well as on reaction conditions (pH, temperature, solvent, time) (Frazier *et al*., 2010). Tannins are multidentate ligands, binding to proteins mainly by hydrophobic interactions and hydrogen bonds (Ozdal *et al.,* 2013).

The main groups of tannins are hydrolyzable tannins and condensed tannins (Loomis, 1974; McSweeney *et al.,* 2001). Tannins from higher plants are subdivided into two classes: hydrolysable tannins and non-hydrolysable or condensed tannins (also known as proanthocyanidins (Haslam, 2007). Hydrolysable tannins are based on gallic or ellagic acid moieties, while condensed tannins are based on flavan structures (Theisen *et al*., 2014). Condensed tannins are oligomeric or polymeric flavonoids, also known as pro-anthocyanidins (Lacombe, 2012). Condensed tannins or pro-anthocyanidins consist of coupled flavan-3-ol units that can appear as isolated dimers and compounds with high polymerization grade (Kovinich *et al.,* 2012). Free or hydrolysable tannins are either gallotannins (glucose core surrounded by 5 or more galloyl ester groups) or ellagitannins (containing hexahydroxydiphenic acid).

Tannins are mainly found in buds, foliage tissues, seeds, barks, roots and sapwood. Tannins are also found in both mature and immature leaves of plants (Merina, 2015). Condensed tannins are made of catechin units (Vermerris and Nicholson, 2008). Condensed tannins have attracted interest because of their anti-oxidant and other potentially health-promoting qualities (Paredes-López *et al*., 2010, Sumbul *et al*., 2011). Condensed tannins are oligomeric or polymeric flavonoids, also known as proanthocyanidins (He *et al*., 2008; Dixon *et al*., 2013). They act as feeding deterrents against some insects such as,gypsy moth (*Lymantria dispar* (L.)),brown tail moth (*Euproctis chrysorrhoea* (L.)) and winter moth beetle (*O. Brumata)*(Achakzai *et al*., 2009). Tannins have a strong deleterious effect on phytophagous insects and affect the insect growth and development by binding to the proteins, reduce nutrient absorption efficiency, and cause midgut lesions (Sharma *et al.,* 2009; Mohamed and Abd-El Hameed 2014).

Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests (War *et al.,* 2012). They precipitate proteins non specifically (including the digestive enzymes of herbivores), by hydrogen bonding or covalent bonding of protein Amino (Achakzai *et al*., 2009) groups and also chelate the metal ions, thereby reducing their bio-availability to herbivores (Haldhar *et al*., 2015). When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. The role of tannins in plant is defense against various stresses and their induction in response to insect damage (Achakzai *et al*., 2009).

Anti-oxidants refer to substances which have the ability to neutralize free radicals by donating their own electrons (Ahmad *et al*., 2013). Antioxidant activity is found in the leaves, bark, fruits and the flowers of plants (Salazar *et al*., 2008). The major natural anti-oxidants include; vitamin E, vitamin C (ascorbic acid), polyphenols, bio-flavonoids and carotenoids. Anti-oxidants can fight against free radicals and protect against numerous diseases. They produce their action either by scavenging the reactive oxygen species or protecting the anti-oxidant defense mechanisms (Ahmad *et al*., 2013). Anti-oxidants also exist in the form of anti-oxidative flavonoids that are among the chemicals that have been reported to regulate oviposition and feeding. Naringenin, hesperetin-7-*O*-rutinoside and quercetin-3-*O*-rutinoside, along with other active compounds, stimulated oviposition in swallowtail butterfly *Papilio* on young leaves of citrus plants (Mierziak 2014). Insects have evolved a complex antioxidant mechanism to overcome the toxic effects of reactive oxygen species (Jithesh *et al*., 2006; Vallet-Gely *et al*., 2008).

2.3 Effectiveness of pawpaw leaves in the control of storage pests

Plants produce a wide variety of allelo-chemicals that are not directly involved in primary metabolic processes of growth and development (Merina , 2015). Papaya leaves contain secondary metabolites which are the most prominent active constituents responsible for the botanical insecticide properties. These are alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, reducing sugars, steroids, phenolics, and cardenolides (Khan, 2017). Carpaine, an alkaloid compound, is also found in the unripe fruit and young leaves of papaya (Yogiraj *et al*., 2014). Alkaloids are found in leaves of the pawpaw plant (Thakuria *et al.,* 2018). Phenols are mostly found in the fruits and leaves of pawpaw plants. Flavonoids are also found in the leaves and in the fruits of pawpaw plants (Merina , 2015). Antioxidants are found in young leaves, unripe fruit, ripe fruit and seeds of pawpaw plants (Maisarah *et al*., 2013).

Antioxidant flavonoids are abundant in pawpaw leaves and (Nugroho *et al*., 2017). Antioxidant flavonoids of pawpaw leaves are comprised of quercetin and kaempferol as an important flavonoid of pawpaw leaves because of its abundance and strong antioxidant activity (Nugroho *et al.,* 2017). Papaya seeds and pulp contain benzyl-glucosinolate that is hydrolyzed by the enzyme myrosinase to produce benzyl-isothiocyanate (Williams *et al.,* 2013). The pawpaw seeds contain sinigrin and caricin, both glycosides, and the enzyme myrosin which are secondary metabolites in nature (Onyema, 2013). Alkaloids form salts that can degrade the cell membrane resulting into cell damage (Gill and Tuteja, 2010). An insecticidal compound 'Papain' has been reported in papaya and it was first characterized in 1968 as the most important enzyme (Kovendan *et al.,* 2012).

Papaya leaf extracts can be used as an insecticide. They are safe for the environment and human consumption (Rasmana *et al.,* 2015). Pawpaw leaf extracts are used to control the following pests in eggplant and okra; aphids (*Aphis gossypii*), flea beetles (*Podagrica spp*), white flies (*Bemisia tabaci*), fruit borers (*Earias sp*), cotton strainers (*Dysdercus superstitiosus*), variegated grasshoppers (*Zonocerus variegatus L*.), shoot and fruit borers (*Leucinodes orbonalis Gn*) (Ojo *et al.,* 2014). Carica papaya seed extracts are used to control fall armyworm in maize crop (*Spodoptera frugiperda*) due to their toxic effect and mortality on their larvae and adult insects. This is achieved at a corrected mortality percentages between 50.0 and 73.6% in concentrations of 10, 100 and 1000ppm of the extracts (Figueroa *et al.,* 2011). Pawpaw seed extracts are effective in controlling larvae of *Spodoptera frugiperda* in water melon plant. Pawpaw leaf extracts are also effectively used in the control of white flies in okra (Zobayer and Hasan, 2013).

Root dust of papaya is used to control maize weevils, and it very effective for adult mortality (Ratnasekera and Rajapakse, 2012). Water extracts of C. papaya seeds repel various kinds of insects. The extracts obtained by pressing the papaya roots destroy the nematodes in soil and the extracts from the immature fruit controls termites effectively (Buhner, 2000). Papaya leaf extracts possess remarkable larvicidal, pupicidal, adulticidal and repellent activity against various species of vector mosquitoes (Kovendan *et al.,* 2012). Pawpaw leaf extracts are used to reduce the severity of early blight disease in tomatoes (Abiodun *et al*., 2017). Pawpaw leaf extracts are potentially used in the control of malaria vector mosquitoes (Oladimeji *et al.,* 2012; Sesanti *et al*., 2014).

Insects breathe by means of trachea that usually opens at the surface of the body through spiracles (Adedire *et al*., 2011). The spiracles can be blocked by the plant powders thereby leading to suffocation of adult bruchids (Ileke *et al.,* 2012). The plant powders also prevent oviposition, adult emergence, reduction in weight loss and seeds damage on treated bean seeds (Getu, 2009). These powders inhibit insect locomotion which affects mating activities and sexual communication as well as deterring females from laying eggs. The powders cause complete suppression of the developmental stages of insects (Adeniyi, 2010; Ileke and Oni, 2011; Ashamo, 2012,).

2.4 Effects of plant powders on the seed germination in storage

The common bean, *Phaseolus vulgaris* can be infested by the common bean weevil, *Acanthoscelides obtectus* both in fields and stores (Thakur and Sharma, 2014). Plant powders are used in storage to protect seeds from insect damage and these powders have negative or positive effects on seed germination (Adedire *et al*., 2011). Seed germination is an internally regulated process influenced by genotype. However external factors such as; light, temperature, moisture, and the presence of certain chemical compounds (phyto-hormones or organic acids) also strongly influence this process (Lazcano *et al.,* 2010). Seed soaking in botanical extracts can soften a hard seed coat and also leach out any chemical inhibitors in the seed, which may prevent germination (Arancon *et al*., 2012).

Treatment of seeds with cinnamon powder improves on germination speed index of seeds. This is because cinnamon powder is reliable to control fungi (Soares *et al*., 2015). The powder causes the suppression of the incidence of the seed borne storage fungi (*Aspergillus spp* and *Penicillium*) that kill the embryo of the seeds leading to viability loss (Asha, 2012). The interference of allelo-chemicals on seed germination depends on the concentration used (Khanh *et al.,* 2007). Essential oils from chamomile (*Matricaria recutita* and *M. Chamomilla)* have been used to inhibit the growth of saprophytic and opportunistic fungi like *Aspergillus flavus, A. fumigatus, A. Niger, Trichoderma harzianum* and *Fusarium oxysporum* which inhibit seed germination (Tolouee *et al., 2010;* Jamalian *et al.,* 2012). Similarly, liquid extracts or powder from the species thorn apple (*Datura stramonium),* crown flower (*Calotropis gigantean),* and neem (*Azadirachta indica*) have been used to control *Fusarium mangiferae* (Usha *et al*., 2009). Another factor that may influence results is the water holding capacity of the substrates. The appropriate humidity percentage in the substrate will induce seed germination without favouring the damping-off

disease (Fajardo-Mejía *et al*., 2016). Plant botanical powders of neem leaf and bishkatali are effective in improving on the germination of stored seeds for up to two years. This is because these plant powders lower and maintain the moisture content of seeds in storage as compared to seeds that are not treated with powders. Untreated seeds absorbs in moisture that lowers the seeds germination in storage (Khatun *et al*., 2011).

The powdered leaves and extracts of nishinda (*Vitex negundo*), eucalyptus (*Eucalyptus globules*) and bankalmi (*Ipomoea sepiaria*), at a 3% mixture, provide good protection for black gram seeds by reducing insect oviposition, first filial generation adults' emergence, and grain infestation rates. The oil treatment of black gram does not show adverse effects on germination capability of seeds, even after three months of treatment (Rahman and Talukder, 2006). Higher plants like neem have also been used as antimicrobials against storage pests because of their relatively safe status and they are widely accepted by the consumers with no effect on seed germination (Anjorin *et al*., 2008).

The root extracts of swallow root *(Decalepis hamiltonii)* which is used as botanical insecticides against the stored product pests does not affect the germination of the treated grains after 3-4 months of storage (Rajashekar *et al.,* 2010). The powdered leaves of hook (*Dalbergia saxatilis)* treated with seeds does not affect the germination and viability of the cowpeas seeds during storage (Okwute *et al*., 2009). The powders of myrtle grass and clove offer great protection of stored rice seeds for six months through reducing on the number of lesser grain borers and grain moths seed while white derris tent to reduce red flour beetle with no effect on seed germination (Kengkanpanich *et al*., 2014).

