POSTHARVEST LOSSES AND POPULATION DYNAMICS OF BRUCHIDS IN RESISTANT COMMON BEAN (*Phaseolus vulgaris* L.) GENOTYPES

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EXTENDED ABSTRACT

Introduction

Tanzania is a leading country in common bean production in Africa, and it ranks 5th among top producers in the world. Dry beans are essential sources of human dietary protein, calories, vitamins, micronutrients and income. Postharvest losses are relatively high due to infestation by storage insect pests (40-80%). The bean bruchids namely *Acanthoscelides obtectus* and *Zabrotes subfasciatus* inflicts economic losses in stored dry common beans. This study aimed at reducing postharvest losses by evaluation of resistant bean genotypes against major bean bruchids *Acanthoscelides obtectus* and *Zabrotes subfasciatus.* Specifically, the study sought to: (i) determine the effect conferred by resistant bean lines on the life stages and population dynamics of major bean bruchids and (ii) evaluate selected common bean lines for resistance to major bean bruchids under the farmer's storage conditions.

Methods

Determination of the effect conferred by resistant bean lines on the life stages and population dynamics of major bean bruchids, the study was laid out in a 2x7 factorial experiment arranged in a completely randomized design (CRD), with two (2) factors and four (4) replications. Factor A entailed the backcrossed common bean genotypes with seven (7) levels i.e., five (5) resistant lines; AO-1012-29-3-3A, 65/44-30-2-3A-1, ROBC (8-54) AA, 30/59-96-2-3A-1, ROBC (14-34) AA CIAT and two (2) susceptible control lines: 44-NJANO and 59-SOYA. While Factor B included the major bruchids species of common bean with two (2) levels i.e., *A. obtectus* and *Z. subfasciatus*. The quantitative data were subjected to analysis of variance. Mean separation test was conducted using Duncan's Multiple Range Test ($\alpha = 0.05$).

The promising bean lines AO-1012-29-3-3A and ROBC (8-54) AA which had the lowest number of holes per grain and emerged adults were on farm evaluated for the period of 90 days as per specific objective 2.

Evaluation of selected common bean lines for resistance to major bean bruchids under the farmer's storage conditions, the study was laid out in a 2x4 factorial experiment arranged in randomized complete block design (RCBD) with two (2) factors and five (5) replications (randomly selected farmers per agroecological zone). Factor A entailed the common bean genotypes at four (4) levels i.e., two (2) resistant lines; AO-1012-29-3-3A, ROBC (8-54) AA, and two (2) susceptible lines; 44-NJANO and 59-SOYA as control. While Factor B consisted the Agroecological zones at two (2) levels namely; Southern Highlands Zone (Nambala village in Mbozi district, Songwe region) and Northern Zone (Mungushi village in Hai district, Kilimanjaro region). Each bean genotype weighing 0.75 kg was placed in a 1 kg brown paper bag and stored in farmers' warehouse under natural infestation of bruchids. The quantitative data for number of holes per grain, number of emerged adults, percentage grain damage and percentage weight loss were subjected to analysis of variance. Mean separation test was conducted using Duncan's Multiple Range Test ($\alpha = 0.05$).

Findings

The results showed that, bruchids infesting AO-1012-29-3-3A had significantly ($p<0.001$) highest number of days from larva to pupa (31), and pupa to adult emergence (17) at 90 days after infestation (DAI) than other genotypes. AO-1012-29-3-3A and ROBC (8-54) AA had significantly ($p<0.001$) lowest number of holes per grain (0.3; 0.4), number of emerged adults (0.3; 0.4), emerging adults' percentage (0.3%; 0.4%), surviving adults (0.3; 0.4), and percentage adult survival (0.3%; 0.4%) at 90 DAI than other genotypes.

The highest number of emerged *A. obtectus* adults occurred the earliest (at the 6th week $\approx$ 42 days) in bean genotypes 44-NJANO (64 adults) and 59-SOYA (48 adults). While the lowest number of emerged *A. obtectus* adults were observed (at the 8th week $\approx$ 56 days) in the bean genotype AO-1012-29-3-3A (2 adults) followed by ROBC (8-54) AA (4 adults) and 65/44-30-2x3A-1 (7 adults). The highest number of emerged *Z. subfasciatus* adults occurred the earliest (at the 5th week $\approx$ 35 days) in bean genotypes 44-NJANO (133 adults) and 59-SOYA (108 adults). While the lowest number of emerged *Z. subfasciatus* adults were observed (at the 8th week $\approx$ 56 days) in the bean genotype ROBC (8-54) AA (4 adults). Regardless of the agroecological zones, the bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA had significantly lowest weight loss (0.23%; 0.65%), seed damage (4.1%; 4.25%), number of emerged adult bruchids (2.4; 2.9) and number of holes per grain (0.42; 0.3), respectively than 44-NJANO and 59-SOYA. While the bean genotype 59-SOYA had significantly highest weight loss (11.5%), seed damage (45.0%), number of emerged adult bruchids (111.9) and number of holes per grain (8.9).

Conclusions

The study aimed at reducing postharvest losses by evaluation of resistant bean genotypes against *A. obtectus* and *Z. subfasciatus.* Out of seven bean genotypes evaluated for bruchids resistance under laboratory conditions, genotype AO-1012-29-3-3A and ROBC (8-54) AA were observed to have highest resistance to *A obtectus and   Z subfasciatus* by delayed lava development, reduced number of hole and number of survived adults as compared to the control variety 44-NJANO and 59-SOYA Under farmer's storage conditions, resistant bean lines AO-1012-29-3-3A, and ROBC (8-54) AA presented lowest means for number of holes per grain, minor means of emerged bruchids, lowest seed damage and percentage weight loss, indicating antibiosis type of resistance as compared to susceptible checks 44-NJANO and 59-SOYA in a period of three months of storage.

Recommendations

This work demonstrates the superior resistance of the common bean lines AO-1012-29-3-3A, and ROBC (8-54) AA on the life stages and population dynamics of the major bruchids pests (*A. obtectus* and *Z. subfasciatus*) of common bean. The results from this study on the performance of bruchid resistant beans is important strategy for reduction of postharvest losses of stored common beans in Tanzania and other bean growing regions where bruchid is a problem. With these high qualifications, the results of the recent trials under farmers' storage conditions both at Nambala village in Mbozi district, Songwe region (Southern Highland Zone) and Mungushi village in Hai district, Kilimanjaro region (Northern Zone) indicate that major bruchids-resistant common bean lines developed at SUA can be stored for long at different agro-ecological zones and give farmers food security and excess for sale at favorable price. Thus, likely to bring significant benefits (towards reduction of postharvest losses) to bean farmers in Tanzania where these bruchid species are major damaging pests. Hence, further efforts may be of importance to ensure a significant access of these common bean lines to farmers in Tanzania and other bean growing regions facing similar constraints linked to the major bruchid species globally.

DECLARATION

I, JOEL GEORGE KAMINYOGE do here declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work which has been done within the period of registration and that it has neither been submitted nor concurrently submitted for a degree award in any other institution.

_____   _____

Joel George Kaminyoge   Date

(MSc Candidate)

The above declaration is confirmed;

_____   _____

Prof. Paul Mbogo Kusolwa   Date

(Supervisor)

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Lastly but not least I thank my wife Teofrida Maurice Mayaya, my daughters Nancy, Ntuli, Nelusigwe, and my sons Nsajigwa, Nuhu and Noel for their prayers and mutual support during the whole period of my study may Almighty God bless them.

DEDICATION

I dedicate this dissertation to my beloved parents Mr. Menard George Kaminyoge (Passed away in June 1996) and Mrs. Loina John Bukuku for bringing me up with affection and for sending me to school.

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LIST OF ABBREVIATIONS AND SYMBOLS

CNSL   Cashew Nut Shell Liquid

CRD   Completely Randomized Design

FAOSTAT   Food and Agriculture Organization Statistics

MT   Metric Tons

PHLs   Postharvest losses

SUA   Sokoine University of Agriculture

Yr   Year

SSA   Sub Saharan Africa

Ca   Calcium

IPM   Integrated Pest Management

Fe   Iron

Mg   Magnesium

Zn   Zinc

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

The common bean (*Phaseolus vulgaris* L.), is an important leguminous crop that is directly consumed worldwide, particularly in the Latin America and Sub-Saharan Africa (Padgham *et al.,* 1992; Broughton *et al*., 2003). Common bean is a native of the South and Central America, whereby it is also the origin of domestication (Purseglove, 1988 cited in Kifle, 2017). The crop is widely cultivated throughout the world in various countries in regions that lie from 35º S to 50 º N, and altitude of up to 3000 meters above sea level (Fivawo and Nchimbi-Msolla, 2011). It thrives in a wide range of environments in temperate, sub tropic and tropical regions. The common bean was introduced from America and carried into high altitude regions in Tanzania approximately 300 years ago (Fivawo and Nchimbi-Msolla, 2011).

Common beans production is constrained by several factors including abiotic and biotic (diseases and insect pest) stresses, which contribute to the reduction of yield and seed quality (Hillocks *et al.,* 2006; Oliveira *et al.,* 2008). Moreover, the major bruchids namely; common bean bruchid, *Acanthoscelides obtectus* (Say), and Mexican bean bruchid, *Zabrotes subfasciatus* (Boheman) are the most important pests contributing to common bean storage losses (Keneni *et al.,* 2011; Tigist *et al*., 2017).

The bruchids specie *A. obtectus* has high fecundity and is more aggressive compared to *Z. subfasciatus* in stored common bean (Kananji, 2007). The risk of attack by these two species is the principle reason of farmers' failure for storing beans (Schoonhoven, 1976; Belmain and Stevenson, 2001). Many of the bruchids species have crossed the

geographical boundaries and have become cosmopolitan in distribution through human-mediated migrations and import/export of food grain (Southgate 1979; Thakur, 2012).

Generally, the great damage by this major bruchids starts by ovipositing process, whereby after mating the adult female bruchids lay their eggs on the surface of mature pods or directly on the seed testa, and they hatch into larvae. However, as soon after being hatched, *A. obtectus* larvae burrow through the pods or seed, and finally feed in nutritious bean cotyledons in contrast to *Z. subfasciatus* larvae that burrow directly into the seed to reach the nutritious cotyledon. For the whole period during metamorphosis, the larvae remain in the seed until when they emerge to continue with the life cycle. The damage is associated with the number of larvae that hatch and burrow into, and feed profusely within the seed (Howe and Currie, 1964; Southgate, 1979; Mwila, 2013).

Environmental manipulations to discourage the growth, development and reproduction of storage insect pests are among the options that have proved effective control. The control measures like the use of chemical and botanical insecticides, cultural, physical, and biological control methods are among the manipulations that can be employed. Synthetic chemical pesticides are effectively used against storage insect pests but are inseparably allied with a number of drawbacks including high costs and concerns about environmental pollution and food safety (Abate and Ampofo, 1996; Cork *et al*., 2009; Baributsa *et al.,* 2014). Moreover, the storage structures under small-scale production conditions in the Tropics and Sub-tropics are built within the same houses where families live. This reduces the suitability of using insecticides for control of storage insect pests. There is no economic threshold level for storage insect pests, unlike field insects. Hence, insecticides are applied as a prophylactic measure which aggravates the problem by destroying the population of predators and parasitoids of the target insect pests (Keneni *et al.,* 2011).

Guzzo *et al*. (2015) reported that, seed-based technologies are easier to transfer to small-scale farmers than more complex knowledge-based agronomic and crop protection practices. Therefore, for the effective control of the major bruchids, host resistant varieties are highly required. Once the cultivars resistant to storage insect pests become available, their adoption by the majority of resource-poor farmers would be expected because their use involves little additional cost (Keneni *et al.,* 2011; War *et al*., 2017).

1.2 Economic importance of common beans

The global bean production was approximately 26 8000 000 MT (Celmeli *et al*., 2018). Annually, Africa produces 4 800 000 MT and approximately 70% are produced from Sub-Saharan Africa region (Binagwa *et al.,* 2018). Tanzania is the leading common bean producer in Africa with an estimated average annual production of 1 140 444 MT, and ranks the 5th among top producers of common bean in the world (Table 1.1; FAOSTAT, 2019). According to Karane (2016), in Tanzania the average bean productivity is around 594.45 kg/ha for local varieties and 695.44 kg/ha for improved bean varieties.

Table 1.1: Common bean production in EAC and other big producers in the world

		Global Dry Bean production (in Metric Tons)										
S/N	Country	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
1	Brazil	3169356	3461194	3486763	3158905	3435366	2794854	2892599	3294586	3090095	2615832	3033017
2	Burundi	205 196	189 661	207 272	201 551	200 673	205 944	225 003	251 761	282 978	371 892	379 861
3	India	3930000	3010000	2430000	4890000	4330000	3710000	3630000	4230000	4260000	3897611	6390000
4	Mexico	993 943	1122720	1041350	1156 51	567 779	1080857	1294634	1273957	969 146	1088767	1183868
5	Tanzania	889 293	570 750	773 720	867 530	675 948	1199267	1113541	1114500	1201922	1140605	1140444
6	Uganda	435 000	912 000	925 000	949 000	915 445	869 607	941 182	1011435	1012446	1008410	1024742
7	Kenya	429 839	265 006	465 363	390 598	577 674	622 759	714 492	615 992	765 000	728 160	846 000
8	Rwanda	329 000	308 000	326 532	327 497	331 166	432 857	438 236	415 259	434 077	437 673	455 822
9	DRC.Congo	112 250	113 240	114 239	115 237	238 124	247 196	248 075	248 957	249 405	242 739	225 1 35
10	Colombia	156 236	160 883	147 017	136 626	129 672	138 324	142 163	149 112	136 847	108 867	117 498

Source: FAOSTAT (27/06/2019)

1.3 Nutritional importance of common beans

Bean is among the important food legumes consumed worldwide (Wortman *et al*., 1998). It has a great impact on food security and nutrition to people both in rural and urban households in developing countries, especially in Sub-Saharan Africa (SSA) (Namugwanya, 2014). Globally, dry beans play a great role as essential source of human dietary protein (20-25%), calories (60-65%), vitamins, and are the major sources of micronutrients (Ca, Fe, Mg and Zn) necessary for the health of communities (Geil and Anderson, 1994; Hillocks *et al.,* 2006; Larochelle *et al*., 2015; Celmeli *et al*., 2018). Regular intake of common beans has medical benefits such as; lowering the risks of cancer, diabetes, heart diseases and helps with weight management (Heller, 2019).

1.4 Common bean production in Tanzania

In Tanzania, common bean is mainly grown in medium to high altitude areas. The major production areas are situated in the Northern zone (Arusha, Manyara, Kilimanjaro and Tanga regions); the lake zone and the western regions (Kagera and Kigoma regions) and the southern highlands (Mbeya, Songwe, Rukwa, Katavi, Njombe and Iringa regions) (Katungi *et al*., 2009).

Common bean is an important crop mainly for smallholder farmers in Tanzania, for home consumption and cash income (Letaa *et al*., 2015). It is roughly estimated that, smallholder farmers mainly women operate 1 to 5 acres, and over 70% of the national bean production in Tanzania is for own consumption and about 40% of the harvests are marketed by households to earn money for various daily uses (Wortman *et al*., 1998; Binagwa *et al*., 2018).