Plant powders protect seeds from infestation by bruchids, by protecting it from damage hence maintaining the viability of seeds (Mehdi, 2012b). A number of plant powders inhibit germination of storage seeds. Some plants like clover powder have a high level of oil content 14-23% that tends to cover the seeds and suppresses them from oxygen intake leading to viability loss (Reddy *et al.,* 2010). Serious infestation of weevils in the seeds occur where little and no powder is applied therefore pre disposing insect to lay eggs, so that larvae hatch from the eggs, penetrate the seeds feed and survive and makes the seeds loose viability. Larvae penetration of weevils occur in seeds, develops and feeds on the cotyledon before adult emerges which causes decrease of germination capacity of the seeds (Oliveira *et al.,* 2009). Reduction in seed germination is related to the number of emergence holes and seed size; small bean seeds damaged by up to two bruchid emergence holes have a 7.1% reduction

in germination, while large bean seeds with a similar number of emergence holes have 25% reduction in germination (Ebinu *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Pawpaw leaves contain a number of secondary metabolites with insecticidal properties that provide seeds with protection against insect damage. These different secondary metabolites vary in concentration in the pawpaw leaves. The pawpaw leaves can be prepared into bio-pesticides in form of powder and liquid extracts to control bean weevils during storage. The purpose of this study was to determine the concentration of secondary metabolites and the most effective concentration of pawpaw leaf ethanol, water and powder extracts as bio-ration over a specific time period was determined.

3.2 Research study area

The research was carried out at National Crops Resources and Research Institute, (NaCRRI), Namulonge. Namulonge is located in Wakiso district, 11km from Gayaza. The research was done in the department of Bio-Science at the Nutrition and Bio-analytical Laboratory. NaCRRI lies on GPS location "0.52165^0N, 32.62757^0E″ (GPS reading). Its altitude is 1,144 meter above the sea level. Annual rainfall is 1270mm and with average daily temperature of 27^0C. NaCRRI has a bimodal rainfall with two seasons; the first season starts from March to May while the second one begins from July to October. The soil type is sandy loam (Kisembo, 2014).

3.3 Sample collection

The pawpaw leaf samples whose ethanol, water and powder extracts were used in the study were collected from Paidha, Nebbi and Namulonge. The pawpaw plants used were the land races whose genotype diversity has not been established in Uganda. Therefore, different morphotypes were used and these were classified using phenotypic traits such as; morphotype one (M-1), morphotype two (M-2), morphotype three (M-3) morphotype seven to M-7 (Table 1).

Table 1: The different pawpaw leaf accessions analysed

	Phenotypic traits			
Morphotypes	**Plant height (meters)**	**Leaf length (cm)**	**Shape of fruit**	**Place of sample collection**
Morphotype one (M-1)	4.3	38cm	Big and Round shape,(25cm long and 12cm wide)	Paidha
Morphotype two (M-2)	7.5	30cm	Smaller fruits(15cm long and 8cm wide)	Paidha
Morphotype three (M-3)	3.8	80cm	Oval shape, yellow when ripe (24cm long and 13cm wide)	Nebbi
Morphotype four (M-4)	3.3	80cm	Long shape(30cm long and 16cm wide)	Nebbi
Morphotype five (M-5)	3.6	35cm	Oval shape, semi-long (23.5cm long and 13cm width)	Namulonge
Morphotype six (M-6)	3	70cm	Long fruits (27.5cm long, 13cm wide)	Namulonge
Morphotype seven (M-7)	1.1	35cm	Oval shape (18cm long, 13.5cm wide)	Namulonge

3.4 Determination of the concentration of Secondary metabolites in pawpaw leaves

3.4.1 Determination of the secondary metabolites in pawpaw leaf powder

3.4.1.1 Preparation of pawpaw leaf powder

Pawpaw leaves used were picked from the middle part of the plant canopy that were neither old or too young. The fresh pawpaw leaves were cleaned to remove impurities and air dried on a raised platform for two weeks under room temperature to preserve the chemical substances in the leaves. The dried pawpaw leaves were separately ground using a warring commercial blender into fine powders and sieved using a 0.25mm pore sieve. The pawpaw leaf powder was packed into air tight containers and stored in a dark place until further analysis (Mulungu *et al*., 2007; Onoja, 2015).

3.4.1.2 Determination of total phenol content of pawpaw leaf powder

A powder sample of 0.1g was placed into a falcon tube and 2.5ml of Methanol-Hydrochloric acid solution was added and boiled in a water bath at 80^0 C for 30 minutes. This mixture was then allowed to cool. From this mixture, 1ml solution was transferred into a fresh tube and 2ml of distilled water were added. Thereafter, 0.5 ml of Folin-Ciocalteu reagent was added and mixed well for 3 minutes. Then 2ml of 20% Sodium carbonate was added and allowed to stand for 60 minutes in the dark. Total absorbance was read at 560nm using a UV-vis Spectrophotometer (Biowave 11+ 18v DC, England). The total concentration was expressed in percentage of total phenolics (Chan *et al.,* 2007).

3.4.1.3 Determination of total flavanoid content of pawpaw leaf powder

A powder sample of 0.1g was homogenized with 2.5 ml of 80% methanol. Then 1 ml of clear extract was transferred into a clean test tube and 4 ml of distilled water added. To this mixture, 0.3ml of 5% Sodium nitrite ($NaN0_2$) solution were mixed well and allowed to stand at room temperature on the bench for 5 minutes. Then, 0.3mls of 10% Aluminium chloride was added and mixed and left to stand for 6 minutes at room temperature and then 2mls of 1M Sodium hydroxide (1M NaOH) was added. Immediately, 2.4ml of distilled water was added and mixed well and the mixture was pink in colour. Total absorbance was read at 510nm against the water blank using a UV-vis Spectrophotometer (Biowave 11+ 18v DC, England) (Zhishen *et al*., 1999). The total flavonoid concentration was expressed as a percentage.

3.4.1.4 Determination of total alkaloids of pawpaw leaf powder

In determination of total alkaloid content, 5g of pawpaw leaf powder sample was ground in 20mls of 90% methanol and boiled at 80^{o}C for 30 minutes. The extract was filtered and the filtrate evaporated to dryness at 45^{o}C using vacuum concentrator plus (eppendorf AG 5305, Germany). The residue was dissolved into 3ml of phosphate buffer and transferred into a separating funnel and mixed with bromocresol green solution and left to stand for 30 minutes. To this extract, 5mls of chloroform was added and shaken for 2 minutes allowing for the separation of organic chloroform layer from the methanol layer. The lower layer was separated off after 10 minutes and the organic chloroform layer (upper) stored. This procedure was repeated twice and the chloroform extracts were mixed and used for spectrophotometric analysis. The absorbance was read at 418nm using a UV-vis Spectrophotometer (Biowave 2+ plus, England). The methanol extract of 90% was used as the blank. Alkaloid content was expressed as a total concentration (Ajanal *et al*., 2012).

3.4.1.5 Determination of total free tannins of pawpaw leaf powder

In determination of total free tannins, 0.1g powdered sample was placed into a 1.5ml eppendorf tube and 0.5ml of Acetone 70% (mixture of acetone 70% and 1% ascorbic acid) was added. This was shaken in orbital shaker (Heidilph, Unimax 1010 DT, Germany) for 20 minutes. To this extract, 0.5ml of petroleum ether was added, shaken and the petroleum ether was left to evaporate. 0.3ml of distilled water was added and centrifuged (Hermle Z 300K) at 1000rpm for 10 minutes. To this mixture 0.1 ml of an aliquant from the supernatant was picked and 2.4ml of hydrochloric acid–butanol solution was added (5%, 95%). This was placed in a water bath for 70 minutes at 80^{o}C. Total absorbance was read at 550nm (Mrudula *et al.,* 2014).Total free tannins were expressed as a total concentration.

3.4.1.6 Determination of total condensed tannins of pawpaw leaf powder

A powdered sample 0.1g was weighed into a 1.5ml Eppendorf tube and 0.5ml of Acetone of 70% Acetone and 1% Ascorbic acid(70%:1%) and was shaken in orbital shaker for 20 minutes. To this extract, 0.5ml of petroleum ether was added, shaken and the petroleum ether was left to evaporate. 0.3ml of distilled water was added and centrifuged at 1000rpm for 10 minutes. From the sample, excess solution was drained off and 0.4ml distilled water was added and 2.6ml of acid-butanol solution were added and boiled in a waterbath at 80^{o}C for 70minutes and the reading was taken at 550nm.Total free tannins were expressed as concentration of total condensed tannins (Mrudula *et al.,* 2014).

3.4.1.7 Determination total anti-oxidant activity of pawpaw leaf powder

Total anti-oxidant activity was determined using iron-reducing capacity. A powdered sample of 0.1g was put into 1.5ml eppendorf and 1meal of methanol/water/Acetic acid (80:19:1) mixture were added and shaken on an orbital shaker for four hours. To this mixture, 0.1ml of the clear extract was pippeted and 2.5meals of a 0.2M phosphate buffer (PH 6.6-7.0) was added to it. This was followed by adding 2.5ml of 1% Potassium Ferric cyanide and incubated at 50^{o}C in a waterbath for 20 minutes. From this sample, 1meal was pippetted and the following were added to this extract; 1meal of 10% Trichloro-acettic acid, 0.2ml of 0.1%Ferricchloride (Iron III Chloride) and 1ml of distilled. This was incubated for 10 minutes at 50^{o}C and the reading was taken 700nm in a spectrophometer (Benzie and Strains, 1996).

3.4.2 Determination of the concentration of secondary metabolites in pawpaw liquid extracts

3.4.2.1 Preparation of pawpaw leaf liquid extracts

The methanolic extract was prepared by adding 2.5 ml of methanol to 0.5 g of fresh leaf sample contained in a 50 ml centrifuge tube, and shaken continuously for 1 hour at room temperature. The mixture was centrifuged at 3,000 rpm for 10 min, and then the supernatant (subsequently referred to as methanolic extract) was collected and stored at -4^{o}C until analysis (Chan *et al.,* 2007). This was used for determining the total concentration of phenolics and flavanoids in the liquid extract forms.

Tannins are more soluble in Acetone and ascorbic acid mixture than methanolic extract. Therefore, Acetone/ascorbic acid mixture was prepared and used instead of methanolic extract. For fresh leaf samples, a leaf disc from the lamina (avoiding the midrib) was put into a 1.5ml Eppendorf tube and 0.5ml of Acetone 70% (mixture of acetone 70% and 1% ascorbic acid) was added and shaken in orbital shaker for 20 minutes. To this extract, 0.5ml of petroleum ether was added, shaken and the petroleum ether was left to evaporate. 0.3mills of distilled water was added and centrifuged at 1000rpm for 10 minutes (Chan *et al.,* 2007; Mrudula *et al.,* 2014).

3.4.2.2 Determination of total phenol content of pawpaw leaf liquid extract

From the methanolic extract, 0.2ml solution was transferred into fresh tubes and 2.8mls of distilled water added to it. Thereafter, 0.5ml of Folin–Ciocalteu reagent was added and mixed well for 3 minutes. To this extract, 2ml of 20% Sodium carbonate was added and allowed to

stand for 60 minutes in the dark. Total absorbance was read at 560nm. Total phenolic content was expressed as a total concentration of total phenolics (Chan *et al.,* 2007).

3.4.2.3 Determination of total flavanoids content of pawpaw leaf liquid extracts

From the methanolic extract, 1ml of solution was picked and transferred into clean test tube and 4 mls of distilled water was added. To this extract, 0.3ml of 5% Sodium nitrite ($NaN0_2$) solution were mixed well and allowed to stand at room temperature on the bench for 5 minutes. To this mixture, 0.3mls of 10% Aluminium chloride was added, mixed and left to stand for 6 minutes at room temperature. This mixture was added on 2 mls of 1M sodium hydroxide. Immediately, 2.4mls of distilled water was added and mixed well. Total absorbance was read at 510nm against the water blank (Zhishen *et al.,* 1999). The total flavonoid was expressed as a concentration of total flavanoids.

3.4.2.4 Determination of total alkaloid content in pawpaw leaf liquid extracts

A sample weighing 5g of fresh leaves were ground in 20mls of 90% methanol and boiled at 80^oC for 30 minutes. The extract was filtered and the filtrate evaporated to dryness at 45^oC using vaccum concentrator plus (eppendorf, Germany made). The residue was dissolved into 3ml of phosphate buffer and transferred into separating funnel and mixed with bromocresol green solution and left to stand for 30 minutes. To this extract, 5mls of chloroform was added and shaken for 2 minutes and the lower layer was separated after 10 minutes, this was repeated twice. The absorbance was read at 418nm. The methanol extract of 90% was used as the blank. The values were expressed as a concentration of total alkaloids (Ajanal *et al.,* 2012).

3.4.2.5 Determination of total free tannins content of pawpaw leaf liquid extracts

In determination of total free tannins, 0.1 mls of aliquots from the supernatant of Acetone and ascorbic acid mixture was picked and 2.4mls of hydrochloric acid–butanol solution was added (5%, 95%). This was placed in water bath for 70 minutes at 80^oC. Total absorbance was read at 550nm. Total free tannins were expressed as total concentration of total free tannins (Mrudula *et al.,* 2014).

3.4.2.6 Determination of total condensed content of pawpaw leaf liquid extracts

In the determination of total condensed tannins for fresh leaf samples for liquid extracts, a leaf disc from the lamina (avoiding the midrib) was placed into a 1.5mls Eppendorf tube and 0.5ml of Acetone of 70% Acetone and 1% Ascorbic acid(70%:1%) and was shaken in orbital shaker for 20 minutes. To this extract of an aliquot from the supernatant of Acetone and

ascorbic acid mixture, 0.5mls of petroleum ether was added, shaken and the petroleum ether was left to evaporate. 0.3mls of distilled water was added and centrifuged at 1000rpm for 10 minutes. From the sample, excess solution was drained off and 0.4ml distilled water was added and 2.6mls of acid-butanol solution were added and boiled in a waterbath at 80^{o}C for 70minutes and the reading was taken at 550nm (Mrudula *et al.*, 2014). Total free tannins were expressed as tannic acid equivalents from a standard curve.

3.4.2.7 Determination of total anti-oxidant activity of pawpaw leaf liquid extracts.

Total anti-oxidant activity was determined using iron-reducing capacity. Fresh pawpaw leaf samples of 0.1g were weighed into 1.5ml eppendorf tube and 1ml of methanol/water/Acetic acid (80:19:1) mixture were added and shaken on an orbital shaker for four hours. 0.1ml of this clear extract was pippeted and 2.5ml of a 0.2M phosphate buffer (PH 6.6-7.0) was added. 2.5ml of 1% potassium ferric cyanide was added and incubated at 50^{o}C in a waterbath for 20 minutes. From this sample, 1ml was pippetted after incubation. The following were added; 1ml of 10% Trichloro-acettic acid, 0.2ml of 0.1% Ferric chloride (Iron III Chloride) and 1ml of distilled water were added. This was incubated for 10 minutes at 50^{o}C and the reading was taken 700nm in a spectrophometer (Benzie and Strains,1996).Total anti-oxidant activity was expressed as a percentage.

3.4.3 Data analysis for the concentration of secondary metabolites in pawpaw leaf powder and liquid extracts.

Data was analysed using Minitab software to generate means, standard deviations and superscripts to determine the significance difference at 5%.Values with the same superscripts along each row are not significantly different from each other at $P > 0.05$.Values that share superscripts are intermediary. The results from the data analysis were used to generate tables, graphs and figures.

3.5 Determination of the effectiveness of pawpaw leaf powder and liquid extracts as bio-pesticides in bean storage.

3.5.1 The bean variety

The clean, undamaged bean seeds of NABE 6 were placed in a cold deep freezer at a temperature of -60^{0}C for 2 weeks so as to sterilize the seeds (Mulungu *et al.*, 2007). NABE 6 is a white small seeded crop with diameter ranging from 0.5-0.9cm which is quite susceptible to pest and disease attack.

3.5.2 In-vitro rearing of the bean weevils

Bean weevil infested legumes used for the study were obtained from National Crops Resource and Research Institute (NaCRRI), Namulonge in Wakiso Uganda. Dried legumes were sieved to remove dirt fine dust and broken or shrivelled seeds. Bean weevils were reared in one litre jar containing 1000 grams of uninfected whole bean grain. The jar was labelled, covered and left at room temperature. It was moved into the dark rearing area for one week for adults to develop. The adult weevils were removed from the jar using 2.0 mm and 0.7 mm opening sieves and discarded. The beans were then returned to the culture jar, covered and allowed to stand for four weeks for adult weevil to emerge. The rearing of the insects was done in the laboratory at room temperature (Mining *et al.*, 2014).

3.5.3 Extraction of pawpaw leaf powder

The fresh pawpaw leaves from different morphotypes were cleaned to remove impurities and were air dried for two weeks on a raised plate under room temperature to preserve the secondary metabolites in the leaves. The dried pawpaw leaves were separately ground using a warring blender into fine powders and sieved using a 0.25mm pore sieve and packed into air tight containers and stored in a dark place until usage(Mulungu *et al*., 2007; Onoja, 2015).

3.5.4 Preparation of different concentration of pawpaw leaf powder

The amount of powder mixed with bean seeds were calculated based on the weight of the seeds to determine the different percentages of 0% control, 5%,10% and 15% (weight of powder over weight of bean seeds= (w/w) (Thakur and Sharma, 2014). In determination of the different percentages desired, the weight of the sample was divided by a hundred percentage and multiplied by fifty, the weight of the bean seeds introduced for the study. The application levels were;

$$\% \text{ Powder} = \frac{\text{Sample weight}}{\text{Hundred percent}} \text{ x weight of beans}$$

$$\text{Control (0\%)} = \frac{0}{100} \text{ x } 50 = 0 \text{ g of plant powder}$$

$$5\% = \frac{5}{100} \text{ x } 50 = 2.5\text{g}$$

Where,

5%=treatment level or concentration used which was expressed over 100;

50g= the weight of the bean sample used

2.5g=the weight of pawpaw leaf powder applied to beans.

The calculated percentage of pawpaw leaf powder was then introduced and mixed thoroughly with the 50g of bean seeds introduced into the plastic containers. These were mixed for five minutes before introducing the bean weevils to them. This was done in four replicates for each of the different pawpaw leaf powder application levels.

3.5.5 Extraction of pawpaw liquid extracts

Fresh leaf extracts were prepared in ethanol and water at a varying concentration of 5%, 10%, 15% and control (0%)-with liquid extract application and stored in a refrigerator until needed. Concentrations used were adopted from studies by (Thakur and Sharma, 2014) in assessing insecticidal properties and seed protective effects. The fresh leaf samples ethanol and water extracts were prepared by weighing the fresh leaf samples at 5g, 10g, 15g for the different concentrations of 5%, 10% and 15%.

The different pawpaw leaf liquid extracts prepared were water and ethanol extracts. In preparation of water extract, distilled water was used as a solvent to avoid impurities and contamination. The ethanol used for the study was diluted to 10% which is similar to the local brews concentration. These were prepared at the different concentrations of 0% (control), 5%, 10% and 15% concentration levels. In preparation of the different concentration of liquid extracts, the fresh pawpaw leaf sample were weighed and divided by 100 percent and multiplied by 100 mls of solvent used (ethanol or water). These were as below;

$$\%\ \text{Concentration} = \frac{\text{Sample weight (g)}}{\text{Solvent weight (g)}} \text{ x } 100\%$$

$$\text{Control} = \frac{0}{100} \text{x} 100 = \text{leaf sample (0g)}$$

$5\% = \frac{5}{100} \text{x } 100 = 5 \text{ g of fresh leaves}$

$10\% = \frac{10}{100} x\ 100 = 10\ g\ of\ fresh\ lea$

$15\% = \frac{15}{100} \text{x } 100 = 15 \text{ g of fresh leaves}$

The weighed leaf samples were pounded with a pestle and a mortar until a homogenous mixture was obtained. To this well macerated paste, 100 mls of the solvent (water or ethanol) was poured and mixed well. These were prepared into the different concentration levels of 5%, 10% and 15% and the liquid extracts were packed in the test tubes with lids tightly closed. The different prepared treatments at varying concentrations were then taken to a centrifuge machine and were centrifuged at 1000 rpm for four minutes at a temperature of four degrees centigrade to avoid chlorophyll that contains the chemical metabolites from dissociating. The extracts were then decanted to form fine solvents and were stored in refrigerator of -5^0 C until usage.

3.5.6 Application of the pawpaw leaf powders and liquid extracts to control weevils.

A complete Randomized Design (CRD) with four replicates was used for the study .Control treatment (0%) was set with no pawpaw leaf powder and liquid extracts used. The bean seeds were treated with pawpaw leaf powder applications levels at 5%, 10 % and 15%. Control was 0% where no powder was applied to the bean seeds. The ethanol and water extract, the bean seeds were treated with 5%, 10% and 15% concentrations of pawpaw leaf liquid extracts. Control was 0% where no plant extract was applied to the bean seeds.

NABE 6 bean samples were weighed at 50g and were dipped into the extracts of pawpaw leaf ethanol and water extracts at varying concentrations of 5%, 10%, 15% and control (0%) for one minute and dried under shade for ten hours. For powder treatment, 50g seeds of *P. vulgaris* (NABE 6) were weighed and placed into each of the plastic containers, of 5%, 10%, 15% and control (0%) of powders were mixed thoroughly for 5 minutes with the containers leads tightly closed and then 20 bean weevils were released in different plastic containers containing the plant powder, water and ethanol extracts. The lids were covered with

transparent cloth and to allow gaseous exchange and also prevent weevils from escaping in the containers (Mulungu *et al.,* 2007; Thakur, 2014).

3.5.7 Data Collection parameters

The set up was left to stand for a period of 13 weeks. For the powder applications, during data collection, the content of each jar were poured out into 0.25 mm sieve and mechanically shaken to separate the seeds from both the insects and the plant powders. The seeds were thereafter weighed. For liquid extracts the data was collected directly on the parameter. The dead weevils were removed and discarded off after recording the data collected. Data was collected after week one, five, nine and week 13 from the time of experimental set up. The first data was collected after one week on insect mortality (dead weevils). This was done by counting the number of dead weevils subtracted from the initial number of weevils introduced to the plastic containers. The initial numbers of weevils introduced were 20 weevils per plastic container. The formula was; Insect mortality= live weevils introduced-dead weevils after a week. In the other weeks of the study (week 5 to 13), the number of dead weevils were directly determined by physically counting the dead weevils and recording them. The weevils were considered dead when touched and did not move, also when air is blown towards them, they didn't move away

Other data collected were the number of F1 Progeny emergence (live weevils). Data on F1 progeny, the newly emerged weevils (live weevils) was collected after 5, 9 and 13 weeks from the time of weevils' introduction. This was done by physically counting the number of live weevils and recording them. Live weevils were also determined by touching them or blowing air towards them, and their movement showed they were alive. Numbers of damaged seeds were determined by physically counting the number of seeds with holes created by larvae of the weevils. Seeds were considered damaged as long as there was any hole caused by larvae on it. The final weight of bean seeds were determined by subtracting the final weight of bean seeds from the initial weight of bean seeds. The initial weight of bean seeds introduced was 50grams per each plastic container. The formula used was;

Weight difference of bean seeds (W_0) = Initial weight of bean seeds (W_1) - Final weight of bean seeds (W_2)

$W_0=W_1-W_2$.