Common bean is cultivated primarily for its green, shelled and dry seeds (Fivawo and Nchimbi-Msolla, 2011). Although, young tender leaves are often used as fresh vegetables. Also, bean has diverse uses, such as it supplements cereals in staple foods including maize, sorghum, rice and others (Gepts *et al*., 2008). Apart from its dietary potential, the crop has also gained importance as a source of income where the surplus is being marketed as fresh or dry bean. It also helps in shortening the hunger periods as well as providing quick cash (Wortman *et al*., 1998; Binagwa *et al*., 2018).

1.5 Constraints of common bean storage

Postharvest losses (PHLs) of common beans are higher without postharvest management. The PHLs in common bean are attributed to various factors such as physical (during harvesting and processing), technical (poor crop produce handling), poor storage structures, and biological factors (including storage pests and diseases), with the latter being the principle cause of these losses (Keneni *et al.,* 2011). Storage insect pests such as the major bruchids namely; common bean bruchid (*Acanthoscelides obtectus*), and Mexican bean bruchid (*Zabrotes subfasciatus*) are amongst the most serious constraints to common bean production at global level, in the field and storage, particularly in the humid tropic and subtropic countries (Keneni *et al.,* 2011; Thakur, 2012; Tigist *et al*., 2017).

Storage insect pests may cause an estimated dry weight loss of 10–40% and up to 80% grain damage/quality loss of which makes the beans less suitable for human consumption (Kiula and Karel, 1985; Paul *et al*., 2009). Previously, losses ranging between 7% and 73% were reported in Colombia, Kenya and Tanzania (Songa and Rono 1998: Mwila 2013). Usually, the risk of attack by these two major bruchid species is the principle reason for farmers' and store owners' failure to store beans (Schoonhoven, 1976; Belmain and Stevenson, 2001). The effect of both species is to reduce the quality and quantity of

beans, rendering them unfit for human consumption and germination (Kananji, 2007; Thakur, 2010).

1.6 The storage insect pest of common bean

Bruchids belong to the order Coleoptera in the family Bruchidae. Bruchids occur in several genus and species, namely, *Acanthoscelides obtectus, Zabrotes subfasciatus, Callosobruchus maculatus, C. rhodesianus, C. analis* and *C. chinensis* (Abate and Ampofo, 1996). They inflict damage to dry beans both in the field and storage (Thakur, 2012). Also, they are normally found in all major land masses except the Antarctica and New Zealand (Southgate, 1979). The internal feeding style of bruchids enhances their probability of introduction in new places. Major Bruchid species may be transported across the geographical boundaries as hidden pest in legume import/export consignments (Thakur, 2012). According to Southgate (1979) and Thakur and Renuka (2014), among the old world (Asia and Africa) species that have reached the Americas in seeds are those of the successful genus *Callosobruchus,* and those spread from Central and South America include *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say). However, in Southern Africa *C. maculatus* and *C. rhodesianus* are the most prevalent (Southgate, 1979).

The *Z. subfasciatus* infests dry bean in storage, while *A. obtectus* infests beans both in the field and store. The larvae of these major bruchids feed on the seeds which leads for seed grains to be unfit for human consumption, reducing their quality and hence marketability furthermore destroying them or reducing germination capacity (Misangu *et al.,* 2007; Blair *et al.,* 2010). *Acanthoscelides obtectus* prefers cooler climates at higher altitudes, where it is the dominant specie. However*, Zabrotes subfasciatus* prefers warmer climates in the lower altitudes and therefore, it is more important in the tropics and subtropics

(Schoonhoven, 1976; Abate and Ampofo, 1996; Thakur and Renuka, 2014). *Zabrotes subfasciatus* and *Acanthoscelides obtectus* are more prevalent and of high economic importance for common bean farmers throughout the world, particularly in certain Sub-Saharan countries such as Malawi and Tanzania (Kananji, 2007). They are characterized by a high rate of reproduction and a short developmental period, enabling them to multiply rapidly and inflict damage (Southgate 1979; Thakur and Renuka, 2014).

The common bean bruchid *A. obtectus* is a small, brown, slightly striped insect, covered with short hairs. The adult is only about 3-5 mm long. It has dark mottling, while its legs and antennae are slightly reddish. The wing cases are short and do not quite reach the tip of the abdomen (Thakur, 2012). Adults of *A. obtectus* measure 3.81±0.39 mm long and 2.05±0.36 mm wide with greyish brown colour. Its head is greyish brown and mouth parts blackish in colour. Antennal segments 1 to 4 filiform, segments 5 to 10 broadened and more serrated, and the segment 11 non-serrated and acute apically. Its segments are 1-5 grey, 6-10 dark blackish, and segment 11 red orange in colour (Thakur, 2012). The morphological features of *A. obtectus* are illustrated in (Plate 1.1).

Plate 1.1: Morphological features of *A. obtectus*
Source: SICTA (2010)

The Mexican bean bruchid *Z. subfasciatus* is about 1.8 to 3.5 mm in body length, which makes it to be the smallest of the bruchids commonly infesting stored legume grain seeds (Credland and Dendy, 1991). The adults of *Z. subfasciatus* have strong sexual dimorphism. The elytra are short, relatively broad and together are somewhat square in shape. The elytra of the female are strongly marked with a pattern of white and pale grey setae on a dark (almost black) background, while that of the male has rather uniform light grey-brown pubescence (sometimes mottled with darker brown) over a dark-grey cuticle. On the apex of the tibia of each hind leg there are two movable spurs, called calcaria, which are reddish in color and equal in length (Kifle, 2017). The males are small in size with the length ranging from 2.44 to 2.83 mm, and female ranges from 3.23 to 3.69 mm (Arora, 1977). The morphological features of *Z. subfasciatus* are illustrated in (Plate 1.2).
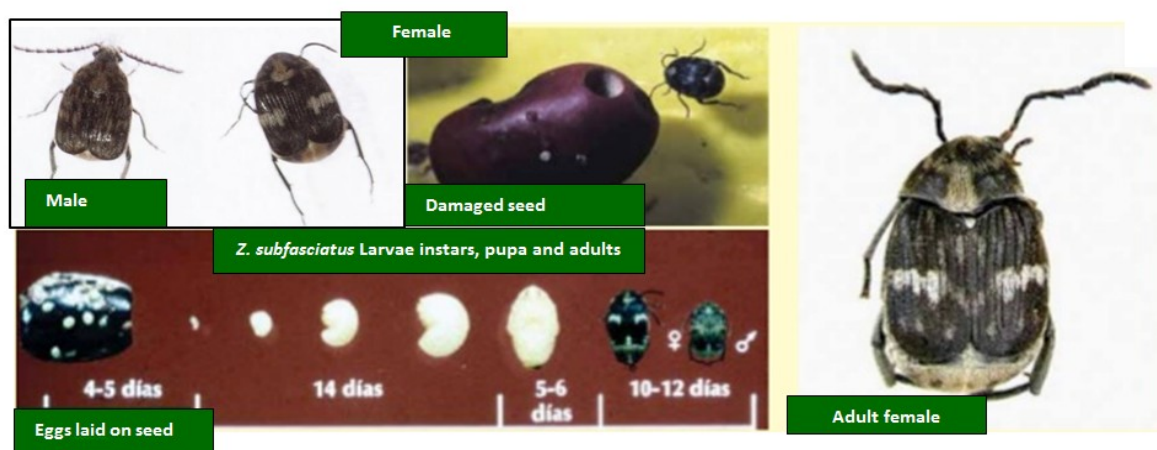
Plate 1.2: Morphological features of *Z. subfasciatus*

Source: SICTA (2010)

1.7 Common beans bruchids control strategies

1.7.1 Cultural control

The cultural practices make the host environment less attractive and unconducive for the growth and multiplication of the major bruchid species (Mishra *et al*., 2017). According to

Van Huis (1991); Prakash *et al.* (2016), despite the success of chemical methods of storage pests control, still farmers are using traditional methods of storage because such practices are not only user-friendly but also increases shelf life of food grains and seeds. Also, are within the technical and financial means of the small-scale farmers.

In most cases, it involves timely harvesting, sun drying at weekly interval, and removal of infested grains before storage, removal of eggshells and dead larvae and proper cleanness of the crop products. Moreover, maintain store hygiene structures and facilities through fumigation and disinfestation. Also, white-washing, painting the walls, floors and ceilings of empty stores with insect repellent paints can be used to control bruchids (Cork *et al.*, 2009; Mishra, *et al.*, 2017).

Some inert dust like rock phosphate, lime, sand, wood ash, tobacco, saw dust, and clays, are extensively used worldwide. Inert dust fills the spaces between stored seeds of the crop. Although considered dirt, inert dust restricts number of progeny emergence and finally death (Tripathy, 2016; War *et al.*, 2017).

1.7.2 Biological control

The biological control agents include; predators, parasitoids and pathogens, and the control strategies focus on the importation, augmentation and conservation. Moreover, various species are presented in formulations of natural enemies of the pests. Beneficial biological agents, as formulated products, are applied to the grains infested with stored pests, but has not been successful with time and space (Mishra *et al.*, 2017).

According to Zaugg *et al.* (2013), fewer and smaller bruchids emerged in the presence of parasitoids. The noted parasitoid species that have shown an effective and beneficial

control of the bruchids includes; Hymenoptera (*Dinarmus spp*) and wasps (*Anisopteromalus clandrae*) and wasps in the genus Horismenus, like, *H. missouriensis, H. depressus* and *H. butcheri* are also inclusive (Yamane, 2013; Kenyon, *et al.,* 2015; Soundararajan *et al.,* 2012). However, smallholder farmers face a number of difficulties in usage of biological method on storage insect pests, as it requires high skills and is costly (Kananji, 2007). In most cases the combination of biological agents and host plant resistance in a compatible manner with due consideration of the ecology in the control of major bruchid species, *A. obtectus* and *Z. subfasciatus* gave extremely promising results (Schmale *et al.,* 2003; Velten *et al.,* 2008).

1.7.3 Chemicals

1.7.3.1 Pesticidal plants

The use of plant extracts or biocides in control against storage insect pests is an old practice especially for resource poor farmers in the entire tropic regions across the world (Rajapakse and Van Emden. 1997; Shaaya and Kostyukovysky, 2006). Furthermore, Okwute (2012) insisted that, the plant-based products are cheap and bio-degradable. Therefore, are environmentally friendly. However, Okwute (2012) reported that, the need to use plant-based products arises from the fact that the synthetic pesticides are harmful to humans and the entire ecosystem following inappropriate uses. They also associated with high toxicity, persistence and too expensive for the resource poor farmers in the least economy countries of the world. Neem oil and Cashew Nut Shell Liquid (CNSL) of plant origin are as effective as insecticides in bruchids control and will not cause any residual effect on the seed. Nevertheless, it has high degradation effect of about 96% within 28 days only (Raja, 2015). The use of dust, wood ashes in spaces between seeds provide some control of bruchids, although not highly effective, and relatively labourious for resource-poor farmers (Murdock *et al.,* 2003). In addition, botanical extracts to some

extent affect non-targeted organisms, but bring less effect as compared to if someone is using synthetic chemicals (Tripathy, 2016; War *et al.*. 2017).

According to Cork *et al*. (2009), the use of ash or other explants dusty biocides are associated with tainting and discolorations of seeds. All these types of admixtures are inconvenient as they require cleaning of the beans before cooking or marketing. Stevenson *et al*. (2016) reported that, *Zanha africana* root bark is used by small scale farmers throughout sub-Saharan Africa including Tanzania, to protect stored cowpea grain from bruchids, such as *Callosobruchus maculatus*. Chloroform, methanol and water extracts of *Z. africana* root bark inhibited oviposition and caused significantly higher mortality of *C. maculatus* at a rate of application equivalent to that applied by farmers compared to control insects (Stevenson *et al.*, 2016). This method is probably most appropriate for the storage of pulses for planting (Kiula and Karel, 1985).

In other hand, the efficacy of plant materials is highly variable even within plant species depending on variety, season and location. Also, tends to break down when exposed to sunlight example azadirachtin and pyrethrin. In some cases when frequently used, leads to pesticides residue accumulation and bitter taste of the oil discouraged farmers from its application. Insect pests' resurgence and ultimate resistance, cause detrimental effects to non-targeted beneficial insects and other living organisms that are in place (Cork *et al.*, 2009; Okwute, 2012; Raja, 2015).

1.7.3.2 Synthetic chemicals

According to Abate and Ampofo (1996), synthetic insecticides have been recommended for the control of storage insect pests however, their use is limited under resource poor farming community conditions due to high costs, and infrequent supply. In a broad sense,

the industrial made insecticides are harmful, and their usage has detrimental effects to human health, especially when used where storage compartments are built within family living houses (Keneni *et al*., 2011; Okwute, 2012).

Other results (Cork *et a*l., 2009; War *et al*., 2017) have indicated that, although the control of insect pests relies heavily on the use of synthetic insecticides such as methyl bromide or phosphine, carbon sulfide and other formulations intended for fumigation, and insecticides used by small scale farmers in households such as Actellic supper dust (Pirimiphos-methyl + Thiamethoxam) and Shumba super dust (Fenitrothion /organophosphate + Deltamethrin/pyrethroid). Their intensive use has led to the development of direct and residual toxicity to predators, pollinators, fish, man, and the ozone layer (Cork *et al.,* 2009).

Apart from being harmful to non-targeted organisms, if one type of chemical is used continuously against storage insect pest, undesirable consequences may result. These includes; genetic resistance by insect pest leading to pest resurgence on that particular chemical, and accelerates the damage to stored crops (Williams and Hammitt, 2001; Bruce, 2010). With these drawbacks associated with the use of industrial synthetic insecticides host plant resistance through the use of improved common bean cultivars against bruchids offers a sustainable environmentally friendly solution (Keneni *et al*., 2011). Host plant resistance, is a principal method for insect pest control which is effective, practical, and low cost to farmers. High levels of resistance to bruchids have recently become available in newly developed bean genotypes (Kusolwa *et al*., 2014). Resistance is simply inherited dominant gene that can be rapidly backcrossed into local varieties of common beans (Kifle, 2017).

1.7.4 Bruchids host resistance and the mechanisms

Resistance phenomenon in insect pests has a specific number of resistance mechanisms, and is usually due to antixenosis, antibiosis, tolerance, non-preference and escape. Tolerance and escape are resistance mechanisms applicable for field infestations but not for storage insect pests of grain crops (Keneni *et al.,* 2011; Mwila, 2013). Previous research work concerning the resistance mechanisms of plant tissues against bruchids strongly suggest many strategies that are used by seeds to protect themselves against insects, that is; seed may be too hard for newly hatched larva to penetrate, may physically be too small or with an inconvenient shape for the larva to reach full size, seed may contain too little food to support the larva or the seed may contain toxins or other substances that can impair or inhibit the larval development (Kashiwaba *et al.,* 2003). The mechanisms of antibiosis and other forms of resistance are discussed in detail below.

1.7.4.1 Antibiosis

Antibiosis is the phenomenon where host plant resists insect attack and has an adverse effect on the binomics of the insect pest. This adversely affects the development and reproduction of insects. This is related to the chemical and biological constituents of the seed such as the presence of certain amino acids which are linked to the trait of resistance to the bruchids (Schoonhoven and Voysest, 1991 cited in Mwila, 2013). Dry bean contains significant amounts of seed storage proteins used for embryo and seedling development, as well as for defense against seed pests. Some of the well-known and important storage proteins in common bean seeds includes: phaseolin, lectins, phytohaemagglutinins (PHA), trypsin inhibitors, and lectin-like proteins that include arcelins and α-amylase inhibitors. Antibiosis properties of arcelins are anticipated to be due to the lysis of epithelial cells of the intestines. Arcelins bind to the carbohydrate moieties of these proteins as well as poorly digestible by gut proteases of the bruchids (Janarthanan *et al.,* 2002). Antibiosis

may not only be explained as a bio-chemical phenomenon, but it also involves physical components. These are known as the surface texture and structure of the seed coat, which affects larval penetration. Bruchids prefers smooth coated seeds to wrinkled seeds for oviposition, and more first instar larvae successfully penetrate the seed coat in smooth than in rough seeds (Kitch *et al*., 2011).