Where, W_0= Weight difference,

W_1= Original weight (before infestation)

W_2=final weight (after infestation).

The percentage weight loss of beans was determined by subtracting the final weight from original weight of bean seeds introduced.

$$\% \text{ weight loss} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} \text{ x } 100$$

The data collection parameters were the; final weight of bean seeds, number of damaged seeds (holes), percentage weight loss of seeds, number of dead weevils and live ones (Onoja, 2015). Data collection was on monthly basis for a period of three months.

3.5.8 Data analysis

The data was analysed using the Minitab to generate table of means, standard deviations, Superscripts to determine the level of significance at 5%. Graphs and figures were generated using Microsoft Excel.

3.6 Establishment of the effect of pawpaw leaf powders on the seed germinability of beans after storage.

3.6.1 Bean seed varieties used

The clean, undamaged bean seeds of NABE 6, NABE 4 and golden brown used were placed in a cold room at a temperature of -80^{0}C for 2 weeks so as to sterilize the seeds (Mulungu *et al*., 2007). NABE 6 is a white small seeded crop with diameter ranging from 0.5-0.9cm which is quite susceptible to pest and disease attack. A golden brown bean seed with a golden colour, a local variety collected from Zombo was used due to its palatability and it is a medium sized bean with diameter ranging from 1.0cm-1.5cm. It is moderately susceptible to bean weevils. NABE 4 is a large seeded crop with diameter ranging from 1.6-2.0cm and is quite tolerant to the bean weevils attack due to its size. All the bean varieties in Uganda are susceptible to bean weevils' infestation except the large seeded crops are quite tolerant to the pest attack compared to the small seeded crops (Ebinu *et al*., 2016).

3.6.2 Treatment of beans in storage

The pawpaw leaves were cleaned and air dried for two weeks on a raised plate and then separately ground using a warring blender into fine powders and sieved using a 0.25mm pore sieve and packed into air tight containers and stored in a dark place until usage. Pawpaw leaf powder prepared at 10% was used for dressing with the different bean varieties for maximum

of three months in storage. In preparing the 10% powder concentration, the amount of powder mixed with bean seeds were calculated based on the weight of the seeds. (Weight of powder over the weight of bean seeds (w/w) (Thakur and Sharma, 2014; Onoja, 2015).

$$\%\ Powder\ = \frac{Sample\ weight}{Hundred\ percent} x\ weight\ of\ beans$$

$$10\%\ Powder\ = \frac{10}{100} x\ 50 = 5g\ \text{ of powder}$$

The weighed powder was thoroughly mixed with the bean seeds of 40grams in a plastic container and was shaken vigorously for 5 minutes and then 14 live weevils were introduced. The plastic containers were covered with transparent cloths to allow gaseous exchange and to prevent the weevils from escaping.

3.6.3 Experimental layout

The effect of pawpaw leaf powder bio-pesticide on seed germinability was done on the different bean varieties including; NABE 6, NABE 4 and golden brown. This was after the bean seeds were treated with the powders for a period of two months. The research design used was a completely randomised design. Four replicates were used for each of the bean variety sample planted. The bean samples were treated with pawpaw leaf powder at 10% concentration and each of the sample set there was a control experiment (0%). Plant powders effect on seed germinability was done after 8 week of beans in storage for NABE 6, NABE 4 golden brown beans. Ten seeds from each of the bean seed varieties of the three varieties were picked randomly and planted into individual plates filled with sterilized tissue paper for 10 days to test their ability to germinate. Seedlings that showed well-developed essential structures of the root system shoot axis, cotyledons and terminal buds were considered "normal germination" and their proportion were recorded as per variety (Demissie *et al*., 2008; Ebinu *et al*., 2016).

3.6.4 Data collection

Data collected included number of germinated seeds, un-germinated seeds and percentage germination of seeds. The number of germinated seeds was done by physically counting the germinated seeds after 10 days. Percentage seed germination was done by diving the number of germinated seeds over ten seeds put multiply by hundred percent.

$$\% \text{ germination} = \frac{\text{No. of germinated seeds}}{\text{Total no. of seeds on petridish}} \text{ x } 100$$

Number of un-germinated seeds was counted and percentage germination determined. The formula for the un germinated seeds as below;

$$\text{Ungerminated seed } \% = \frac{\text{No. of ungerminated seeds}}{\text{Total no. of seeds on petri dish}} \text{ x } 100$$

3.6.5 Data analysis

Data was analysed using Minitab software to generate means, standard deviations and superscripts to determine the significance difference at 5%.

CHAPTER FOUR

RESULTS

4.1 Introduction

The different pawpaw leaf accessions were analysed for the concentration of selected secondary metabolites and these were; tannins, flavanoids, alkaloids, phenolics and total anti-oxidant activity. These were analysed in liquid extracts and powdered forms. The concentration of the secondary metabolites varied significantly ($P<0.05$) for the different pawpaw accessions used. The concentrations of secondary metabolites were higher in pawpaw leaf powder than pawpaw leaf liquid extracts. Pawpaw leaf powder did not have a significant effect ($P<0.05$) on seed germination for different bean varieties for up to three months in storage. Pawpaw leaf powder was the most effective in causing highest insect mortality. Water extract had the least effect in causing insect mortality. The effectiveness of the treatments was dose dependant. These trends were observed for both seasons (Figure 1).

4.2 Concentration of selected secondary metabolites in pawpaw leaf powder and liquid extracts

Generally, the concentration of secondary metabolites varied significantly ($P<0.05$) between the powder and liquid extracts as well among the different morphotypes used. Such differences could explain the variation in efficacy of the two extracts when applied in controlling weevils. Pawpaw leaf powder had the highest concentration of most of the secondary metabolites analysed.

4.2.1 Concentration of selected secondary metabolites in pawpaw leaf powder extracts

There was a significant difference ($P<0.05$) in the concentration of the secondary metabolites of the different pawpaw leaf morphotypes (Table2). Total free tannins were the least abundant metabolites in all accessions (Table 2). Pawpaw morphotype three had the lowest concentration of most of the secondary metabolites in the powdered extract (Table 2). Morphotype two had the highest concentration of total free tannins at 0.64% in pawpaw leaf and the lowest was recorded in morphotype 3 at 0.252%. Morphotype two had the highest concentration of 4.60% of total condensed tannins in while morphotype three had the lowest concentration of condensed tannins at 2.45%.

Total flavanoid concentration was highest in pawpaw morphotype two at 3.468% whereas the lowest concentration was found in pawpaw morphotype three at 2.10% which was

significantly lower (P<0.05) than other morphotypes. The highest concentration of phenolics was found in pawpaw leaf morphotype two at 3.10% whereas the lowest concentration for total phenolics was registered in pawpaw morphotype three at 2.24% (Table 2). Alkaloid content was highest in pawpaw morphotype three at 1.50% and lowest in pawpaw morphotype 1 at 0.68%. Total anti-oxidant activity was found highest in pawpaw morphotype 3 at 11.59% whereas the lowest concentration was found in pawpaw morphotype 2 at 6.01% (Table 2).

Table 2. Concentration of the different secondary metabolites in the different pawpaw leaf powder accession extracts

	Pawpaw accessions		
Secondary metabolites (%)	**M-1**	**M-2**	**M-3**
Free tannins	0.498±0.01[a]	0.642±0.060[b]	0.252±0.006[c]
Condensed tannins	3.427±0.02[a]	4.601±0.069[b]	2.448±0.026[c]
Total flavanoids	2.796±0.05[a]	3.468±0.039[b]	2.100±0.060[c]
Total phenolics	2.759±0.01[a]	3.102±0.010[b]	2.239±0.007[c]
Total alkaloids	0.683±0.003[a]	1.492±0.009[b]	1.503±0.004[b]
Total anti-oxidants	10.140 ±0.05[a]	6.099±0.016[b]	11.586±0.027[c]

Means that do not share a letter are significantly different. M-1=Morphotype one, M-2=Morphotype two, M-3=Morphotype three

4.2.2 Concentration of secondary metabolites in pawpaw leaf liquid extracts

Pawpaw leaf liquid extracts had significantly varying (P<0.05) concentrations of the selected secondary metabolites in the different pawpaw morphotypes. Morphotype five had the highest concentration of flavonoids at 5.52% while Morphotype seven had the lowest at 1.37%. Morphotype seven generally had the lowest concentration of the different secondary metabolites analysed. Morphotype 4 registered the highest concentration of total free tannins at 0.23% whereas morphotype seven had the least concentration (0.036%).The highest concentration of condensed tannins was found in pawpaw morphotype five at 2.263% whereas the lowest concentration was in morphotype seven at 0.64% (Table 3).

Total flavanoid concentration was highest in pawpaw morphotype five at 5.52%while the lowest concentration was registered in morphotype seven at 1.37%. The highest concentration for total phenolics was found in morphotype five at 0.29% whereas the lowest concentration was found in morphotype four at 0.26%. Total alkaloids concentration was found highest in morphotype four at 1.55% and the lowest concentration of alkaloids was in morphotype six (1.13%). The highest concentration of total anti-oxidant activity was found in morphotype five (4.36%) and the lowest concentration in morphotype four (3.33%) (Table 3).

Table 3. Concentration of the different secondary metabolites in the different pawpaw leaf liquid extracts accessions

	Pawpaw accessions			
Secondary metabolites (%)	**M-4**	**M-5**	**M-6**	**M-7**
Free tannins	0.230±0.016[a]	0.129±0.040[b]	0.038±0.010[c]	0.036±0.004[c]
Condensed tannins	1.841±0.021[a]	2.263±0.027[b]	1.269±0.020[c]	0.644±0.037[d]
Total flavanoids	4.488±2.272[a]	5.520±0.049[a]	5.206±0.007[a]	1.368±0.069[b]
Total phenolics	0.264±0.012[a]	0.298±0.011[b]	0.283±0.006[c]	0.269±0.008[bc]
Total alkaloids	1.547±0.002[a]	1.518±0.001[ab]	1.127±0.139[c]	1.363±0.132[b]
Total anti-oxidants	3.328±0.009[a]	4.363±0.047[b]	3.786±0.437[c]	3.432±0.008[ba]

Means that do not share a letter are significantly different. M-1=Morphotype one, M-2=Morphotype two, M-3=Morphotype three

4.3 Effectiveness of pawpaw liquid extracts and leaf powder as bio-pesticides against bean weevils over specific time period.

4.3.1 Effect of pawpaw leaf water, ethanol and powder extracts on the number of dead weevils

The number of dead weevils kept on increasing from week one to week thirteen in both seasons and this was observed for all the 3 different treatments (Figure 1). Season two generally had a higher mortality rate of weevils compared to season one. Pawpaw leaf ethanol extract had the highest insect mortality rate among all the treatments compared to controls in both seasons. Powdered treatment had higher mortality rate in the control, compared to the other treatment levels from week one to week nine in both seasons (Figure 1).