1.7.4.2 Phaseolin

Phaseolin is an important source of essential amino acids residing in beans for animal nutrition, and unlike other bean seed storage proteins, it is not associated with an antibiosis effect to insect pests. Phaseolin is among the most extensively studied major storage protein of common beans (Brown *et al*., 1982; Gepts, 1988) and has been used to explain the evolutionary affiliation of different germplasm pools/collections within *P. vulgaris* (Gepts, 1988; Kami *et al*., 1995). The level of phaseolin in the seed coat (16.7%) was found to be sufficient to prevent larval development of bruchids. The expression of a *C. maculatus*-detrimental protein in the testa of non-host seeds suggests that the protein may have played a significant role in the evolutionary adaptation of bruchids to legume seeds (Lattanzio *et al*., 2000; Mwila, 2013).

1.7.4.3 Lectins and lectin like proteins (LLP'S)

In addition to phaseolin, the second most common group of seed proteins in common beans are the loosely called lectins or PHAs, as well as additional lectin-like proteins (Osborn *et al*., 1988b; Chrispeels and Raikhel, 1991). Lectins are groups of proteins possessing at least one non catalytic domain which binds reversibly to a specific mono or oligosaccharide. They have been considered as defensive compounds against cowpea bruchid even if toxic effects of active lectins in some cases could be due to an α- amylase inhibitor presence (Pedra *et al*., 2003).

As seed storage proteins, they accumulate in cotyledons and provide a reserve for amino acids required in seed germination, and seedling development. PHA is the major lectin of beans and functions as a carbohydrate binding protein that defends plants against predation by most organisms, but is less effectively against cow-pea bruchid *C. maculatus* (Murdock *et al.*, 1990). Yet PHA may have a synergistic effect when combined with other anti-nutritional storage proteins in inhibition activity to predatory insects. PHA is an anti-nutritional factor for mammals because it binds to the glycoproteins that line the intestinal tract thus inhibiting nutrient absorption (Broughton *et al.*, 2003). Similarly, protease inhibitors in bruchids were suggested as potential anti-nutritional restraints to larvae of *A. obtectus* and result in delayed growth and development (Campos *et al.*, 2004).

1.7.4.4 Arcelins

A protein arcelin is postulated as the factor responsible for resistance in the wild *P. vulgaris* types. According to Kusolwa (2007), seven arcelin variants have already been described from various accessions of wild common bean based on amino acid sequence. Genetically, the variants are different alleles of the same locus discovered by different researchers: *Arl-1, Arl-2, Arl-3, and Arl-4* (Osborn *et al.*, 1986; 1988b; Hartweck *et al.* 1997), *Arl-5* (Lioi and Bollini, 1989; Goossens *et al.* 1994) *Arl-6* (Santino *et al.* 1991) and *Arl-7* (Acosta-Gallegos *et al.* 1998). Infestation studies were also previously conducted at CIAT using different bean lines developed by the University of Wisconsin, for the presence and absence of the arcelin. Those lines positive for arcelin were resistant to *Z. subfasciatus* but susceptible to *A. obtectus*. Bean lines without arcelin were susceptible to both species (CIAT, 2005).

Different scientists have conducted research on arcelins. The earliest research was performed by Osborn *et al.* (1986) whereby they discovered that arcelins associated with

inhibition of development of some bruchid species. It was first found in a limited number of wild common bean accessions from Mexico (Osborn *et al*., 1988a, Osborn *et al*., 1988b). The discovered showed that arcelins are abundant seed storage proteins and they were associated with inhibition of development of some species of bruchids. The polypeptides for arcelins are closely related to Phytohaemmaglutinin and α-amylase inhibitors. Arcelins have different intrinsic specificity for complex sugars that make it toxic to bruchids (Minney *et al*., 1990). Cardona *et al*. (1990) observed high levels of resistance of Arcelins to bruchids through a delay in adult emergence and larvae mortality especially in first and second instar.

The wild tepary beans were noted to contain variants of arcelin proteins. Furthermore, this protein is absent in cultivated common bean (Chrispeels and Raikhel, 1991). Based on complimentary DNA sequence homology, arcelin can be placed into six variants group into three clusters. The first cluster consists of Arl-1, Arl-2 and Arl-6, the second cluster consists of Arl-3 and Arl-4 and the last one consists of Arl-5. These variants have different levels of resistance, Arl-1 and Arl-5 have high resistance against *Z. subfasciatus* while, Arl-4 and Arl-2 comprise high resistance to *A. obtectus* (Hartweck *et al*., 1997).

Additional and more advanced work on arcelin-like proteins were also achieved. Kusolwa (2007); Mbogo *et al*. (2009); Kusolwa and Myers (2010, 2011, 2012) demonstrated the superior resistance common bean backcross lines to *A. obtectus* conferred by the presence of the APA proteins introgressed from tepary bean. Also, both the interspecific backcross progenies and G40199 contain arcelins and arcelin-like (ARL2) proteins that are co-expressed with other proteins of the arcelin, α-amylase inhibitor and phytohemagglutinin (APA) locus demonstrating the presence of multiple arcelin-like proteins as the major seed storage proteins. The occurrence of these multiple variants of the APA seed storage

proteins in a single accession, presented an important opportunity for exploration of antibiosis proteins in breeding for resistance to bean bruchids, major storage pests in common beans in the tropics.

1.7.4.5 Trypsin inhibitors and tannins

Tannins, hydrolysable tannins and condensed proanthocyanidins are large polyphenolics whose molecular weights range from 500 to 4000 kDa and whose many hydroxyl groups interact with proteins, denaturing and precipitating them from solution (Haslam, 1998). Tannins may affect the growth of insects in three main ways: they have an astringent taste which affects palatability and decreases feed consumption, they form complexes with proteins of reduced digestibility and they act as enzyme inactivators (Swain, 1977). Seed tissues contain tannins located mainly in a layer between the outer integument and the aleurone layer, while α-amylase inhibitors are located in cotyledons (Lattanzio *et al.*, 2000).

1.7.4.6 Lipids

Lipids have been found to be responsible for some resistance in beans. The influence of bean seed surface lipids on infestation of seeds by *A. obtectus* was investigated. The fatty acids and monoacylglycerols groups prevent bean bruchid infestation, while alkanes, sterols acted as attractants (Nietupski *et al.*, 2005).

1.7.4.7 Non-preference

Non-preference is a phenomenon where certain plants are less attractive to the pest for oviposition or feeding because of their texture, colour, odour or taste, seed size, seed coat thickness (Nwanze and Horber, 1976; Brewer and Horber, 1983). This makes the plant unsuitable for colonization or oviposition of an insect.

Previous studies on oviposition preferences of bruchids have shown bruchid species exhibited a marked preference for large seeded materials when mixtures of bean seeds of all sizes were infested. This resulted in many small seeded materials escaping infestation and a bias toward selecting large seeded types (Schoonhoven and Voysest, 1991).

It has been confirmed that physical factors such as seed coat hardness and seed coat roughness confer resistance to bruchids (Giga and Smith, 2002). A hard seed coat may prevent larvae from successfully penetrating the seed, while a rough seed coat provides difficulties in oviposition for *Z. subfasciatus* in particular, because it glues its eggs on the seed testa. Rough seeds are therefore less preferred for oviposition (Nwanze and Horber, 1976; Messina and Renwick, 1985).

1.7.4.8 Antixenosis

Antixenosis refers to non-preference of the insect pest due to unsuitability of the host for oviposition, growth and/or survival due to some morphological or biochemical factors in the host. Morphologically, varieties with smooth, soft and thin seed coats may be more preferable for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Shaheen *et al*., 2006; Ahmed *et al.,* 1986 cited in Keneni, 2011).

1.7.5 Transfer of genetic resistance to host plant

In an effort to enhance resistance to common bean bruchids, evaluation of bean germplasm has identified seed proteins of the lectin-like family, called arcelins, found in wild common beans. High levels of resistance have been reported in four arcelin variants (Arl-1, 2, 4 and 5), resistance levels were only maintained in lines generated from crosses with Arl-2 and Arl-4 parents, but arcelin-1-containing accessions have not been identified as resistant, probably because the arcelin-1 allele occurs at low frequencies in wild

accessions containing this variant (Osborn *et al.,* 1986; Osborn *et al.,* 1988). The discovery of arcelin has facilitated and accelerated the breeding of bruchid resistance in common beans (Hartweck *et al.,* 1997; Hartweck and Osborn, 1997).

Genomic DNA sequences from wild accession G40199 which is highly resistant to the two major bruchid pests of common bean and the interspecific hybrids revealed a high sequence similarity to arcelin and ARL2 and α-amylase (α-AI) genes of *P. acutifolius*. The lectin-related proteins of *P. acutifolius* have been associated with strong resistance to bruchids, thus these proteins alone or in combination with other factors, may contribute to the unclear bruchid resistance mechanism in G40199 (Mbogo *et al.,* 2009).

Arcelin (ARL2) protein from a wild tepary bean accession G40199 which was transferred into common bean cultivars ICA Pijao and Rojo (backcross lines with homozygous expression of arcelin and ARL2 proteins of tepary bean) has shown the clear delay of 63 days for 50% adult emergence, reduction in size, weight and the number of emerged F1 of *A. obtectus* adults (Kusolwa and Myers, 2011).

Presence of multiple arcelin-like proteins as the major seed storage proteins was reported (Kusolwa and Myers, 2012; Kusolwa *et al.,* 2016). Peptide peaks and their amino acids sequences demonstrated that both the interspecific backcross progenies and wild accession (G40199) of *P. acutifolius* contain arcelins and arcelin-like (ARL2) proteins that are co-expressed with other proteins of the arcelin, α-amylase inhibitor and Phytohemagglutinin (APA) locus. A multiple virus and bean bruchid-resistant bean line (AO-1012-29-3-3A) adapted to the humid tropics had the three bands associated with the presence of ARL2, α-AI, and PHA in the complex APA locus. Therefore, the developed bean line with multiple virus and bean bruchid-resistance can help to reduce yield loss during the growing season and avoid seed damage in storage (Kusolwa *et al.,* 2016).

Furthermore, the absence of phaseolin from common bean lines increased arcelin concentration, subsequently improved resistance to Mexican bean bruchid (Hartweck *et al.*, 1997). Seed of bean genotypes with the phaseolin null alleles has also been reported to possess higher levels of cysteine and methionine (Taylor *et al.*, 2008). Previous study showed that, one of the common bean lines (AO-1012-29-3-3A) from parent MARC-2-PN-1 has an arcelin-like seed storage protein with size corresponding to 33kD in tepary bean accession G40199 (Kusolwa *et al.*, 2016). These seed of the common bean bruchid resistant line AO-1012-29-3-3A had 15% more cysteine, methionine (10.7%), at least 10% greater levels of threonine, proline, analine, valine, lysine and crude protein in the seed.

1.7.6 Integrated management of bruchids

The combined use of resistant cultivars, with other approaches like the cultural control techniques such as timely and frequent harvesting, legumes planted away from granaries, crop hygiene and storage in pods, are within the technical and financial means of the small-scale farmers (Van Huis, 1991). This practice may reduce the resistance of storage insect pests and eliminate the need for synthetic insecticides in the management of major bruchid species of *A. obtectus* and *Z. subfasciatus* (Yamane, 2013).

The uses of predators to feed on the bruchid hatched larvae in the stored common bean crops have shown an effective and beneficial control of the bruchids, but the great limitation of this approach is that it require high skills and investments (in raising parasitoids). In other hand it can work well in a combination with host resistant cultivars. Moreover, the use of groundnut, cashew nut shell liquid, or coconut oils has shown success in a control of major storage insect pests although have been noted to inhibit seed germination (Mishra *et al.*, 2017).

The combination of a new technology, Purdue Improved Crop Storage (PICS) triple-layer hermetic storage bags, may provide an improved alternative for insecticide-free, long-term storage of common beans with minimal grain damage. Although, its great drawbacks lie at their availability and cost which become difficult for the resource poor farmers to access them (Mutungi *et al.,* 2015). The combinations of the stated approaches with resistant cultivars would furthermore support Integrated Pests Management against the *A. obtectus* and *Z. subfasciatus* and leads in the reduction of using industrial synthetic insecticides or fumigants (Yamane, 2013).

1.8 Justification of the Study

The long period storage of common bean at small-scale subsistence farming levels in the Sub Saharan African region is limited by various factors including bruchids species *Z. subfasciatus* and *A. obtectus* (Jones *et al*., 2011). Bruchids infestations lead to heavy losses in terms of quality, weight and nutritional value (Thakur, 2010; Keneni *et al.,* 2011). Postharvest losses due to storage pests are both qualitative and quantitative (Kananji, 2007). The losses in dry bean grains due to bruchid damage range from 40% weighty losses and 73 to 80 % quality losses for cultivars which are more susceptible to major bruchids, especially in poor post-harvest management (Songa and Rono, 1998; Silim, 1990 cited in Mwila, 2013).

To save their crops against storage pests, farmers employ several options including the use of bio-pesticides, synthetic insecticides, and selling-off excess beans immediately after harvest (Belmain and Stevenson, 2001; Kifle, 2017). Synthetic insecticides were reported by Okwute (2012); Keneni *et al*. (2011) that, regardless their availability but they are expensive, and their application at higher doses leads to the accumulation of toxic residues in treated products and are environmentally undesirable, as the bean crop is mostly

intended for family consumption (War *et al*., 2017). They are harmful to human health especially when used in storage compartments or structures built within a family living houses (Cork *et al.,* 2009; Keneni *et al*., 2011). According to Evans (1985); Egwuatua (1987); Talukder (2009), the bruchids showed resistance to various pesticides including permethrin, lindane, pirimiphos-methyl, phostoxin, methyl bromide and iodofenphos.

The control of storage insect pest in different legume crops could be achieved by improving the genetic resistance of the host plant as an effective and environment-friendly management option. The use of bruchids resistant cultivars will enable farmers to store their grain longer with less storage costs and reduced post-harvest loss and sell their grain at a high price (Keneni *et al.,* 2011). Resistance may be justifiable if beans can be kept out from bruchid damage for at least 60-90 days or longer after harvest (Kusolwa *et al.,* 2007). Furthermore, successful bruchid-resistant cultivars, result in reduced usage of chemical pesticides and increased activity of natural biocontrol agents, and thus sustained yield by culminating losses in storage. The use of host-plant-resistant cultivars will also form the backbone of integrated bruchid pest management in the future (Mishra *et al.,* 2017).

Cultivars bred for resistance to insect pests has further advantages in that the technical knowledge and the existing farmers' practices may need only some minor modification with the adoption of new cultivars. Seed-based technologies can easily be transferred to small-scale farmers than more complex knowledge-based agronomic and crop protection practices. In addition, host resistance may be used not only as an independent element but also as a component of integrated pest management (IPM), because it is compatible with cultural, chemical, physical and biological control measures (Yamane, 2013; Kifle, 2017).

1.9 Objectives of the study

1.9.1 Overall objectives

This study aimed to reduce postharvest losses inflicted by major bean bruchids in stored dry common beans.