Pawpaw leaf water extract did not have a significant effect ($P>0.05$) on insect mortality from week one to week nine in all the treatment levels (Figure 1). However, the mortality rate significantly increased in week thirteen ($P<0.05$). Pawpaw leaf ethanol extract at all concentrations had a significant effect ($P<0.05$) on insect mortality compared to the control and this was observed from week one to week thirteen of data collection (Figure 1). Pawpaw leaf powder significantly increased insect mortality compared to the control from week one to week thirteen of data collection (Figure 1).

In season one at week one, the highest mortality rate was observed at 15% powder treatment level with 19 dead weevils as compared to other treatments (Figure 1). The lowest insect mortality rate was 14 observed at 5% concentration of the pawpaw water extract treatment (Figure 1).In season one at week five, the highest mortality rate was 12 at 10% pawpaw water

extract treatment. In season one at week five, the lowest insect mortality rate was 0 for a 5% pawpaw water extract treatment (Figure 1). In season one at week nine, the highest mortality rate was31 weevils for the 15% pawpaw water extract (Figure 1). In season one at week nine, the lowest insect mortality rate was one weevil at 10% powdered treatment level (Figure 1). In season one at week thirteen, the highest mortality rate was 73 weevils in pawpaw water extract treatment at 10% concentration (Figure 1).In season one at week thirteen, the lowest insect mortality rate was 1 weevil for the 10% powdered treatment level (Figure 1).

In season two at week one, the highest insect mortality was 19 weevils for powdered treatment at 15% treatment level (Figure 1). In season two at week one, the lowest insect mortality was 13 weevils for 5% water extract. In season two at week five, the highest insect mortality was observed for powdered treatment at 15% treatment which had 25 dead weevils (Figure 1). In season two at week five, the lowest mortality was 3 weevils at a 5% pawpaw ethanol extract. In season two at week nine, the highest insect mortality was 93 weevils in powdered treatment at a concentration of 15%. In season two at week nine, the lowest mortality was 22 weevils at 5% pawpaw water extract treatment (Figure 1). In season two at week 13, the highest number of dead weevils was 394 weevils for powder at 15% concentration. In season two at week thirteen, the lowest insect mortality was 102 for powdered treatment at 10% treatment level (Figure 1).

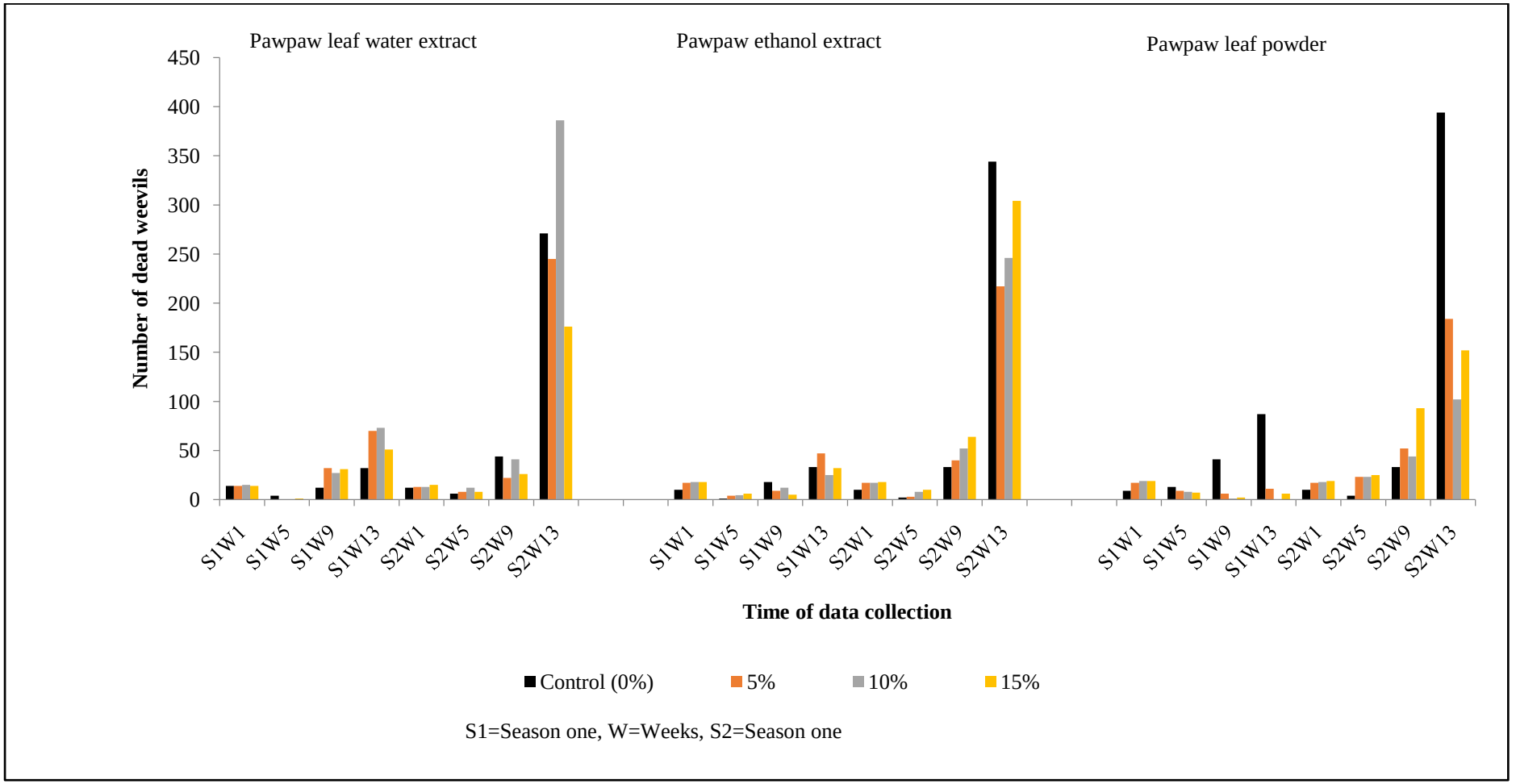

Figure 1 Effect of pawpaw leaf water, ethanol and powder extracts on the number of dead weevils

4.3.2Effect of pawpaw leaf powder, water extract and ethanol extracts on the F1 progeny emergence (number of live weevils) in NABE 6 beans.

The number of live weevils significantly increased ($p<0.05$) from week one to week nine for all treatments in both seasons (Figure 2). Season two generally had higher numbers of live weevils in week five as compared to season one. Pawpaw water extract had the highest number of live weevils at the different treatment levels. The live weevil numbers kept on fluctuating from control to other treatment levels and the trend was observed for both seasons. The powdered treatment had lowest numbers of live weevils as compared to other treatments in both seasons. The effectiveness of both powder and pawpaw ethanol extract increased with increasing concentration for both seasons (Figure 2).

In season one at week one, the highest weevil emergence was 20 weevils for the pawpaw water extract at 5% application rate (Figure 2), while only one live weevil was found in powdered treatment at 15% application rate. In season one at week five, the highest number of live weevils was 14 weevils for pawpaw water extract at 5% concentration (Figure 2), whereas the lowest F1 progeny emergence was zero weevil in powdered treatment levels of 10% and 15% (Figure 2). In season one at week nine, the highest number of live weevils was 177 for water extract at 5% application rate and the lowest weevil numbers at week nine was 0 for powder treatment at 10% application rate (Figure 2).

In season two at week one, the highest number of weevils was 44 for water extract at 10% application rate, while the lowest number of weevils was 25 for powdered extract at 10% application rate (Figure 2). In season two at week five, the highest number of live weevils was 104 for water extract at 5% application rate, whereas the lowest number of live weevils 34 in powdered extract at 10% application rate. In season two at week nine, the highest number of weevils was 48 in pawpaw ethanol extract treatment at 5% application rate, while the lowest weevil number was one in the powdered treatment at 10% application rate (Figure 2).

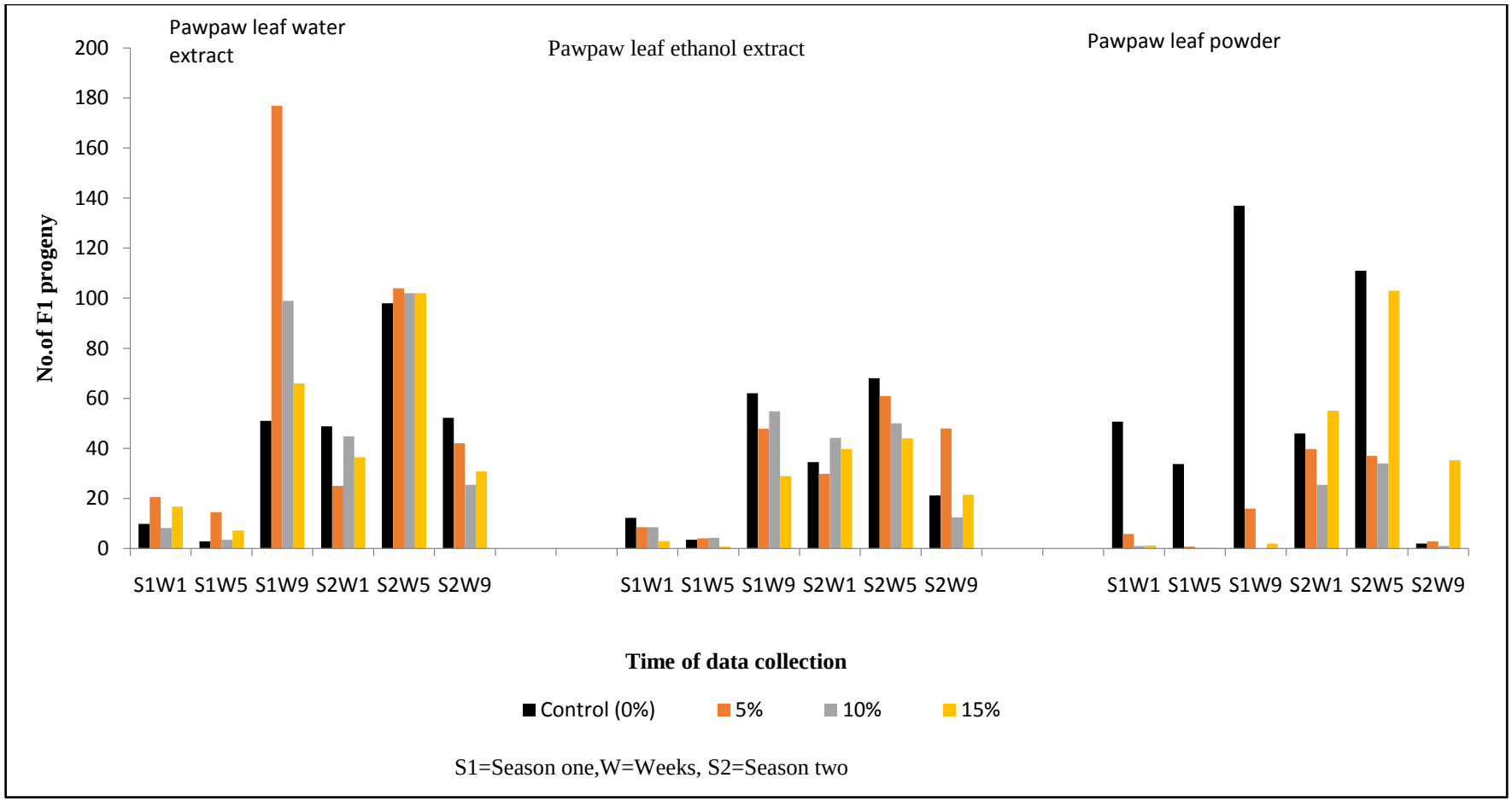

Figure 2 Effect of pawpaw leaf water, ethanol and powder extracts on F1 Progeny emergence (number of live weevils)

4.3.3 Effect of pawpaw leaf powder, water and ethanol extracts on the number of damaged seeds in NABE 6 beans

The number of damaged seeds kept on increasing from week one to week nine of data collection in all treatments for both seasons one and two (Figure 3). Powdered treatments from week one to nine significantly reduced ($P<0.05$) the number of damaged seeds at all treatment levels as compared to the control in both seasons. Powdered treatments were generally the most effective in reducing on the number of damaged seeds while the least effective was the pawpaw water extract. This trend was observed for both seasons. Generally season two had slightly more number of damaged seeds in all the different treatments, compared to season one (Figure 3).