1.9.2 Specific objectives

  (i)   To determine the effect conferred by resistant bean lines on the life stages and population dynamics of major bean bruchids

  (ii) To evaluate selected common bean lines for resistance to major bean bruchids under the farmer's storage conditions

1.10 Organization of this Dissertation

This dissertation is developed in publishable manuscripts format consisting four chapters. Chapter one is general introduction of the dissertation, chapter two and three consisted manuscripts in form of publishable papers. Chapter four is the general conclusion and recommendations.

References

Abate, T. and Ampofo, J. K. O. (1996). Insect pests of beans in Africa: Their ecology and management. *Annual Review of Entomology* 41(1): 45 – 73.

Acosta-Gallegos, J. A., Quintero, C., Vargas, J., Toro, O., Tohme, J., Cardona, C. (1998). A new variant of arcelin in wild common bean, *Phaseolus vulgaris* L., from southern Mexico. *Genetic Resources and Crop Evolution* 45:235 – 242.

Arora, G. L. (1977). Taxonomy of the Bruchidae (Coleoptera) of Northwest India. Part I. Adults. *Oriental Insects* 11(7): 1 – 132.

Baributsa, D., Djibo, K., Lowenberg-DeBoer, J., Moussa, B. and Baoua, I. (2014). The fate of triple-layer plastic bags used for cowpea storage. *Journal of Stored Production Research* 58: 97 – 102.

Belmain, S. and Stevenson, P. (2001). Ethnobotanicals in Ghana: Reviving and modernizing age-old farmer, practice. *Pesticide Outlook* 12: 233 – 238.

Binagwa, P. H., Magdalena, W., Michael, K., Zakayo, E., Mbiu, J., Msaky, J., Mdachi, M., Kasubiri, F., Kisamo, A., Nestory, S. M. and Rubyogo, J. C. (2018). Selian Agricultural Research Institute Released Seven (7) Improved Common Bean Varieties.

Blair, M. W., Prieto, S., Díaz, L. M., Buendía, H. F. and Cardona, C. (2010). Linkage disequilibrium at the APA insecticidal seed protein locus of common bean (*Phaseolus vulgaris* L.). *BioMed Central Plant Biology* 10(1): 79.

Brewer, I. N. and Horber, E. (1983). Evaluating resistance to *Callosobruchus chinensis* L. in different seed legumes. In: Proceedings of 3rd International Working Conference on Stored Products Entomology, KSU., Manhattan, Kansas, 23-28.

Broughton, W. J., Hernandez, G., Blair, M. W., Beebe, S,. Gepts, P. and Vanderleyden, J. (2003). Beans (Phaseolus spp.)-model food legumes. *Plant Soil* 252: 55 – 128.

Brown, J. W. S., Osborn, T. C., Bliss, F. A. and Hall, T. C. (1982). Bean Lectins, Part 1: Relationships between agglutinating activity and electrophoretic variation in the lectin-containing G2/albumin seed proteins of French bean (*Phaseolus vulgaris* L.). *Theory Applied Genetic* 62: 263 – 271.

Bruce, T. J. (2010). Tackling the threat to food security caused by crop pests in the new millennium. *Food Security* 2: 133 – 141.

Campos, J. E., Whitaker, J. R., Yip, T., Hutchens, T. W. and Blanco-Labra, A., (2004). Unusual structural characteristics and complete amino acid sequence of a protease inhibitor from Phaseolus acutifolius seeds. *Plant Physiology and Biochem,* 42: 209 – 214.

Cardona, C., Kornegay, J., Posso, C. E., Morales, F. and Ramirez, H. (1990). Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil Entomology Exp. Applied 56: 197 – 206.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T. and Toker, C. (2018). The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. *Agronomy* 8(9):166.

Chrispeels, M. J. and Raikhel, N. V. (1991). Lectins, lectin genes, and their role in plant defense. *The Plant cell* 3(1): 1–9.

CIAT (2005). Annual Report. http://www.ciat.cgiar,org/beans/pdfs.

Cork, A., Dobson, H., Grzywacz, D., Hodges, R., Orr, A. and Stevenson, P. (2009). *Review of Pre and Postharvest Pest Management for Pulses with Special Reference to East and Southern Africa.* Natural Resources Institute, University of Greenwich, London. 136pp.

Credland, P. F. and Dendy, J. (1991). Development, fecundity and egg dispersion of *Zabrotes subfasciatus*. *Entomologia Experimentalis et Applicata* 59(1): 9 – 17.

Egwuatua R. I. (1987) Current status of conventional insecticides in the management of stored product insect pests in the tropics. *International Journal of Tropical Insect Science* 8: 695–701.

Evans, J. N. (1985). The effectiveness of various insecticides on some resistant beetle pests of stored products from Uganda. *Journal of Stored Products Research* 21: 105–109.

FAOSTAT (2019). Faostat Data.[http://www.fao.org/faostat/en/#data] site visited on 27/6/2019.

Fivawo, N. C. and Nchimbi-Msolla, S. N. (2011). The diversity of common bean landraces in Tanzania. *Tanzania Journal of Natural and Applied Sciences* 2(1): 337 – 351.

Geil, P. B. and Anderson, J. W. (1994). Nutrition and health implications of dry beans: A review. *Journal of American Coll. Nutrition* 13: 549 – 558.

Gepts, P. (1988). Phaseolin as an evolutionary marker. In: *Genetic Resources of Phaseolus Beans*. (Edited by Gepts, P.), Kluwer Academic Publishers, Netherlands. pp. 215 – 241.

Gepts, P., Aragao, F. J. L., de Barros, E., Blair M. W. and Brondani, R. (2008). Genomics of phaseolus beans, a major source of dietary protein and micronutrients in the tropics. In: *Genomics of Tropical Crop Plants*. (Edited by Moore, P. H. and Ming, R.), Springer, Germany, pp.113 – 140.

Giga, D. P. and Smith, R. H. (2002). Comparative life history studies of four Callosobruchus species infesting cowpeas with special reference to *Callosobruchus rhodesianus* (Pic) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 19 (4): 189-198.

Goossens, A., Geremia, R., Bauw, G., Van Montagu, M., Angenon, G. (1994). Isolation and characterisation of arcelin-5 proteins and cDNAs. European *Journal of Biochemistry* 225: 787 – 795.

Goossens, A., Quitero, C., Dillen, W., De Rycke, R., Valor, J. F., De Clercq, J., Van Montagu, M., Cardona, C. and Angenon, G. (2000). Analysis of bruchid resistance in the wild common bean accession G02771: No evidence for insecticidal activity of arcelin 5. Journal of Exp. Bot. 51: 1229 – 1236.

Guzzo, E. C., Vendramim, J. D., Chiorato, A. F., Lourenção, A. L., Carbonell, S. A. M. and Corrêa, O. M. B. (2015). No correlation of morpho-agronomic traits of Phaseolus vulgaris (Fabaceae) genotypes and resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae). *Neotropical Entomology* 44(6): 619 – 625.

Hartweck, L. M. and T. C. Osborn, (1997). Altering protein composition by genetically removing phaseolin from common bean seeds containing arcelin or phytohaemagglutinin. *Theory Applied Genetic* 95: 1012 – 1017.

Hartweck, L. M., Cardona, C. and Osborn, T. C. (1997). Bruchid resistance of common bean lines having an altered seed protein composition. *Theory Applied Genetic* 95: 1018 – 1023.

Haslam, E., (1998). *Practical Polyphenolics. From Structure to Molecular Recognition and Physiological Action*. Cambridge University Press, Cambridge, UK. 61(11): 1454-1455.

Heller, S. (2019). After-40 Nutrition: The surprising health benefits of beans. [http:/www.doctoroz.com/article] site visited on 18/6/2019.

Hillocks, R. J., Madata, C. S., Chirwa, R., Minja, E. M. and Msolla. S. (2006). Phaseolus bean improvement in Tanzania, 1959–2005. *Euphytica* 150: 215 – 231.

Howe, R. W and Currie, J. E. (1964). Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bulletin of Entomological Research* 55(3): 437 – 477.

Janarthanan, S., Seshadri, S. and Ignacimuthu, S. (2002). Arcelins- a potential new age protein anti-metabolite in legume seed defense against stored product Pests. *Journal of Scientific and Industrial Research* 61: 97 – 102.

Jones, M., Alexander, C. and Lowenberg-DeBoer, J. (2011). *Profitability of Hermetic Purdue Improved Crop Storage Bags for African Common Bean Producers*. Department of Agricultural Economics, Purdue University. 29pp.

Kami, J., Velásquez, B. V., Debouck, D. G. and Gepts, P. (1995). Identification of presumed ancestral DNA sequences of phaseolin in Phaseolus vulgaris. *Proceeding National Academic Science* 92: 1101 – 1104.

Kananji, G. A. D. (2007). A study of bruchid resistance and its inheritance in Malawian dry bean germplasm. Thesis for Award of PhD Degree at University of Kwazulu Natal, South Africa, 187pp.

Karane, S. V. (2016). Factors influencing on-farm common bean profitability: the case of smallholder bean farmers in Babati District, Tanzania. (Doctoral dissertation, Egerton): 1-6pp

Kashiwaba, K., N. Tomooka, A. Kaga, O. K. Han and D. A. Vaughans, (2003). Characterization of resistance to three bruchid species (*Callosobruchus* spp., Coleoptera, Bruchidae) in cultivated rice bean (Vigna umbellata). *Journal of Economic Entomology* 96: 207 – 213.

Katungi, E., Farrow, A., Chianu, J., Sperling, L. and Beebe, S. (2009). *Common Bean in Eastern and Southern Africa: A Situation and Outlook Analysis.* International Centre for Tropical Agriculture, sehemu 61pp.

Keneni, G., Bekele, E., Getu, E., Imtiaz, M., Damte, T., Mulatu, B. and Dagne, K. (2011). Breeding food legumes for resistance to storage insect pests: potential and limitations. *Sustainability* 3(9): 1399 – 1415.

Kenyon, S. G., Buerki, S., Hansson, C., Alvarez, N. and Benrey, B. (2015).Uncovering cryptic parasitoid diversity in Horismenus (Chalcidoidea, Eulophidae). *PloS One* 10(9): e0136063.

Kifle, F. (2017). Studies on the management of *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) on Common beans (*Phaseolus vulgaris* L) using Resistant Varieties and Botanicals Powders "College of Natural and computational sciences department of zoological sciences insect sciences stream. Dissertation for Award of MSc Degree at University Addis Ababa, Ethiopia, 69pp.

Kitch, L. W., R. E. Shade and L. L. Murdock, (2011). Resistance to the cowpea weevil (*Callosobruchus maculatus*) larva in pods of cowpea (*Vigna unguiculata*) article. published on 5th April 2011.

Kiula B. A. and Karel A. K. (1985). *Effectiveness of Vegetable Oils and Other Plant Products in Protecting Beans Against Mexican Bean Weevil (Zabrotes subfasciatus) (Boheman)*. Annual Report No. 28. Bean Improvement Cooperation, New York. 5pp.

Kusolwa, P. M. (2007). Breeding for bruchid resistance in common bean (*Phaseolus vulgaris* L.): Interspecific introgression of lectin-like seed proteins from tepary bean (*P. acutifolius* A. Gray), genetic control and bruchid characterization. Thesis for Award of PhD Degree at Oregon State University, Corvallis, Oregon, USA, pp

Kusolwa, P. M. and Myers, J. R. (2011). Seed storage proteins ARL2 and its variants from the apalocus of wild tepary bean G40199 confer resistance to *Acanthoscelides obtectus* when expressed in common beans. *African Crop Science Journal* 19(4): 255 – 265.

Kusolwa, P. M. and Myers, J. R. (2012). Peptide sequences from seed storage proteins of tepary bean (*Phaseolus acutifolius*) accession G40199 demonstrate the presence of multiple variants of APA proteins. *International Journal of Biochemistry and Biotechnology* 1(1): 12 – 18.

Kusolwa, P. M., Mwatawala, M. W., Mwaitulo, S., Msolla, S. N., Mgembe, E. R., Tryphone, G. M. and Myers, J. R. (2014). Inheritance and performance of bruchid resistance into farmers' preferred common bean (*P. vulgaris*) varieties in Tanzania. Bean Improvement Cooperative conference, held 27 – 29 November 2013- Portland – Oregon, USA.

Kusolwa, P. M., Myers, J. R., Porch, T. G., Trukhina, Y., González-Vélez, A. and Beaver, J. S. (2016). Registration of AO-1012-29-3-3A Red Kidney Bean Germplasm Line with Bean Weevil, BCMV, and BCMNV Resistance. *Journal of Plant Registrations* 1-5.

Larochelle, C., Katungi, E. and Beebe, S. (2015). *Disaggregated Analysis of Bean Consumption Demand and Contribution to Household Food Security in Uganda*. International Center for Tropical Agriculture, Cali, Columbia. 31pp.

Lattanzio, V., S. Arpaia, A. Cardinali, D. Di Venere and V. Linsalata, (2000). Role of endogenous flavonoids in resistance mechanism of Vigna to aphids. *Journal Agriculture Food Chemisty* 48: 5316 – 5320.

Letaa, E., Kabungo, C., Katungi, E., Ojara, M. and Ndunguru, A. (2015). Farm level adoption and spatial diffusion of improved common bean varieties in southern highlands of Tanzania. *African Crop Science Journal* 23(3): 261 – 277

Lioi, L., Bollini, R. (1989). Identification of a new arcelin variant in wild bean seeds. *Bean Improvement Cooperative* 32: 28.

Mbogo, P. K., Myers, J. R. and Davis, J. (2009). Transfer of the Arcelin-Phytohemmaglutinin α-Amylase inhibitor seed protein locus from tepary bean (*Phaseolus acutifolius* A. Gray) to common bean (*P. vulgaris* L.). *Biotechnology* 8: 285 – 295.

Messina, F. J. and Renwick, J. A. (1985). Resistance of *C. maculatus* (Coleoptera: Bruchidae) in selected cowpea lines. *Environmental Entomology* 14: 868 – 872.

Minney, B. H. P., Gatehouse, A. M., Dobie, P., Dendy, J., Cardona, C., and Gatehouse, J. A. (1990). Biochemical bases of seed resistance to *Zabrotes subfasciatus* (bean weevil) in *Phaseolus vulgaris* (common bean); a mechanism for arcelin toxicity. *Journal of Insect Physiology* 36(10): 757 – 767.

Misangu, R. N., Chipungahelo, M. S. and SO, R. (2007). The effects of sowing bruchid damaged bean (*Phaseolus vulgaris*) seeds on germination, plant development and yields. *Journal of Entomology* 4(4): 337 – 340.

Mishra, S. K., Macedo, M. L. R., Panda, S. K. and Panigrahi, J. (2017). Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annals of Applied Biology* 172: 4 – 19.

Murdock, L. M., J. E. Huesing, S. S. Nielsen, R. C. Pratt and R. E. Shade, (1990). Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* 29: 85 – 89.

Murdock, L.L., Seck, D., Ntoukam, G., Kitch, L. and Shade, R. E. (2003). Preservation of cowpea grain in sub-Saharan Africa – Bean/cowpea CRSP contributions. *Field Crops Research* 82: 169 – 178.

Mutungi, C., Affognon, H. D., Njoroge, A. W., Manono, J., Baributsa, D. and Murdock, L. L. (2015). Triple-layer plastic bags protect dry common beans (*Phaseolus vulgaris*) against damage by *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) during storage. *Journal of Economic Entomology* 108(5): 2479 – 2488.

Mwila, N. (2013). Inheritance of bruchid (*Callosobruchus maculatus*) resistance in common beans *(phaseolus vulgaris)*. Dissertation for Award of MSc Degree at University of Zambia, Lusaka, 105pp.

Namugwanya, M., Tenywa, J. S., Otabbong, E., Mubiru, D. N. and Masamba, T. A. (2014). Development of common bean (*Phaseolus vulgaris* L.) production under low soil phosphorus and drought in Sub-Saharan Africa: A review. *Journal of Sustainable Development* 7(5): 128 – 139.

Nietupski, M., B. Szafranek, D. Ciepielewska, E. Synak, L. Fornal, and J. Szafranek, (2005). Correlation between bean seed surface lipids and *Acanthoscelides obtectus* Say development. *Journal of Plant Protection Research* 45:2.

Nwanze, K. and E. Horber, (1976). Seed coats of cowpeas affect oviposition and larval development of *Callosobruchus maculatus*. *Environmental Entomology* 5: 213 – 218.

Okwute, S. K. (2012). *Plants as Potential Sources of Pesticidal Agents: A review*. *Pesticides-Advances in Chemical and Botanical Pesticides*. University of Abuja, Nigeria. 26pp.

Oliveira, L. K., Melo, L. C., Brondani, C., Peloso, M. J. D. and Brondani, R. P. V. (2008). Backcross assisted by microsatellite markers in common bean. *Genetics and Molecular Research* 7(4): 1000 – 1010.

Osborn, T. C., Alexander, D., Sun, S. S. M., Cardona, C. and Bliss, F. A.(1988b). Insecticidal activity and lectin homology of arcelin seed protein. *Science* 240: 207 – 210.

Osborn, T. C., Blake, T., Gepts, P. and Bliss, F. A. (1986). Bean arcelin. *Theoretical and Applied Genetics* 71(6): 847 – 855.

Osborn, T. C., Burrow, M. and Bliss, F. A. (1988a). Purification and characterization of arcelin seed protein from common bean. *Plant Physiology* 86: 399 – 405.

Padgham, J., Pike, V., Dick, K. and Cardona, C. (1992). Resistance of a common bean (*Phaseolus vulgaris* L.) cultivar to post harvest infestation by *Zabrotes subfasciatus* (Boheman)(Coleoptera: Bruchidae). I. Laboratory tests. *International Journal of Pest Management* 38(2): 167 – 172.

Paul, U. V., Lossini J. S., Edwards P. J. and Hilbeck A. (2009). Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. *Journal of Stored Products Research* 45: 97–107.

Pedra, J. H. F., Brandt, A., Westerman, R., Lobo, N., LiH, M., Romero- Severson, J., Murdock, L. L. and Pittendrigh, B. R. (2003). Transcriptome analysis of the cowpea weevil bruchid: identification of putative proteinases and α-amylases associated with food breakdown. *Insect Mol Biol* 12:405–412.

Prakash, B. G., Raghavendra, K. V., Gowthami, R. and Shashank, R. (2016). Indigenous practices for eco-friendly storage of food grains and seeds. *Advance Plants Agriculture Research* 3(4): 1 – 7.

Raja, K. (2015). Comparative performance on insecticidal and oviposition deterrence of cashew nut shell liquid on bruchids (*Callosobruchus chinensis* L.) in cowpea (*Vigna unguiculata* L. Walp.) seed. *Journal of Biopesticides* 8(2): 147 – 153.

Rajapakse, R. and Emden, H. F. (1997). Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpeas by *Callosobruchus maculatus, C. chinesis* and *C. rhodesianus*. *Journal of Stored Production Research* 33: 59 – 68.

Santino, A., Valsasina, B., Lioi, L., Vitale, A., Bollini, R. (1991). Bean (*Phaseolus vulgaris* L.) seed lectins: a novel electrophoretic variant of arcelin. *Plant Physiology (Life Sciences Advances)* 10: 7 – 11.

Schmale, I., Wäckers, F.L., Cardona, C. and Dorn, S. (2003) Combining parasitoids and plant resistance for the control of the bruchid *Acanthoscelides obtectus* in stored beans. *Journal of Stored Products Research* 39: 401 – 411.

Schoonhoven, A. V. (1976). Pests of stored beans and their economic importance in Latin America.

Schoonhoven, A. V. and Voysest, O. (1991). *Common Beans: Research for Crop Improvement*. Commonwealth for Agriculture Bureau International, Wallingford, pp

Shaaya, E. and Kostyukovysky, M. (2006). Essential oils: potency against stored product insects and mode of action. *Stew*. *Post harv*. *Rev*. 4: 1 – 6.

Shaheen, F. A., Khaliq, A. and Aslam, M. (2006). Resistance of chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. *Pakistan Journal of Botany* 38(4): 12 – 37.

SICTA (2010). Identification and Integrated Management Guide: Bean Pests in Central America. IICA, SICTA Network Project and Swiss Cooperation, Managua-Nicaragua, Central America. 45pp.

Songa, J. M. and W. Rono, 1998. Indigenous methods for bruchid beetle (Coleoptera: Bruchidae) control in stored beans (*Phaseolus vulgaris* L.). *International Journal of Pest Management* 44: 1 – 4.

Soundararajan R.P., Chitra N., Geetha S. and Poorani J. (2012) Biological control of bruchid *Callosobruchus maculatus* (F.) in Blackgram. *Journal of Biopesticides* 5: 192–195.

Southgate, B. J. (1979). Biology of the Bruchidae. *Annual Review of Entomology* 24(1): 449 – 473.

Stevenson, P. C., Green, P. W., Veitch, N. C., Farrell, I. W., Kusolwa, P. and Belmain, S. R. (2016). Nor-hopanes from *Zanha africana* root bark with toxicity to bruchid beetles. *Phytochemistry*, 123: 25-32.

Swain, T. (1977). Secondary compounds as protective agents. *Annual Review Plant Physiology* 28: 479 – 450.

Talukder, F. (2009). Pesticide resistance in stored product insects and alternative biorational management: a brief review. *Agricultural and Marine Sciences* 14: 9–15.

Taylor, M., Chapman, R., Beyaert, R., Hernández-Sebastià, C. and Marsolais, F. (2008). Seed storage protein deficiency improves sulfur amino acid content in common bean (*Phaseolus vulgaris* L.): redirection of sulfur from γ-glutamyl-S-methyl-cysteine. *Journal of Agricultural and Food Chemistry* 56(14): 5647 – 5654.

Thakur, D. R. (2010). Invasion and threats of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) to kidney beans in India-a first record. In: *Proceedings of the 10th International Working Conference on Stored Product Protection* pp. 193 – 196.

Thakur, D. R. (2012). Taxonomy, distribution and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae)-A New Record. *Pakistan Journal of Zoology* 44(1): 189 – 195.

Thakur, D. R. and Renuka (2014). Biology and biointensive management of *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae)–a pest of kidney beans worldwide. In: *Proceedings of the 11th International Working Conference on Stored Product Protection* pp. 1 – 13.

Tigist, S. G. (2017). Genetic studies on host plant resistance to Mexican Bean Weevil (*Zabrotes subfasciatus* Boheman) in Ethiopian Common Bean (*Phaseolus vulgaris* L.) Germplasms. Dissertation for Award of MSc Degree at Alemaya University of Agriculture, Ethiopia, 185pp.

Tripathy, S. K. (2016). Bruchid resistance in food legumes-an overview. *Research Journal Biotechnology* 7: 98–105.

Van Huis, A. (1991). Biological methods of bruchid control in the tropics: review. *International Journal of Tropical Insect Science* 12(3): 87 – 102.

Velten, G., Rott, A.S., Conde-Petit, B.J., Cardona, C. and Dorn, S. (2008) Improved bruchid management through favorable host plant traits and natural enemies. *Biological Control* 47: 133 – 140.

War, A. R., Murugesan, S., Boddepalli, V. N., Srinivasan, R. and Nair, R. M. (2017). Mechanism of Resistance in Mungbean [*Vigna radiata* L. R. Wilczek var. radiata] to bruchids, *Callosobruchus* spp. (Coleoptera: Bruchidae). *Frontiers in lant Science* 8(1031): 1 – 11.

Williams, P. R. and Hammitt J. K. (2001). Perceived risks of conventional and organic produce: pesticides, pathogens, and natural toxins. *Risk Analysis* 21: 319 – 330.

Wortman, C. S., Kirby, R. A., Eledu, C. A. and Allen, D.J. (1998). Atlas of common bean (Phaseolus vulgaris L.) production in Africa, Cali Columbia: CIAT publication No. 297.

Yamane, T. (2013). Biorational control methods for protection of stored grain legumes against bruchid beetles. *Agricultural Sciences* 4(12): 762 – 766.

Zaugg, I., Benrey, B. and Bacher, S. (2013). Bottom-up and top-down effects influence bruchid beetle individual performance but not population densities in the field. *PLoS One* 8(1): 1 – 10.

CHAPTER TWO

2.0 THE EFFECT CONFERRED BY RESISTANT BEAN LINES ON THE LIFE STAGES AND POPULATION DYNAMICS OF MAJOR BEAN BRUCHIDS

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2.1 Abstract

Common bean bruchid (*Acanthoscelides obtectus*) and Mexican bean bruchid (*Zabrotes subfasciatus*) are among major storage constraints to common bean (*Phaseolus vulgaris* L.). This study aimed to determine the effectiveness of resistant bean lines on the life stages and population dynamics of the major bean bruchids. The study was laid out as a 2x7 factorial experiment arranged in a completely randomized design (CRD), with two factors and four replications. Factor A entailed the backcrossed common bean genotypes with seven levels i.e., five resistant lines; AO-1012-29-3-3A, 65/44-30-2-3A-1, ROBC (8-54) AA, 30/59-96-2-3A-1, ROBC (14-34) AA CIAT and two susceptible control lines: 44-NJANO and 59-SOYA. While Factor B included the major bruchids species of common bean with two levels i.e., *A. obtectus* and *Z. subfasciatus*. The quantitative data were subjected to analysis of variance. Mean separation test was conducted using Duncan's Multiple Range Test (α = 0.05). The results showed that, AO-1012-29-3-3A had significantly (*p*<0.001) highest number of days from larva to pupa (31), and pupa to adult emergence (17) at 90 days after infestation (DAI) than other genotypes. AO-1012-29-3-3A and ROBC (8-54) AA had significantly (*p*<0.001) lowest number of holes per grain (0.3; 0.4), number of emerged adults (0.3; 0.4), emerging adults' percentage (0.3%; 0.4%),

surviving adults (0.3; 0.4), and percentage adult survival (0.3%; 0.4%) at 90 DAI than other genotypes. The highest number of emerged *A. obtectus* adults occurred most quickly (at the 6th week ≈ 42 days) in bean genotypes 44-NJANO (64 adults) and 59-SOYA (48 adults). While the lowest number of emerged *A. obtectus* adults occurred more delayed (at the 8th week ≈ 56 days) in the bean genotype AO-1012-29-3-3A (2 adults) followed by ROBC (8-54) AA (4 adults) and 65/44-30-2-3A-1 (7 adults). The highest number of emerged *Z. subfasciatus* adults occurred most quickly (at the 5th week ≈ 35 days) in bean genotypes 44-NJANO (133 adults) and 59-SOYA (108 adults). While the lowest number of emerged *Z. subfasciatus* adults occurred more delayed (at the 8th week ≈ 56 days) in the bean genotype ROBC(8-54)AA (4 adults). This work demonstrates the superior resistance of the common bean backcross lines on the life stages and population dynamics of the major bruchids pests (*A. obtectus* and *Z. subfasciatus*) of common bean. The results from this study on the performance of bruchid resistant beans is important strategy for reduction of postharvest losses of stored common beans in Tanzania and other bean growing regions where bruchid is a problem.

Keywords: *Phaseolus vulgaris*, *Acanthoscelides obtectus*, *Zabrotes subfasciatus*, Life Stages, Population Dynamics, Host resistance

2.2 Introduction

Bruchids are the major problems affecting common bean (*Phaseolus vulgaris* L.) seed and grain in storage. The common bean bruchid *Acanthoscelides obtectus* (Say) and the Mexican bean bruchid *Zabrotes subfasciatus* (Boheman) are the two major species of bruchid pests of stored beans (Schoonhoven and Cardona, 1982; Guzzo *et al.*, 2015).

These bruchids multiply fast, depending on ecological conditions the larval and subsequent pupal stages together take about 23 days to complete. Adult bruchids do not

feed, they are short-lived (normally, *Z. subfasciatus* lives shorter than *A. obtectus*) and are weak flyers (Howe and Currie, 1964; Schoonhoven, 1976). Their life cycles last about 28 days for *A. obtectus* and 24 days for *Z. subfasciatus* (Kornegay and Cardona 1991; Mutungi *et al*., 2015). The generalized bruchids life cycle in pods and seeds of dry common beans is illustrated in (Fig. 2.1).
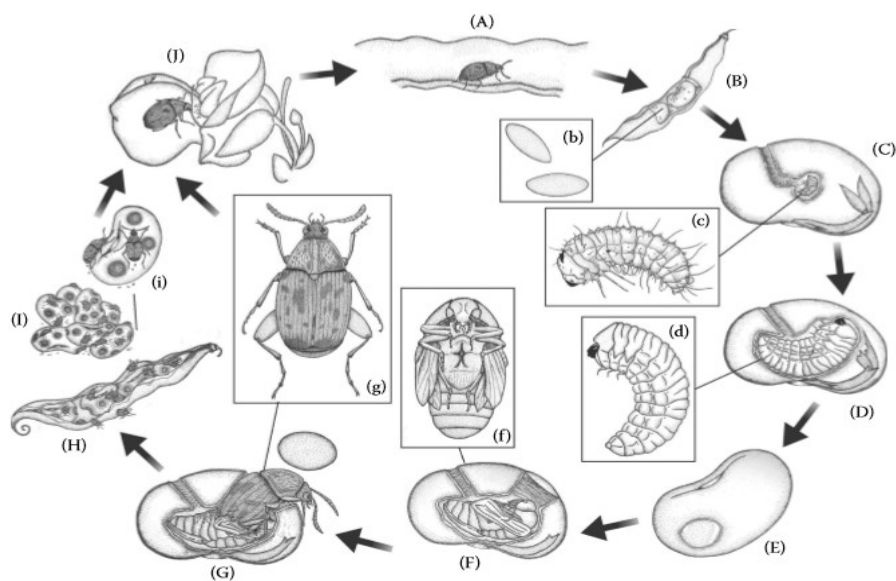
Figure 2 1: **Generalized bruchids life cycle in pods and seeds of dry common beans. (A) Female adults ovipositing on the ventral pod suture. (B) Single eggs, freely laid inside the pod and/or attached to the seeds; b, Eggs. (C) Part of seed with entrance hole and tunnel mined by the first instar larvae; (c) First instar larvae penetrating the seed. (D) Larval growth and modifications after the first molt; (d) Larvae. (E) Demarcation of operculum by larvae. (F) Pupa inside the larval feeding chamber as well as larval entrance and exit hole; (f) Pupa. (G) Emergence of adult; (g) Adult. (H) Emergence of adults likely to reinfest the seeds and pods in the field. (I) Emergence of adults from stored grains; (i) infested grain with more than three holes. (J). Adults in the field feeding on flowers (pollen and nectar).** Source: **Ribeiro-Costa and Almeida (2012)**

Usually, *Z. subfasciatus* do coexist with *A. obtectus* and have similar biology, but one characteristic that differentiates *A. obtectus* from *Z. subfasciatus* is the ability of the former to oviposit on maturing pods in the field and continues to inflict damage to beans in storage, whereas the latter does so, on the shelled beans and scarcely attacks beans in the field (Howe and Currie, 1964; Abate and Ampofo 1996; Blair *et al.*, 2010; Thakur, 2012).