In season one at week one, the highest number of damaged seeds was 17 for water extract at 15% application rate (Figure 3). The lowest number of damaged seeds was 1 for powdered extract at 15% application rate (Figure 3). In season one at week five, the highest number of damaged seeds was 33 in the pawpaw water extract treatment at 5% application rate, while at 10% treatment level there were no damaged seeds (Figure 3). In season one at week nine, the highest number of damaged seeds was 122 in the pawpaw water extract treatment at 5% application rate. On the other hand, the lowest number of damaged seeds was 2 in powdered extract treatment at 10% application rate (Figure 3).

In season two at week one, the highest number of damaged seeds was 40 in the control experiment (Figure 3) and the lowest number of damaged seeds was 26 in the powdered extract treatment at 5% application rate. In season two at week five, the highest number of damaged seeds was 92 in the pawpaw water extract treatment at 5% application rate and the lowest number of damaged seeds was 44 in powder extract treatment at 10% application rate (Figure 3). In season two at week nine, the highest number of damaged seeds was 169 for the pawpaw water extract treatment at 10% application rate and the lowest number of damaged seeds was 99 in the powdered extract treatment at 5% application rate (Figure 3).

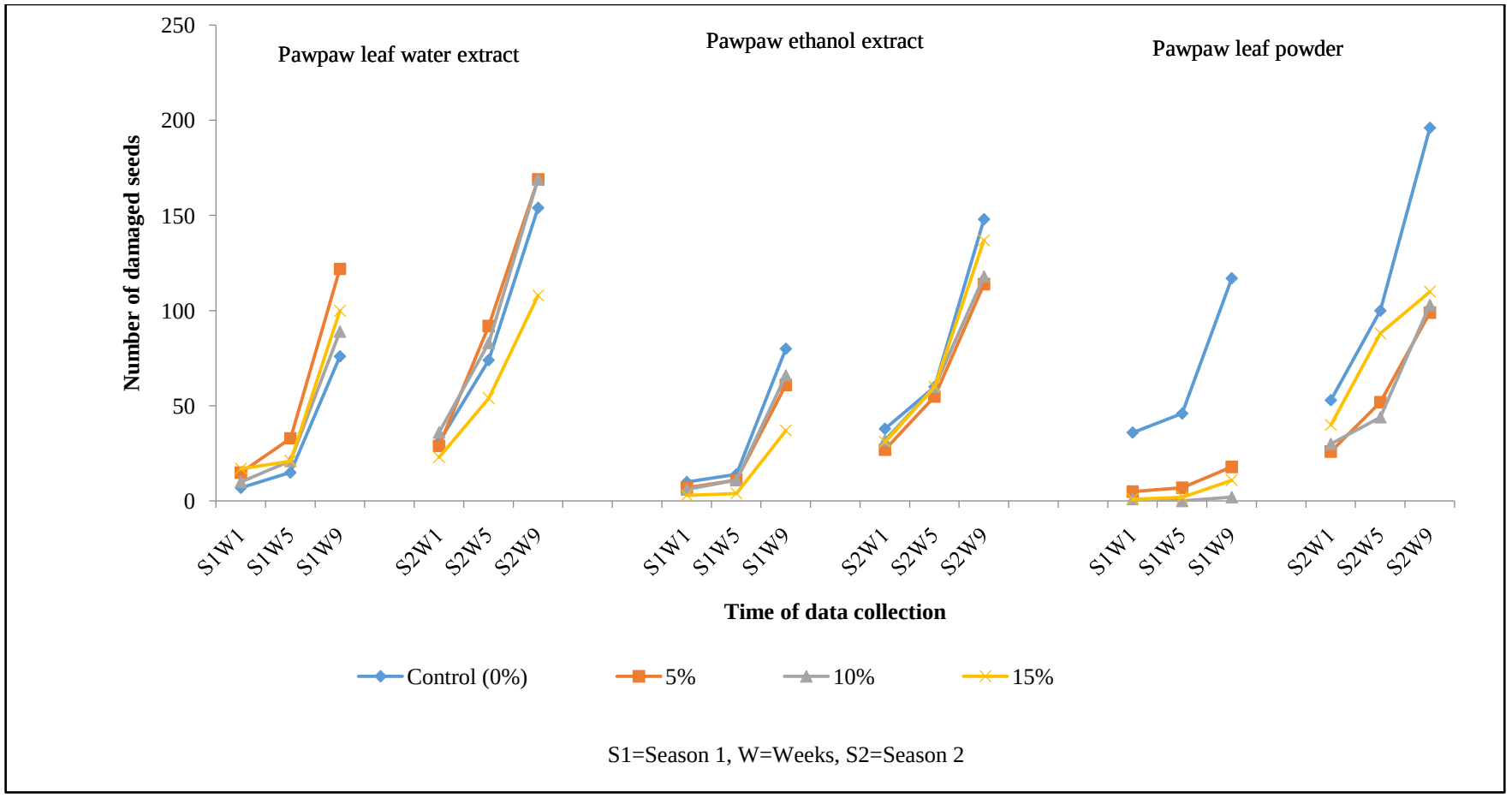

Figure 3: The effect of pawpaw leaf water, ethanol and powder extracts on the number of damaged seeds.

4.3.4 Effect of pawpaw leaf powder, water and ethanol extracts on the final weight of bean seeds

The final weight of beans kept on dropping from week one to week nine in all the three treatments for both seasons. Generally, the final weight of bean seeds treated with pawpaw leaf powder had the least weight loss recorded, compared to other treatments. This was followed by ethanol extract and the least effective against seed weight loss was the water extracts. This trend was observed for both seasons. Season two generally had a lower final weight as compared to season one for all the weeks of the study (Figure 4).

In season one at week one, the highest final weight was 50g for powder extract treated seeds at 15% application rate and the lowest final weight was 47.4g in the pawpaw water extract treated seeds at 5% application rate (Figure 4). In season one at week five, the highest final weight was 50g registered for seeds treated with pawpaw powder extract at 10% and 15% application rates whereas the lowest final weight was registered in week five (44.2g) for seeds treated with pawpaw water extract at 5% application rate (Figure 4). In season one at week nine, the highest final weight was 50g for seeds treated with pawpaw powder extract at 10% application rate and the lowest final weight was 40.7g in seeds treated with pawpaw water extract at 5% application rate (Figure 4).

In season two at week one, the highest final weight was 49.7g in seeds treated with water extract at 15% application rate and the lowest final weight was 49.1g for seeds treated with pawpaw powder extract at 10% application rate (Figure 4). At week five of season two, the highest final weight was 47.5g for seeds treated with powder extract at 10% application rate and the lowest final weight was 42.9g for seeds treated with water extract at 10% application rate (Figure 4). In week nine of season two, the highest final weight was 45.6g for seeds treated with pawpaw powder at a 5% application rate and the lowest final weight was 37.4g for seeds treated with pawpaw water extract at 5% application rate (Figure 4).

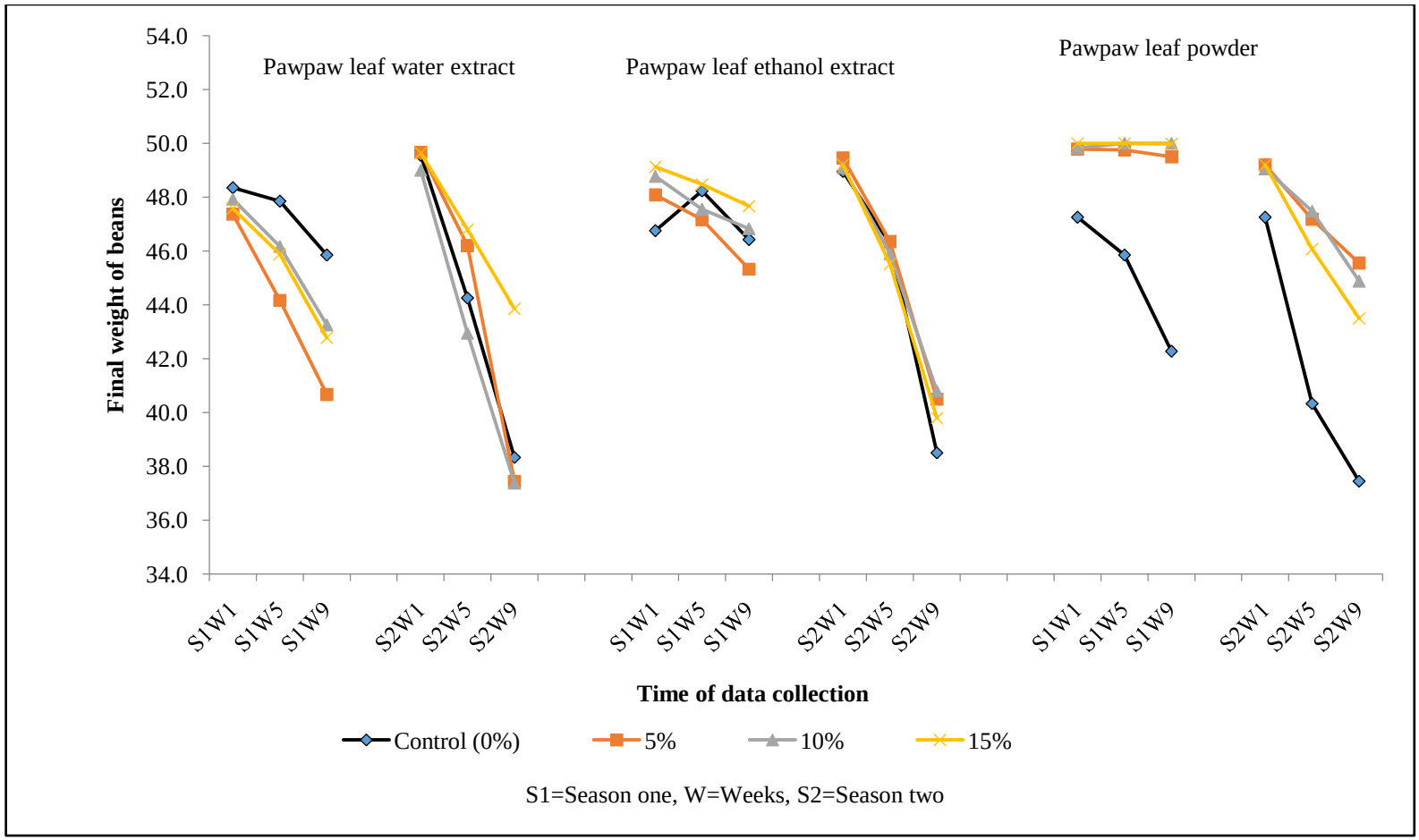

Figure 4 Effect of pawpaw leaf water, ethanol and powder extracts on the final weight of beans

4.3.5 Effect of pawpaw leaf powder, water and ethanol extracts on the percentage weight loss of bean seeds in NABE 6 beans

Generally the percentage weight loss for all the treatments in season two was slightly higher than for season one. The weight loss kept on increasing from week one to week nine. The least weight loss was recorded from seeds treated with pawpaw leaf powder. The highest weight loss was observed for beans treated with pawpaw water extract. The effectiveness of powdered and liquid extracts improved with the increasing concentrations (Figure 5).