The common bean bruchid *Acanthoscelides obtectus* (Say) is bruchid specie of Neotropical origin, and is specialized on beans since the domestication and diffusion of dry bean grains across the world (Thakur, 2012; Alvarez *et al.*, 2005). According to Howe and Currie (1964), bruchid specie *A. obtectus* have a direct economic importance as major pests of the seeds of legumes, one of the most important groups of plants serving as human food and animal fodder. The *A. obtectus*, causes great economic losses since it inflict damages on dry bean crops when both in the field and storage (Howe and Currie 1964; Baier and Webster, 1992).

The adult of *A. obtectus* are short lived and do not feed on crop produce. The freshly emerged adults copulate at any time within 24 hours after their emergence. Copulation lasts for 4-5 minutes, during this period male normally raises its fore and middle legs to hold the female. *A. obtectus* female lay an average of 40-60 eggs in a loose group of ten or more in holes chewed in the developing pod or among the stored seeds and their eggs lasts 6-7 days. *A. obtectus* females do not attach eggs individually to host seeds but scatter them irregularly among potential hosts (Howe, 1964; Randome *et al.*, 2016). Normally freshly eggs are milky white in colour and ellipsoidal in shape (Howe and Currie, 1964; Schoonhoven, 1976; Cardona, 1989; Randome *et al.*, 2016; Maro, 2017).

The oviposition of *A. obtectus* lasts for 7-10 days and the incubation period is 8-10 days. Since most of the eggs were not glued onto the seeds it is essential for the freshly hatched first instar larva to find and select the host seeds for the remaining stages of development and food requirements. Development of successive larval instars completes inside the host seeds (Thakur and Renuka, 2014).

All the larval instars of *A. obtectus* are voracious feeders. The last larval instar prepares an emergence window before molting to pupal stage. Larval development completes in 14-20 days. The pupal stage also completes development inside the host seeds and pupal development takes 14-17 days. The total life cycle requires 44-54 days. The first larva feeds inside the pods and usually this stage can last for 2 weeks to 6 months, most commonly takes two to eight weeks depending on the climate (Thakur and Renuka, 2014).

Before the *A. obtectus* bruchid pupates inside the bean, it cuts a small operculum, a hard flap used as a kind of door on the surface of the bean. This operculum keeps the bruchid safe inside the bean and later the fully developed insect exits the pod through this opening. The time for pupation can vary between 12 and 25 days, and as soon as the temperature is right, the fully developed bruchid becomes active and exits the pod (Inra, 2019).

The Mexican bean bruchid *Zabrotes subfasciatus* (Boheman) Coleoptera: Bruchidae is native to central and South America but has now been spread to many other areas of the world (tropical and subtropical) through bean seeds (Southgate, 1979). It commonly infests the seeds of lima bean (*Phaseolus lunatus*) and common bean (*Phaseolus vulgaris*) (Credland and Dendy, 1992).

Adults *Z. subfasciatus* mate within an hour after coming out from seed and mating lasts 5 to 8 minutes, although the insects mate several times but to ensure egg laying only one

mating is sufficient (Talekar, 1988 cited in Kifle, 2017). *Z. subfasciatus* female lay an average of 36 to 56 eggs and the eggs lasts for 5-6 days. Because the eggs of *Z. subfasciatus* are glued to the bean testa, they are easily visible (Paul *et al.,* 2009). Generally, Larvae and pupa stages take 23 days whereby, different larvae instar takes 14 days, pupa stage 6-7 days and adults live 10-14 days (Cardona, 1989; Maro, 2017).

In Tanzania, common bean losses of up to 40% due to the major common bean bruchids have been reported whereby; their damage reduces the weight, quality and viability of bean seed (Kiula and Karel 1985). The degree of loss due to bruchid damage is quite variable and depends on the storage period and storage conditions. The risk of bean damage by bruchids reduces farmers' inspiration to increase area of production and productivity per unit area (Kifle, 2017). Farmers fear that stored beans will be attacked by bruchids so they sell-off most of their beans soon after harvesting to avoid large storage losses (Syombua, 2015). Therefore, this work aimed to evaluate the effectiveness of selected bean genotypes for resistance to the major bean bruchids using no choice test in the laboratory.

2.3 Materials and Methods

2.3.1 Description of the study area

The study was conducted in Morogoro region from September to December, 2018 at the Horticulture Section of Sokoine University of Agriculture (SUA), located at latitude 6º50'S, longitude 33º 9'E and altitude of 520 meters above sea level. The University falls within a climatic zone where highest mean maximum temperatures are above 31ºC during the months of November to February and mean minimum temperature below 16ºC in June to August (SUA, 2016). There are two distinct rainy seasons i.e., which is the short rainy season during November and December, and the long rainy season during March to May

(SUA, 2016). The study area was chosen because it contains the required facilities for the experiment.

2.3.2 Description of bean genotypes

AO-1012-29-3-3A is a multiple virus (possesses I and bc-12 genes that confer resistance to BCMV and BCMNV), and bean bruchid resistant line. It is a dark red kidney seed type from the cross of the BC3F4 lines 'Rojo'*3///SMARC-2-PN-1// 'ICA Pijao'*2/G40199. ROBC (14-34) AA CIAT and ROBC (8-54) AA is a Rojo backcross line with genes for APA resistance to bruchids developed from crosses between Rojo and RAZ lines with arcelins 2, and introgressed with APA proteins from tepary beans G40199. Bean genotype 30/59-96-2-3A-1 is a cross between 59-SOYA and AO-1012-29-3-3A while 65/44-30-2-3A-1 is a cross between 44-NJANO and AO-1012-29-3-3A. 44-NJANO and 59-SOYA are the landraces and common market and farmers preferred beans all are susceptible to bean bruchids (Kusolwa *et al*., 2016). The common bean lines used in the present study are illustrated in (Appendix 1)

2.3.3 Description of common bean bruchid species

Adults of *A. obtectus* and *Z. subfasciatus* emerging from the seeds collected from infested bean samples were reared using 1kg of susceptible bean seeds. Plastic bottle containers with a perforated lid to allow for ventilation, while preventing the escape of insects were used as feeding and rearing units. The emerged insects were passed through the sieve with 5mm diameter holes. Sieved individuals were considered as dead if insect appendages did not move when prodded after sieving, and again after 5 min (Jovanović *et al*., 2007).

2.3.4 Experimental design

The experiment was conducted as described by Golob and Kilminster (1982). The study was laid out as a 2x7 factorial experiment arranged in a completely randomized design

(CRD), with two (2) factors and four (4) replications as illustrated in (Plate 2.1). Factor A entailed the common bean genotypes with seven (7) levels i.e., five (5) resistant lines; AO-1012-29-3-3A, 65/44-30-2-3A-1, ROBC(8-54)AA, 30/59-96-2-3A-1, ROBC(14-34)AACIAT and two (2) susceptible control lines: 44-NJANO and 59-SOYA. While Factor B included the major bruchids species of common bean with two (2) levels i.e., *A. obtectus* and *Z. subfasciatus*.

Plate 2.1*:* **Layout of the experiment set at SUA Laboratory**

2.3.5 Data collection and processing

2.3.5.1 Determination of biological characteristics and population dynamics of bruchids

Fifteen (15) beans from each genotype were placed in 56 separate plastic containers. Each container was covered with perforated lids and allowed to be infested for 24hrs with five (5) couples of four (4) days old matured adult bruchids each from *A. obtectus* and *Z. subfasciatus*, respectively. The bruchids entailed those of the cohort that were obtained through technique described in section 2.3.3. Then the beans were observed until the appearance of windows.

The 'windows' assisted to mark the area under the testa eroded by the developing larva. Hence, the total number of larvae and the number of days from larvae to pupae stage were

counted and recorded. The 'windows' also helped to understand the beginning of the pupal stage and adult emergence during the incubation period (90 days), thus the number of days from pupa to adult stage as well as the number of pupated larvae (pupa) was counted and recorded (Golob and Kilminster, 1982). The percentage pupation was calculated using formula presented in equation 2.1.

$$Pupation(\%) = \frac{Number\ of\ pupated\ larva}{Total\ number\ of\ larva} \times 100 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

In order to establish the lifespan (longevity) of each species of major bruchids (*A.obtectus* and *Z. subfasciatus*), the day to day observation of emerged adults was carried out. The number of emerged adults, number of days which an adult took from its emergence to death and number of surviving adults was counted and recorded. The percentage adult emergence and survival were calculated using formula presented in equation 2.2 and 2.3.

$$Percentage\ adult\ emergence(\%) = \frac{Number\ of\ pupa\ turned\ into\ adults}{Total\ number\ of\ pupa} \times 100 \ \ldots\ldots\ldots (2.2)$$

$$Percentage\ adult\ survival(\%) = \frac{Number\ of\ surviving\ adults}{Total\ number\ of\ adults\ emerged} \times 100 \ \ldots\ldots\ldots\ldots (2.3)$$

2.3.5.2 Number of eggs laid and hatched per female per day

Five (5) beans from each genotype were placed in separate plastic containers covered with perforated lids and allowed to be infested for 24 hours with a pair of virgin adults bruchids less than a day old, each from *A. obtectus* and *Z. subfasciatus* of the cohort that were obtained through technique described in section 2.3.4. Thereafter, every day the beans were removed and replaced by fresh beans until the female died or up to 10 days. The number of eggs laid and hatched were examined daily, counted and recorded (Golob and Kilminster, 1982). The hatching percentage was calculated using formula presented in equation 2.4.

$$Hatching\,(\%)= \frac{Number\,of\,hatched\,eggs}{Total\,number\,of\,laid\,eggs} \times 100 \,\ldots\ldots\ldots\ldots..\ldots\ldots.\ldots\ldots.. \,(2.4)$$

2.3.5.3 Number of days from laying eggs to adult emergence

Ten (10) pairs of virgin adults of less than a day old from each species *A. obtectus* and *Z. subfasciatus* of the cohort that were obtained through technique described in section 2.3.4 were placed on 100g of beans seeds for each of the genotypes in the plastic container covered with a perforated lid for 48hrs. After 30 days, emergence of offsprings was daily observed and recorded (Golob and Kilminster 1982).

2.3.6 Data analysis

The data collected were subjected to Analysis of Variance (ANOVA) using Genstat Statistical Software 16th Edition (VSN International, UK). Mean separation test was conducted using Duncan's Multiple Range Test (DMRT) at 5% significance level.

2.4 Results

2.4.1 Weather condition during the study period in the year 2018

The weather condition during the study period from August to December in the year 2018 was as follows; the mean air temperature was lowest in August to September and highest in November to December. The Mean monthly maximum air temperature ranged from 29 °C to 34°C while the average monthly minimum air temperature ranged from 17°C to 22°C. The mean monthly maximum relative humidity ranged from 59 to 68% (Fig. 2.2).
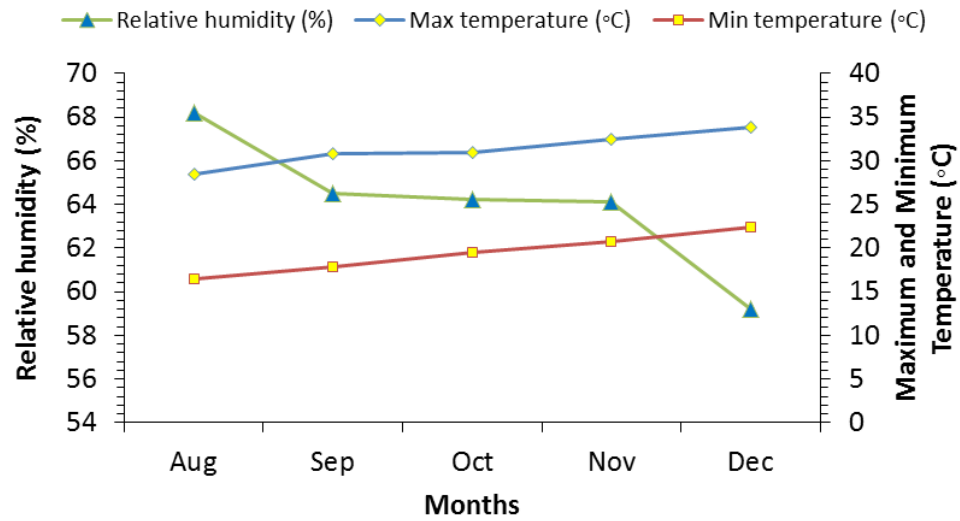
Figure 2.2: **Weather condition during the study period from August to December 2018**

2.4.2 Effect of bean genotypes and major bruchids on eggs laying and hatching

The number of eggs laid by the bruchids species was significantly different ($p<0.001$) among bean genotypes and bruchid species (Table 2.1). The highest number of eggs laid was recorded on the bean genotype 44-NJANO (27.25) while the bean genotype 65/44-30-2-3A-1 had the lowest number of eggs laid (7.75) compared to other bean genotypes. The bruchid specie *Z. subfasciatus* laid the highest number of eggs (20.36) than bruchid specie *A. obtectus* (8.96).

The number of eggs hatched was significantly different ($p<0.001$) among bean genotypes and bruchid species (Table 2.1). The highest number of eggs hatched was recorded on the bean genotype 44-NJANO (25.12) while the bean genotype 65/44-30-2-3A-1 had the lowest number of eggs hatched (5.75) compared to other bean genotypes. The bruchid specie *Z. subfasciatus* hatched the highest number of eggs (18.71) hatched than bruchid specie *A. obtectus* (6.75).

The hatching percentage was significantly different among bean genotypes ($p = 0.021$) and bruchid species ($p<0.001$) (Table 2.1). The highest hatching percentage was recorded on the bean genotype AO-1012-29-3-3A (86.34%) while the bean genotype 65/44-30-2-3A-1 had the lowest hatching percentage (73.65%) compared to other bean genotypes. The bruchid specie *Z. subfasciatus* laid the highest hatching percentage (88.6%) than bruchid specie *A. obtectus* (75.2%).

Table 2.1: **Effect of bean genotypes and major bruchids on eggs laying and hatching**

	No. of eggs laid	No. of eggs hatched	Hatching (%)
Factor A: Bean genotype			
AO-1012-29-3-3A	13.75b	12.12b	86.34b
ROBC (8-54) AA	14.62b	12.88b	86.16b
65/44-30-2-3A-1	7.75a	5.75a	73.65a
ROBC (14-34) AA	8.12a	6.38a	77.33ab
30/59-96-2-3A-1	16.00b	14.12b	84.60b
44-NJANO	27.25c	25.12c	84.24b
59-SOYA	15.12b	12.75b	80.79ab
Mean	14.66	12.73	81.9
S. E	1.951	1.857	4.05
CV%	6.7	8.6	6.5
P. value	<0.001	<0.001	0.021
Factor B: Bruchid species			
A. obtectus	8.96	6.75	75.2
Z. subfasciatus	20.36	18.71	88.6
Mean	14.66	12.73	81.9
S. E	1.043	0.993	2.16
CV%	6.7	8.6	6.5
P. value	<0.001	<0.001	<0.001

Means bearing the same letter(s) within the column are insignificantly ($p<0.05$) different according to Duncan's Multiple Range Test (DMRT)

There was a significant ($p<0.001$) difference on the number of eggs laid with respect to the interaction between bean genotypes and bruchids species (Fig. 2.3). The bean genotype 44-NJANO recorded the highest number of eggs laid (45.5) by bruchid specie *Z. subfasciatus* compared to the genotype 65/44-30-2-3A-1 with the lowest number of eggs laid (6.75) by the bruchid specie *Z. subfasciatus*. Bean genotypes 30/59-96-2-3A-1 and 59-SOYA recorded the highest number of eggs laid by bruchid specie *A. obtectus* (9.5)

compared to the genotype ROBC (8-54) AA with the lowest number of eggs laid (8.25) by

the bruchid specie *A. obtectus* (Fig. 2.3).
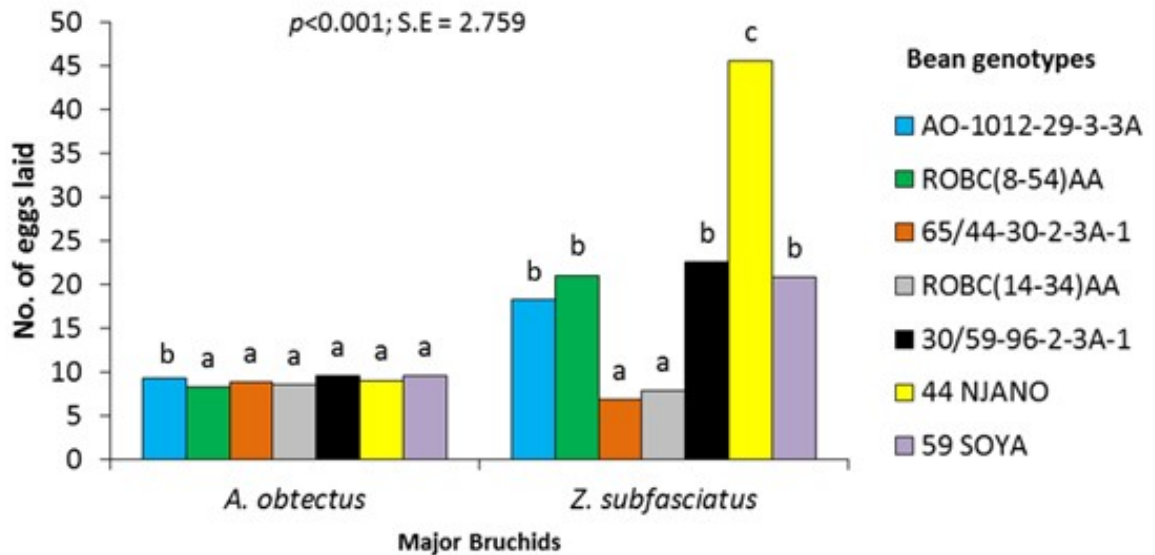
Figure 2.3: **Interaction effect between bean genotypes and bruchid species on the**
number of eggs laid

The interaction between bean genotypes and bruchids species had a significant ($p<0.001$)

effect on the number of eggs hatched (Fig. 2.4). The bean genotype 44-NJANO recorded

the highest number of eggs hatched (43.75) by bruchid specie *Z. subfasciatus* compared to

the genotype 65/44-30-2-3A-1 with the lowest number of eggs hatched (5.25) by the

bruchid specie *Z. subfasciatus*. Bean genotypes AO-1012-29-3-3A and 30/59-96-2-3A-1

recorded the highest number of eggs hatched by bruchid specie *A. obtectus* (7.25)

compared to the genotype 65/44-30-2-3A-1 and ROBC (14-34) AA with the lowest

number of eggs hatched (6.25) by the bruchid specie *A. obtectus* (Fig. 2.4).
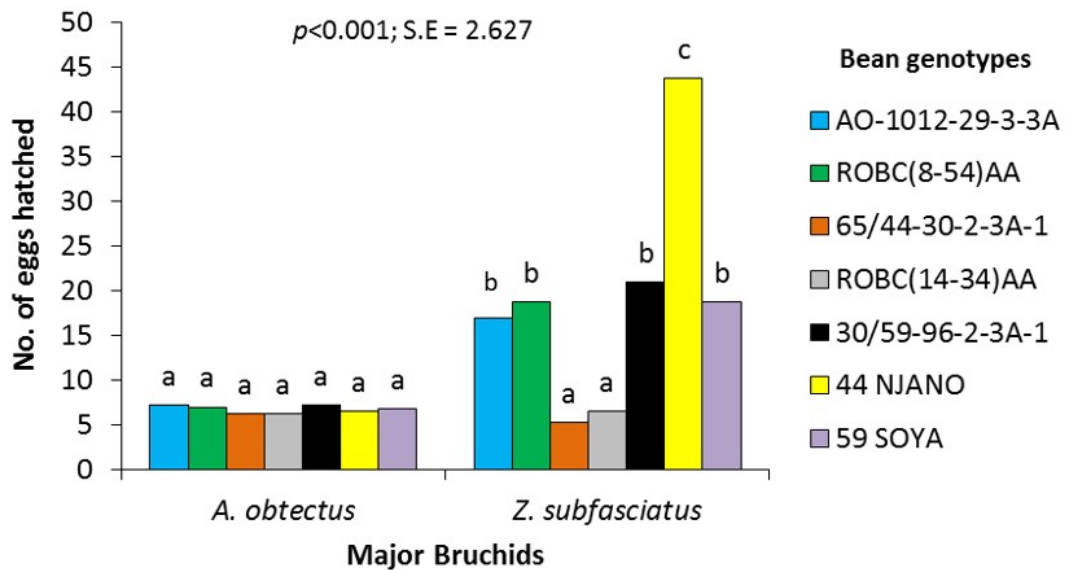
Figure 2.4: **Interaction effect between bean genotypes and bruchid species on the number of eggs hatched**

There was no significant ($p = 0.233$) difference on the hatching percentage with respect to the interaction between bean genotypes and bruchid species (Fig. 2.5). The bean genotype 44-NJANO recorded the highest rate of eggs hatched (96.14) by bruchid specie *Z. subfasciatus* compared to the genotype 65/44-30-2-3A-1 with the lowest rate of eggs hatched (77.06) by the bruchid specie *Z. subfasciatus*. Bean genotypes ROBC (8-54) AA recorded the highest rate of eggs hatched by bruchid specie *A. obtectus* (83.54) compared to the genotype 65/44-30-2-3A-1 with the lowest rate of eggs hatched (70.25) by the bruchid specie *A. obtectus* (Fig. 2.5).
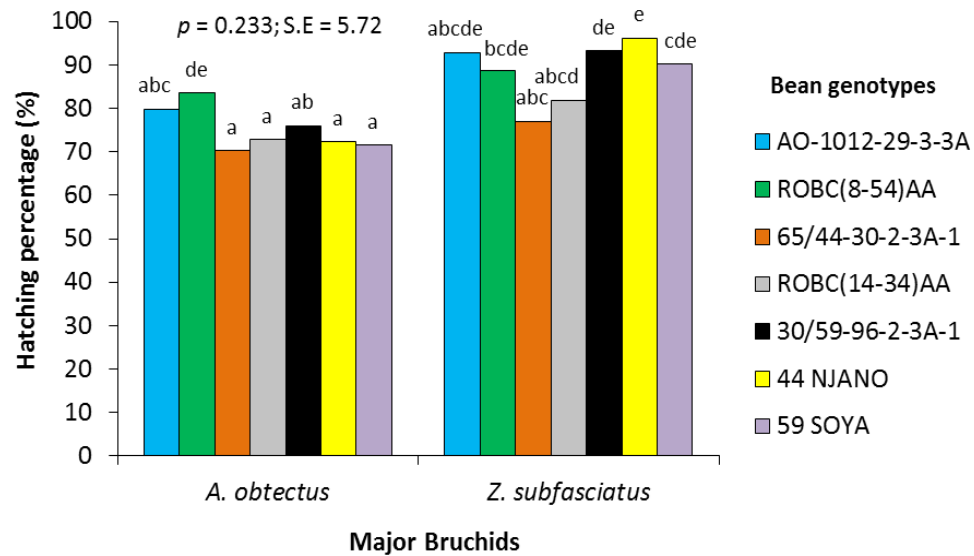
Figure 2.5: Interaction effect of bean genotypes and bruchids species on the hatching percentage

2.4.3 Effect of bean genotypes on the number of days from larva to pupa and adult emergence

The number of days from larva to pupa was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.2). The highest number of days from larva to pupa was recorded on bean genotype ROBC (8-54) AA (29) while bean genotype 44-NJANO had the lowest number of days from larva to pupa (13.5) compared to other bean genotypes. The bruchid specie *A. obtectus* laid the highest number of days from larva to pupa (20.5) than bruchid specie *Z. subfasciatus* (17.6).

The number of days from pupa to adult emergence was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.2). The highest number of days from pupa to adult emergence was recorded on the bean genotype AO-1012-29-3-3A and ROBC (8-54) AA (15.5) while the bean genotype 59-SOYA with the lowest number of days from pupa to adult emergence (13.25) compared to other bean genotypes. The

bruchid specie *A. obtectus* hatched the highest number of days from pupa to adult emergence (15.1) than bruchid specie *Z. subfasciatus* (13.7).

The percentage pupation was significantly different ($p<0.001$) among the bean genotypes ($p<0.001$) and bruchid species ($p = 0.019$) (Table 2.2). The highest percentage pupation was recorded on the bean genotype 44-NJANO and 59-SOYA (100%) while the bean genotype AO-1012-29-3-3A had the lowest percentage pupation (41.67%) compared to other bean genotypes. The bruchid specie *Z. subfasciatus* had the highest percentage pupation (83.2%) than bruchid specie *A. obtectus* (76.5%).

Table 2.2: **Effect of bean genotypes and major bruchids on the number of days from larva to pupa and adult emergence**

	No. of days from (larva – pupa)	No. of days (pupa – adults)	Pupation (%)
Factor A: Bean genotype			
AO-1012-29-3-3A	28.00e	15.50e	41.67a
ROBC (8-54) AA	29.00f	15.50e	51.04a
65/44-30-2-3A-1	16.50d	14.00c	75.18a
ROBC (14-34) AA	16.50d	14.50d	91.88b
30/59-96-2-3A-1	16.00c	14.50d	99.31c
44-NJANO	13.50a	13.50b	100.00c
59-SOYA	13.75b	13.25a	100.00c
Mean	19.1	14.4	79.9
S. E	0.0908	0.0908	5.07
CV%	1.0	7.0	14.50
P. value	<0.001	<0.001	<0.001
Factor B: Bruchid species			
A. obtectus	20.5	15.1	76.5
Z. subfasciatus	17.6	13.7	83.2
Mean	19.1	14.4	79.9
S. E	0.0485	0.0485	2.17
CV%	1.0	7.0	14.50
P. value	<0.001	<0.001	0.019

Means bearing the same letter(s) within the column are insignificantly ($p<0.05$) different according to Duncan's Multiple Range Test (DMRT)

There was a significant (p<0.001) difference on the number of days from larva to pupa with respect to the interaction between bean genotypes and bruchids species (Fig. 2.6). The bean genotype ROBC (8-54) AA recorded the highest number of days from larva to pupa (30) by bruchid specie *Z. subfasciatus* compared to the genotype 44-NJANO with the lowest number of days from larva to pupa (13) by the bruchid specie *Z. subfasciatus*. Bean genotypes AO-1012-29-3-3A recorded the highest number of days from larva to pupa by bruchid specie *A. obtectus* (31) compared to the genotype 44-NJANO with the lowest number of days from larva to pupa (14) by the bruchid specie *A. obtectus*.
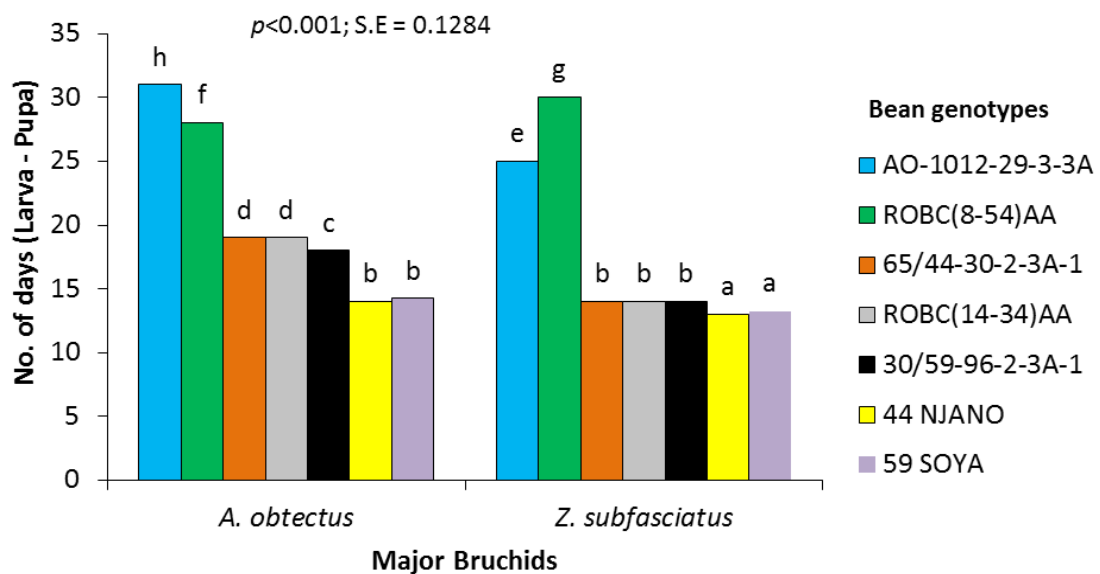
Figure 2 6: **Interaction effect between bean genotypes and bruchid species on the number of days from larva to pupa**

The interaction between bean genotypes and bruchids species had a significant (p<0.001) effect on the number of days from pupa to adult emergence (Fig. 2.7). The bean genotype ROBC (8-54) AA recorded the highest number of days from pupa to adult emergence (17) by bruchid specie *Z. subfasciatus* compared to the genotype 44-NJANO with the lowest number of days from pupa to adult emergence (12.75) by the bruchid specie *Z.*

subfasciatus. Bean genotypes AO-1012-29-3-3A recorded the highest number of days from pupa to adult emergence (17) by bruchid specie *A. obtectus* compared to the genotype 44-NJANO with the lowest number of days from pupa to adult emergence (13.75) by the bruchid specie *A. obtectus*.
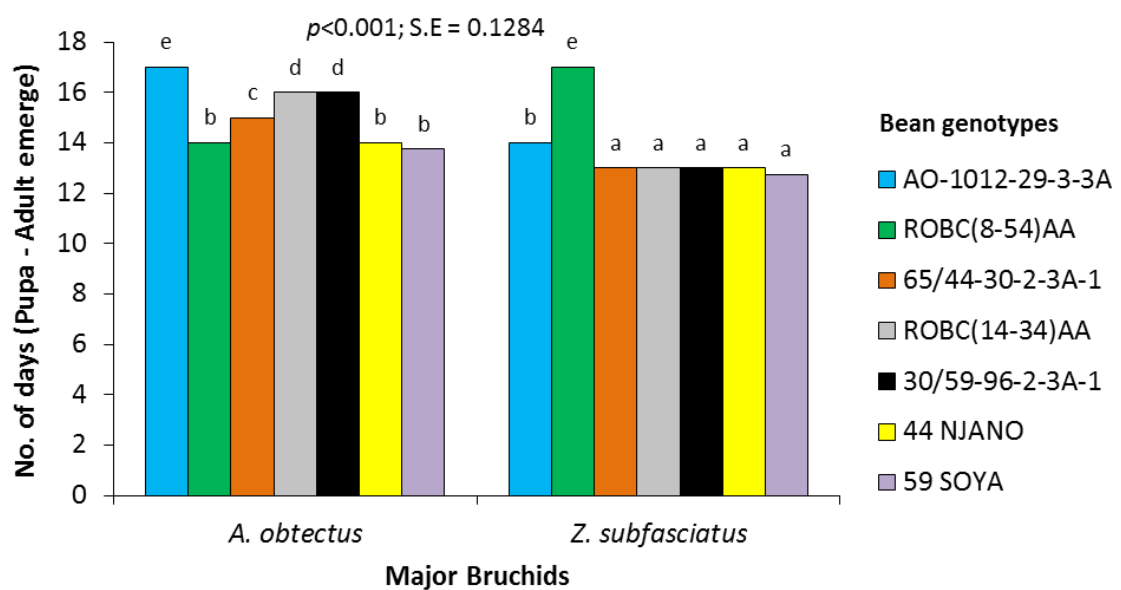
Figure 2.7: **Interaction effect between bean genotypes and bruchid species on the number of days from pupa to adult emergence**