In season one at week one, the highest percentage weight loss was 2.6% in seeds treated with pawpaw water extract at 5% application rate, while the lowest percentage weight loss was 0 in seeds treated with pawpaw powder extract at 15% application rate (Figure 5). In week five of season one, the highest percentage weight loss was 5.8 % in water extract treatment at 5% application rate, while the lowest percentage weight loss was 0% for seeds treated with pawpaw powder extracts at 10% and 15% application rates. In week nine of season one, the highest percentage weight loss was 9.2% in seeds treated with pawpaw water extract at 5% application rate and the lowest percentage weight loss of 0% was in seeds treated with pawpaw powder at a 10% application rate.

In season two at week one, the highest percentage weight loss was 1% and this was in seeds treated with pawpaw leaf water extract at 10% application rate and the lowest percentage weight loss for week one was 0.35% in seeds treated with pawpaw leaf water extract at 10% application rate (Figure 5). In week five of season two, the highest percentage weight loss was 7% in seeds treated with pawpaw leaf water extract at 10% application rate and the lowest weight loss was 2.5% in seeds treated with pawpaw powdered extract at 10% application rate (Figure 5). In week nine of season two, the highest weight loss was 12.6% in seeds treated with pawpaw water extract at a 10% application rate while the lowest percentage weight loss was 4.5% in seeds treated with pawpaw powdered extract at a 5% application rate (Figure5).

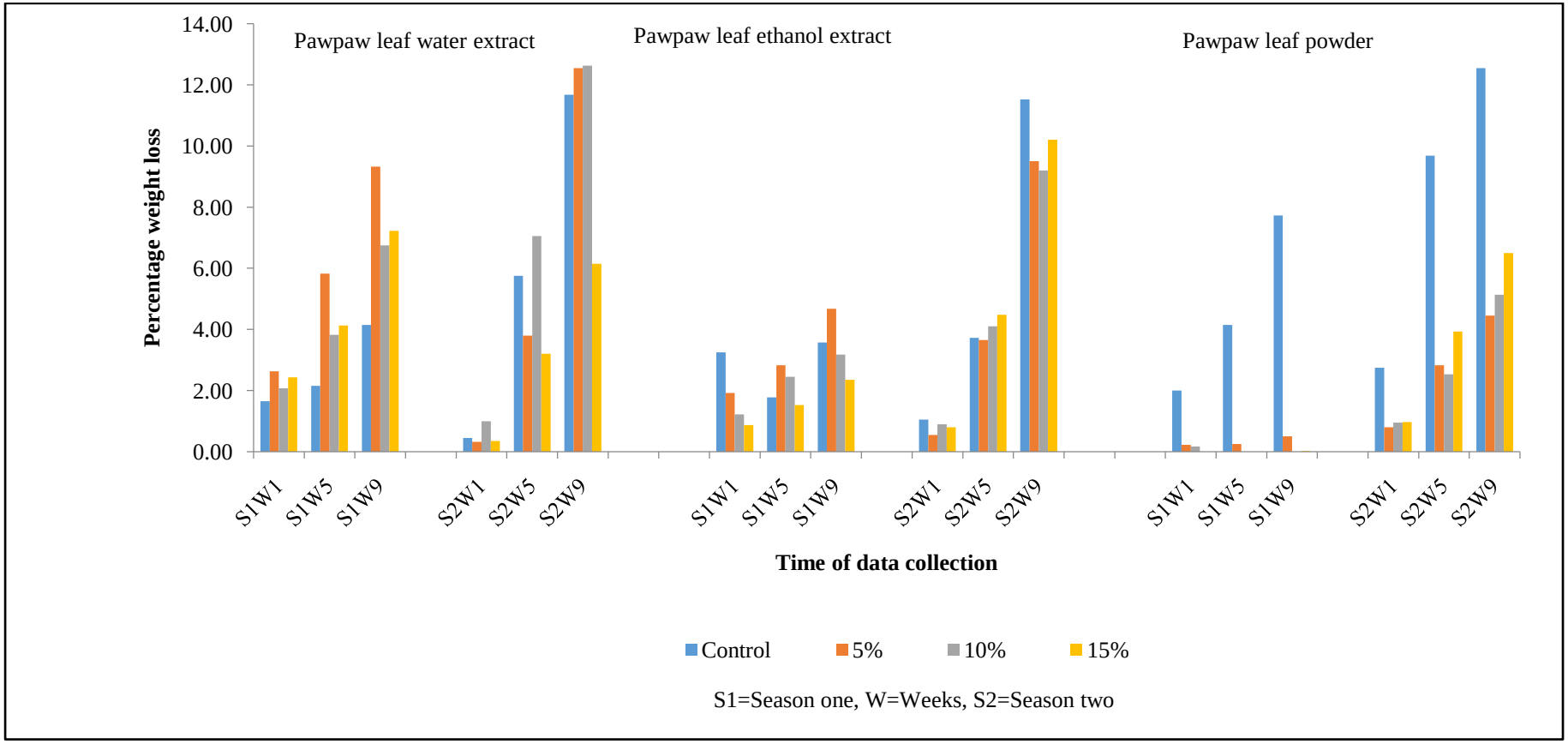

Figure 5: Effect of pawpaw leaf water, ethanol and powder extracts on the percentage weight loss of beans

4.4 Effect of pawpaw leaf powder on germination of selected bean seed varieties after storage.

Seeds treated with pawpaw leaf powder did not show any significant variation (P>0.05) in seed germination of the different bean varieties and control (Table 4). There were no much variation in the seed germination in both the treated seeds and controls for both season one and two. This was observed in the different varieties of NABE 4, NABE 6 and golden brown varieties. In season one, the highest germination was observed in the control where no plant powders were applied in the NABE 4, NABE 6 and golden brown bean seeds. The germination percentage for the bean seeds were 87.5±15.0 (golden brown), 87.5±9.6 (NABE 4)87.5±5.0 (NABE 6) at three months treatment (Table 4).

The lowest seed germination in season one was 7.75±0.9 for NABE 6 powder treated beans. Pawpaw leaf powder did not have any significant effect (P>0.05) on the seed germination as compared to control where no powder was applied and other bean varieties. No significant difference was also observed in the same variety. In season two, the highest seed germinability was recorded in NABE 4 at 95.0±5.7% and the lowest seed germination was 75.0±10.0% for NABE 6 treated beans. This was not significantly different (P>0.05) with other treated varieties and their controls (Table 4).

Table 4. Effect of pawpaw leaf powder on germination of the different bean varieties over storage time.

Germination of different bean varieties				
Bean variety	Season	No. of germinated Seeds	No. of un germinated Seeds	Germination percentage
Golden brown	1	8.00±0.82[a]	2.0 ± 0.82[a]	80.0±8.16[a]
Golden brown (control)	1	8.75±1.50[a]	1.3±1.50[a]	87.5±15.00[a]
Golden brown	2	7.75±0.50[a]	2.3±0.50[a]	77.5±5.0[a]
Golden brown (control)	2	9.00±1.16[a]	1.0±1.16[a]	90.0±11.55[a]
NABE 4	1	8.00±0.82[a]	2.0±0.82[a]	80.0±8.16[a]
NABE 4 (control)	1	8.75±0.96[a]	1.3±0.96[a]	87.5±9.57[a]
NABE 4	2	9.50±0.58[a]	0.5±0.58[a]	95.0±5.77[a]
NABE 4 (Control)	2	8.75±2.50[a]	1.3±2.50[a]	87.5±25.00[a]
NABE 6	1	7.75±0.96[a]	2.3±0.96[a]	77.5±9.57[a]
NABE 6 (Control)	1	8.37±0.92[a]	1.6±0.92[a]	80.0±15.12[a]
NABE 6	2	7.50±1.00[a]	2.5±1.00[a]	75.0±10.00[a]
NABE 6 (Control)	2	8.50±1.60[a]	1.5 ±1.60[a]	85.0±16.04[a]

Means that do not share a letter are significantly different. Numbers in brackets indicates the time frame (months) the powder was used to preserve the beans

CHAPTER FIVE

DISCUSIONS

5.1Concentration of selected secondary metabolites in pawpaw leaf powder and liquid extracts with bio-pesticide properties.

Tannins (both total free and condensed tannins) have been linked to reduction in protein digestion in feeds (Gupta and Verma, 2011), making them an ideal component of bio pesticides. High tannin concentration such as in pawpaw leaf powder for accession two (M-2) could therefore have been responsible for the reduction of weevil effects on both the final weight and weight loss of bean seeds. Another study also found that tannins acted as feeding deterrents against many insect pests in plants (Achakzai, *et al.*, 2009). When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores (Haruna Ada *et al*., 2015). Low tannin content may therefore not offer sufficient deterrence against weevil damage in beans storage, hence limit the efficacy of the bio-pesticide. This may explain why liquid extracts were less effective in reducing weevil numbers and damage to the seeds compared to powdered treatments (Table 2 and 3).

High concentration of flavanoids such as in the pawpaw liquid extract of plant accession five (M-5) could be linked to higher bio pesticide efficacy against bean bruchids and thus increased seed protection against damage during storage (Figure 1-4). In another study on cotton, high flavonoid content was directly linked to reduced larval development of Lepidoptera pests (Nix and Colgrave, 2017), whereas low flavonoid content allowed the larvae to establish on plants. This suggests that low flavonoid content in bio pesticides renders them ineffective (Mierziak and Kulma, 2014). Flavanoids have adverse effect on the feeding, development and survival rates of beetles, resulting in higher mortality rate in adult beetles of maize (Deepak *et al*., 2012). This was observed in Figure 1 where the highest mortality rate of weevils was recorded due to higher flavanoid concentration in the leaves whereas the lowest concentration of flavanoids such as in plant accession seven (M-7) may have been too low to have any adverse effect on mortality rate of weevils in storage.

The highest concentration of total phenolics in plant accession two of powdered treatments (Figure 1) corresponded to high insect mortality (Figure 1) and feeding deterrence (Figure 3) on weevils in stored beans. This implies that high content of phenolics renders bio-pesticides effective against pests while low phenolic content such as in liquid extracts of accession four

(M-4), corresponded with higher weevil numbers and damage to the bean seeds, implying low bio pesticide efficacy (Mamphiswana and Mdee, 2010). Another study reported the ecological roles of phenolic compounds which induced toxicity and feeding deterrence in insect pests (Napal *et al*., 2010).

The high alkaloid concentration in pawpaw accession (M-4) of the liquid extract (Table 3) could be linked to higher bio pesticide efficacy against been weevils whereas the low alkaloid content such as in pawpaw accession one (M-1) could have reduced the efficacy of the bio pesticide against weevils (Ge *et al*., 2015). In one study, higher concentration of alkaloids caused larval mortality of many pests and this may be due to the effect of alkaloids on protein kinase that is involved in signal transduction and development process of most cells and tissues of insect pests (Lvovich, 2014, Qadir, 2014). Alkaloid extracts from plants have also been found to cause high mortality on the adult insect pests (Habimana and Hakizayezu, 2014). Therefore, alkaloids at a higher concentration were more effective in protecting seeds from weevils' damage, weight loss of seeds (Figure 3 and 5).

The highest concentration of anti-oxidant activity in powdered treatment of pawpaw accession three (M-3) could have contributed to bio pesticide efficacy by regulating oviposition and deterring feeding of bean weevils, whereas low antioxidant activity such as pawpaw accession M-4 may be linked to reduced bio pesticide efficacy against the bean weevils. In another study, Naringenin, hesperetin-7-*O*-rutinoside and quercetin-3-*O*-rutinoside, along with other active antioxidative compounds, reduced oviposition in swallowtail butterfly *Papilio* on young leaves of citrus plants (Mierziak, 2014). Another study also found that extracts of *Curcuma amada* had high antioxidant and corresponding bio pesticide properties (Bera and Dutta, 2015). In this study higher anti-oxidant activity corresponded with reduced weevil damage on bean seeds (Table 3), weight loss (Figure 5) and reduction in F1 progeny emergence (Figure 2). From this study, *Carica papaya* leaves contained alkaloids, Saponins, tannins, and flavonoids which are strong antioxidants, which is in agreement with the work by (Khan, 2017).

5.2 Effectiveness of pawpaw leaf water, ethanol and powder extracts as bio-pesticides against bean weevils over time.

5.2.1 Effectiveness of pawpaw leaf powder and liquid extracts on the number of dead and live weevils.

The highest weevil mortality (19 weevils) observed at the pawpaw leaf powder treatment level of 15% may directly be attributed to contact toxicity on both larvae and adult weevil populations. In a similar study, high concentrations of pawpaw extract were found to be effective against mustard aphids (Uijan and Shazad, 2014). The pawpaw leaf powders have also been found to have detrimental effects on *Callosobruchus maculatus* pests at a high application rate of 7.5%. It was further postulated that higher application rates resulted in higher toxicity to bean weevils due to increasing secondary metabolites, some of which have detrimental effects on the weevils (Ge *et al*., 2015). The findings from this study are also in agreement with Ratnasekera and Rajapakse, 2012 who discovered that root dust of papaya had produced the highest mortality rate on adult maize weevils due to contact. The insect mortality due to higher powder application rates (10 and 15%) could also be attributed to the fact that insects breathe by means of trachea that usually open at the surface of the body through spiracles and these might have been blocked by the plant powders thereby leading to suffocation of adult weevils (Adedire *et al*., 2011).

At low application rates of pawpaw leaf powder (5%), it may be concluded that there was no sufficient contact between the bio-pesticide powder and the weevils, allowing for survival and continued weevil activity. This has also been found in another study where low pawpaw powder application rates resulted minimal deterrence on insect pests (Uijan *et al*., 2014).

On the other hand, pawpaw leaf water extracts had the lowest mortality due to deterrence on bean weevils compared to ethanol, possibly due to the low solubility of toxic secondary metabolites in water. This therefore could have allowed weevils to continue breeding and reproducing, hence causing damage to beans in storage. This was observed in another study, where pawpaw leaf ethanol extracts resulted in higher mortality on termites, than water extracts at similar application rates (Utami and Tuapattinaya, 2017).

A number of authors have also reported the efficacy of pawpaw leaf powder and extracts in the control of insect pests. Pawpaw leaf extracts were effectively used in the control of white flies in okra (Zobayer, 2013). The present observation also corroborate the report by Figueroa-Brito *et al.*,2011 who reported that *Carica papaya* seed extracts are used to control

fall armyworm (*Spodoptera frugiperda*) in maize crop due to their toxic effect and mortality on their larvae and adult insects.

The higher live weevil numbers in the pawpaw leaf water extract experiments could therefore be associated with low concentrations of secondary metabolites in the water extracts, which left the beans with minimal protection (Figure 2). This could possibly be a result of low solubility of toxic compounds from pawpaw leaf into water (Baroacha and Shahzad, 2014). In another study, the ethanol extracts of *Vitex negundo L* and *Momordica charantia L* were found to be more effective against rice weevils than the water extracts, at similar application rates, implying differences in polarity of solvents, hence variations in extracting capabilities (Hasnat and Ahmad). Without any form of detterence, bruchids have the ability to multiply very first and cause serious damage in untreated beans in storage. The weevil numbers were observed to increase with time in the control experiment as well because of minimal deterrence coupled with the high rate of fecundity in weevils with each adult female bruchid capable of laying 60 to 70 eggs in as short period of time (Ebinu *et al.*, 2015).

5.2.2 Effect of pawpaw leaf powder and liquid extracts on the number of damaged seeds, final weight and weight loss in bean seeds

The high number of damaged seeds in both the control and water treatment at 5% indicates lack of sufficient deterrents to the feeding of weevils in both treatments. Moreover, most secondary metabolites have low solubility in water (Mapari *et al*., 2005; Awah *et al.*, 2017), making the 5% application rate very minimal against weevil control. Another study also found that more polar solvents such as methanol, acetone and ethanol had more extracting power on secondary metabolites in plants than water, owing to the high solubility of the metabolites in the polar solvents (Altemimi and Lightfoot, 2017). In this regard, the 5% application rate could have lacked sufficient lethal doses of metabolites to cause significant grievous harm to the weevils, thus reduce on seed damage. In other findings, secondary metabolites were found to be more soluble in methanol than water, making methanolic extracts of *Ocimum basilicum* very effective against weevils (Marie *et al.*, 2017).

Pawpaw leaf ethanol extracts were more effective in reducing the number of damaged seeds, when applied at a 15% rate. This may be attributed to the attainment of lethal doses of secondary metabolites to the bean weevils at that rate. These findings are similar to those where pawpaw leaf ethanol extract efficacy on termites increased with rate of application at 20, 40 and 70% (Utami *et al*., 2017). Another study also confirmed the higher solubility of

secondary metabolites in ethanol than water (Jones and Kinghorn, 2006), implying that it is easier to attain lethal doses of plant extracts when ethanol is used rather than water in bio pesticide making. At 10% treatment level, pawpaw powder provided the best protection on seeds from the weevils' damage possibly due to lethality of inherent metabolites resulting in low feeding pressure on the seeds, hence less damage (Mandudzi and Edziwa, 2016). The efficacy of the pawpaw powder at that application rate (10%) may also be attributed to outright suffocation of the weevils due to blockage of the spiracles (Adedire *et al*., 2011).

The seeds treated with water extracts had low final weights, owing to the unchecked feeding by weevils. This may have resulted from low solubility of insecticidal secondary metabolites in water, resulting in minimal deterrence on weevils (Utami *et al*., 2017). On the other hand, the ethanol extracts were more effective than water extracts in reducing weevil numbers and their larvae, enabling relatively low damage on seeds, hence a stable final weight (Hasnat *et al*.). The pawpaw leaf powder was the most effective in controlling weevils during storage, hence resulting in less damage on seeds and minimal weight loss on the beans, due to high concentration of lethal secondary metabolites (Mandudzi and Edziwa, 2016). This was also found in another study where high alkaloid content in extracts had significant feeding deterrence against the gypsy moth larvae on stored seeds (Vonnie *et al.*, 2008).

The degree of weight loss in beans due to bruchid damage was quite variable and depended on the storage period and treatment used. The highest percentage weight loss of beans from week 5 to week 13 for both the control and water extract at 5% application rate may be attributed to minimal feeding deterrence due to lack of or low concentrations of secondary metabolites (Musa and Adewale, 2014). The lowest weight loss observed in bean seeds could be due the effectiveness of pawpaw leaf powder at the higher application rates (10 and 15%), corresponding with high concentration of lethal secondary metabolites in powder as compared to the liquid extracts. These powders inhibit locomotion, cause suffocation which affects mating activities as well as deterring females from laying eggs; some of the metabolites further suppress the developmental stages of insects as (Ileke and Oni 2011). All these modes of work protect the seeds from damage and eventual weight loss.

5.3 Effect of pawpaw leaf powder on seed germination of selected bean varieties

There was no significant difference in the germination ability of seeds treated with pawpaw leaf powder and the control experiment. However, the control had slightly higher germination percentage than treated seeds which therefore suggests that the pawpaw leaf powder had a

slight effect on seed germination. However, another study refutes these findings and reported significant improvements in germination percentage for bean seeds treated with pawpaw leaf extracts at low application rate than the control (Masangwa and Aveling, 2017). Similarly, Okwute *et al.,* 2009 discovered that the leaf powdered of *Dalbergia saxatilis* treated with seeds does not affect the germination and viability of the cowpeas seeds during storage. Pawpaw leaf powder does not affect germination of seeds because it provided complete protection of seeds from pest damage. This was also reported by Mehdi, 2012 where plant powders provide protection of seeds from infestation by bruchids, by protecting it from damage hence maintaining the viability of seeds. Seed viability maintenance properties of pawpaw leaves could be a result of its inhibitory effect on the seed borne fungi that could limit the germination ability of the seeds (Khanh *et al.,* 2007).

There was some variation in seed germination among the different varieties (Golden brown, Nabe 4 and Nabe 6), though this could be attributed to the differences in vigour among the varieties (Masangwa *et al*., 2017). In addition, the variation in germination could be attributed to differences in resistance to fungi among the varieties, whereby some varieties are more susceptible, hence giving slightly lower germination percentages (Haruna Ada *et al*., 2015). Overall, this study demonstrated the untapped potential that pawpaw leaves have in controlling bean bruchids while maintaining seed viability. The findings have also clearly showed the differences in liquid and papaw powder bio pesticide efficacy in the control of storage pests.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study found that pawpaw leaf powder had the highest concentration of secondary metabolites than the liquid extracts, implying a higher potential for use as bio-pesticides against bean bruchids. The efficacy of ethanol extract in seed protection was short lived possibly due to the volatility of ethanol, which may have affected potency of the bio-pesticides. This implies that ethanol bio-pesticides are not ideal for long term storage of seeds. On the other hand, the pawpaw leaf powder extract had the potential of protecting seeds up to five months with minimal damage in storage, which is comparable to the storage season of seeds by most small holder farmers. The effectiveness of powder and liquid extracts, in seed protection, was observed to be dose dependant with 10% concentration generally being the most effective application rate. Pawpaw leaf powder was most effective in the control of bean weevil populations than the liquid extract possibly due to the higher concentration of secondary metabolites in powder. In addition, the powder extract did not have any significant effect on seed germination, implying that it can be used for protecting with seeds for a complete season. For optimum results, the bean seeds should be treated with powder immediately after harvesting and drying them to avoid the bruchids eggs carry over from the field to the storage. In conclusion, admixing the powder of *Carica papaya* is recommended as affordable, easily available and may provide suitable alternative to the synthetic insecticides for small-scale farmers in rural environment in management of *A. Obtectus.*

6.2 Recommendations

Morphotypes 2, 3 and 5 that had the highest concentration of the different secondary metabolites should be exploited further for making bio-pesticides. The extraction and isolation of these secondary metabolites for commercial bio-bio-pesticides should be done on these selected accessions. A comparative study should be done on the efficacy of pawpaw leaf methanolic extract and powder on insect mortality over different storage times of up to six months. This could form a basis for a commercial bio-pesticide formulation.

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APPENDIX

Efficacy of pawpaw leaf powder and liquid extracts as bio-pesticides in the control of bean weevils in storage.

Pawpaw powdered extracts

Pawpaw leaf powder applications

5% powder 10% powder 15% powder 0% (Control)

Fresh pawpaw leaf extracts

Pawpaw liquid ethanol extract

5% Extract 10% Extract 15% Extract 0% (Control)

Pawpaw liquid water extract

5% Extract 10% Extract 15% Extract 0% (Control)