There was no significant ($p = 0.123$) difference on the pupation percentage with respect to the interaction between bean genotypes and bruchid species (Fig. 2.8). The bean genotype ROBC (14-34) AA, 44-NJANO, 59-SOYA, recorded the highest pupation percentage (100%) by bruchid specie *Z. subfasciatus* compared to the genotype AO-1012-29-3-3A with the lowest pupation percentage (41.67%) by the bruchid specie *Z. subfasciatus*. Bean genotypes 30/59-96-2-3A-1, 44-NJANO and 59-SOYA recorded the highest pupation percentage by bruchid specie *A. obtectus* (100%) compared to the genotype ROBC (8-54) AA with the lowest pupation percentage (39.58%) by the bruchid specie *A. obtectus*.
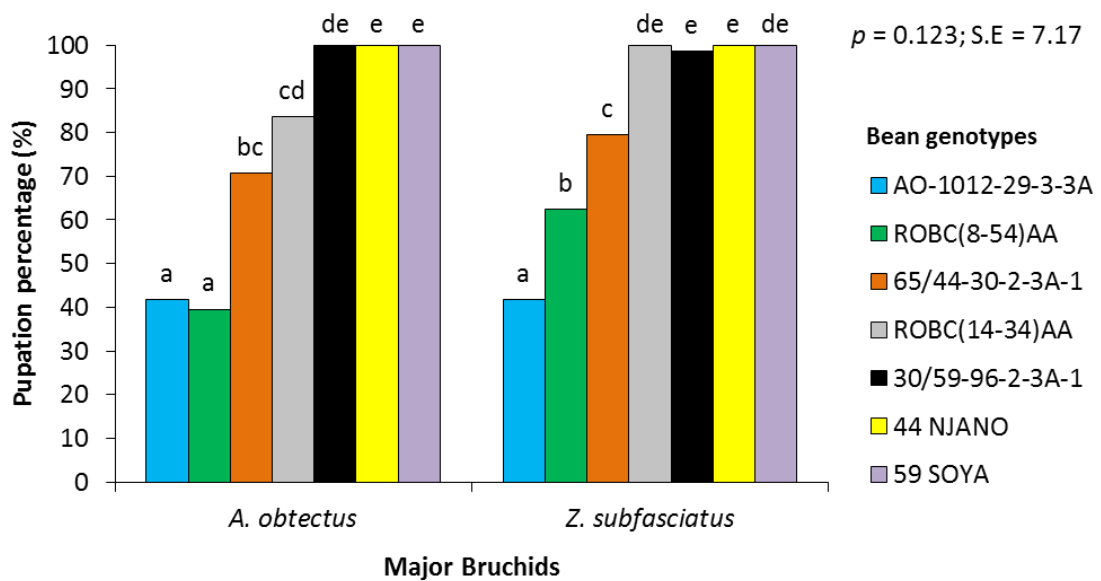
Figure 2.8: **Interaction effect between bean genotypes and bruchid species on pupation percentage**

2.4.4 Effect of bean genotypes and major bruchids on the emergence and survival

The average number of holes per grain was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.3). The highest number of holes per grain was recorded on bean genotype 44-NJANO (7.125) while the lowest number of holes per grain was recorded on bean genotypes AO-1012-29-3-3A (0.5) and ROBC (8-54) AA (0.531). The bruchid specie *Z. subfasciatus* bored the highest number of holes per grain (4.0) than bruchid specie *A. obtectus* (2.0).

The number of emerged adults was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.3). The highest number of emerged adults was recorded on bean genotype 44-NJANO (24.0) while the lowest number of emerged adults was recorded on bean genotype AO-1012-29-3-3A(0.5). The bruchid specie *Z. subfasciatus* recorded the highest number of emerged adults (12.36) than bruchid specie *A. obtectus* (3.46).

The emerging adult percentage was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.3). The highest emerging adult percentage was recorded on bean genotype 44-NJANO and 59-SOYA (100%) while the lowest emerging adult percentage was recorded on bean genotype ROBC (8-54) AA (7.92%). The bruchid specie *Z. subfasciatus* recorded the highest emerging adult percentage (96.2%) than bruchid specie *A. obtectus* (70.5%).

The number of surviving adults was significantly different ($p<0.001$) among the bean genotypes and bruchid species (Table 2.3). The highest number of surviving adults was recorded on bean genotype 44-NJANO (24.5) while the lowest number of surviving adults was recorded on bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA (0.5). The bruchid specie *Z. subfasciatus* recorded the highest number of surviving adults (12.25) than bruchid specie *A. obtectus* (3.39).

The percentage adult's survival differed significantly ($p<0.001$) among bean genotypes and bruchid species (Table 2.3). The highest percentage adults survival was recorded on the bean genotype 44-NJANO and 59-SOYA (100%) while the lowest percentage adults survival was recorded on bean genotypes AO-1012-29-3-3A (0.3%) and ROBC (8-54) AA (0.4%). The bruchid specie *A. obtectus* had the highest percentage adult's survival (65.8%) than bruchid specie *Z. subfasciatus* (51.0%).

Table 2.3: **Effect of bean genotypes and major bruchids on the emergence and survival**

	No. of holes per grain	No. of emerged adults	Emerging adults (%)	No. of surviving adults	Surviving adults (%)
Factor A: Bean genotype					
AO-1012-29-3-3A	0.500a	0.500a	11.88a	0.500a	0.300a
ROBC (8-54) AA	0.531a	0.625a	7.92a	0.500a	0.400a
65/44-30-2-3A-1	1.771b	3.125ab	61.46b	2.875ab	76.04c
ROBC (14-34) AA	1.500b	5.000b	66.31b	4.875b	65.62b
30/59-96-2-3A-1	2.875c	10.750c	71.67b	10.625c	67.25bc
44-NJANO	7.125e	24.00d	100.00c	24.500d	100.00d
59-SOYA	5.250d	10.875c	100.00c	10.875c	100.00d
Mean	2.8	7.9	83.4	7.8	58.4
S. E	0.4	1.266	5.49	1.281	4.80
CV%	30.7	32.0	17.7	32.8	16.4
P. value	<0.001	<0.001	<0.001	<0.001	<0.001
Factor B: Bruchid species					
A. obtectus	2.0	3.46	70.5	3.39	65.8
Z. subfasciatus	4.0	12.36	96.2	12.25	51.0
Mean	2.8	7.9	83.4	7.8	58.4
S. E	0.2	0.677	2.93	0.685	2.57
CV%	30.7	32.0	17.7	32.8	16.4
P. value	<0.001	<0.001	<0.001	<0.001	<0.001

Means bearing the same letter(s) within the column are insignificantly ($p<0.05$) different according to Duncan's Multiple Range Test (DMRT)

The number of holes per bean grain differed significantly ($p<0.001$) with respect to the interaction between bean genotypes and bruchid species (Fig. 2.9). The bean genotype 44-NJANO, had highest of number of holes per bean grain caused by bruchid specie *Z. subfasciatus* (9.0) compared to the genotype ROBC (8-54) AA with the lowest of number of holes per grain by the bruchid specie *Z. subfasciatus* (1.0). Bean genotype 44-NJANO had the highest of holes per grain by bruchid specie *A. obtectus* (5.25) while the lowest number of holes per grain by the bruchid specie *A. obtectus* was recorded on genotype AO-1012-29-3-3A (0.3) and ROBC (8-54) AA (0.4).
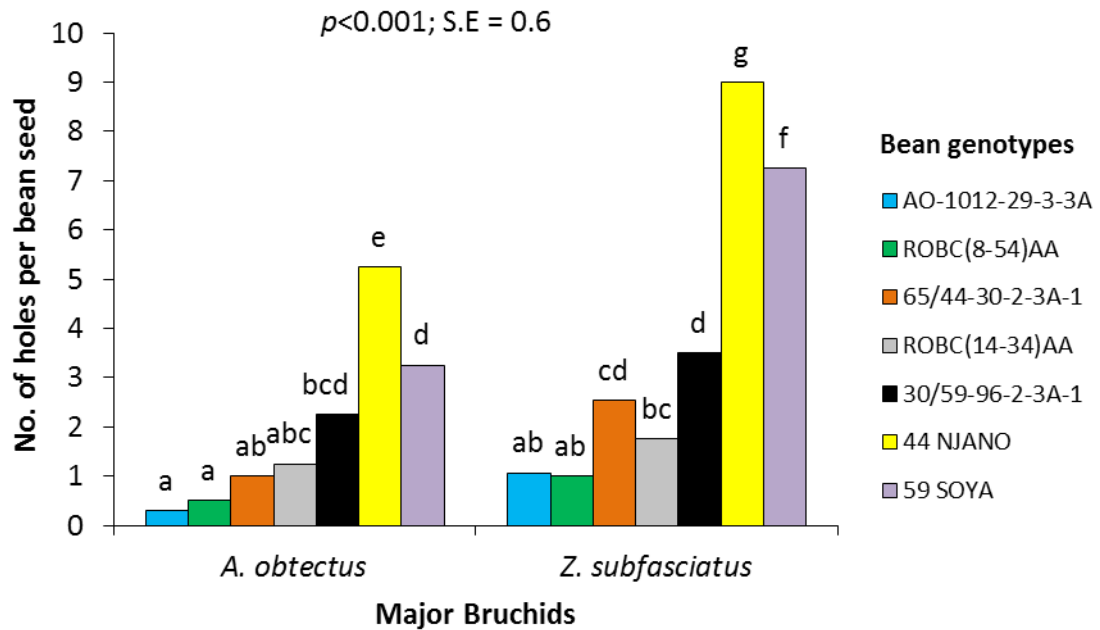
Figure 2.9: **Interaction effect of bean genotypes and bruchids species on mean number of holes per bean grain**

There was a significant (*p*<0.001) difference on the number of emerged adults with respect to the interaction between bean genotypes and bruchids species (Fig. 2.10). The bean genotype 44-NJANO, had the highest of emerged bruchid specie *Z. subfasciatus* adults (42.75) compared to the genotype AO-1012-29-3-3A with the lowest of emerged bruchid specie *Z. subfasciatus* adults (1.0). Bean genotypes 30/59-96-3-3A-1 and 44-NJANO had the highest number of emerged bruchid specie *A. obtectus* adults (6.25) while the lowest number of emerged bruchid specie *A. obtectus* adults was recorded on genotypes AO-1012-29-3-3A (0.3) and ROBC (8-54) AA (0.4).
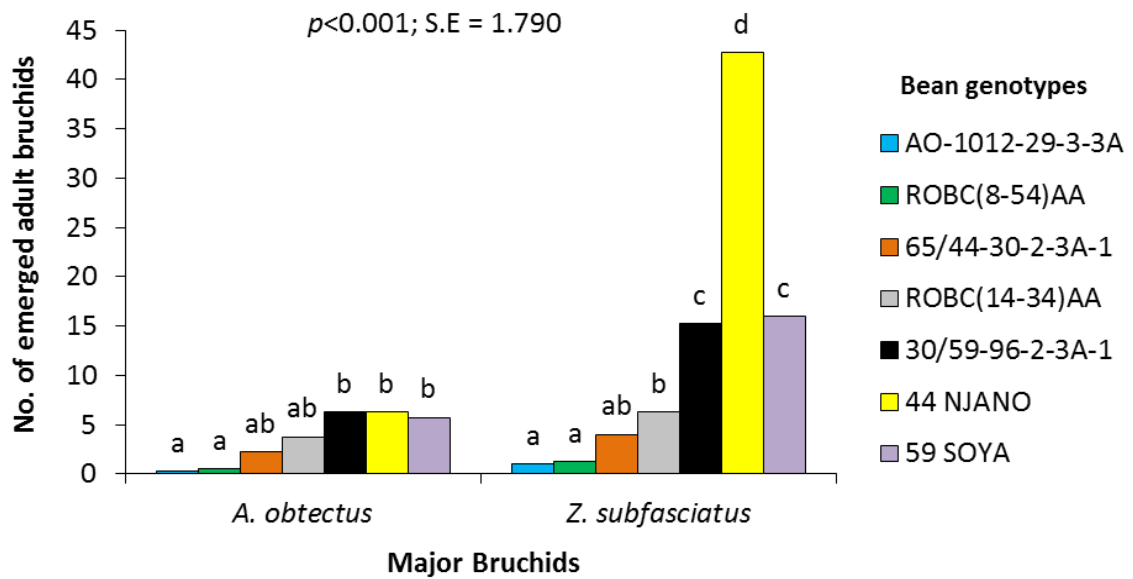
Figure 2.10: **Interaction effect of bean genotypes and bruchids species on the number of emerged adults**

The percentage emerging adults differed significantly ($p<0.001$) with respect to the interaction between bean genotypes and bruchid species (Fig. 2.11). The bean genotype 44-NJANO and 59-SOYA recorded the highest emerging bruchid specie *Z. subfasciatus* adults' percentage (100%) compared to the genotype ROBC (8-54) AA with the lowest of emerging bruchid specie *Z. subfasciatus* adults' percentage (8.33%). Bean genotypes 44-NJANO and 59-SOYA recorded the highest emerging bruchid specie *A. obtectus* adults' percentage (100%) while the lowest emerging bruchid specie *A. obtectus* adults' percentage was recorded on genotypes AO-1012-29-3-3A (0.3%) and ROBC (8-54) AA (0.4%).

Figure 2.11: **Interaction effect of bean genotypes and bruchids species on the number of emerged adults**

The number of surviving adults bruchids differed significantly ($p<0.001$) with respect to the interaction between bean genotypes and bruchid species (Fig. 2.12). The bean genotype 44-NJANO recorded the highest number of bruchid specie *Z. subfasciatus* surviving adults (42.75) compared to the genotype AO-1012-29-3-3A and ROBC (8-54) AA with the lowest number of bruchid specie *Z. subfasciatus* surviving adults (1.0). Bean genotypes 44-NJANO and 30/59-96-3-3A-1 recorded the highest number of bruchid specie *A. obtectus* surviving adults (6.25) while the lowest number of bruchid specie *A. obtectus* surviving adults was recorded on genotypes AO-1012-29-3-3A (0.3) and ROBC (8-54) AA (0.4).
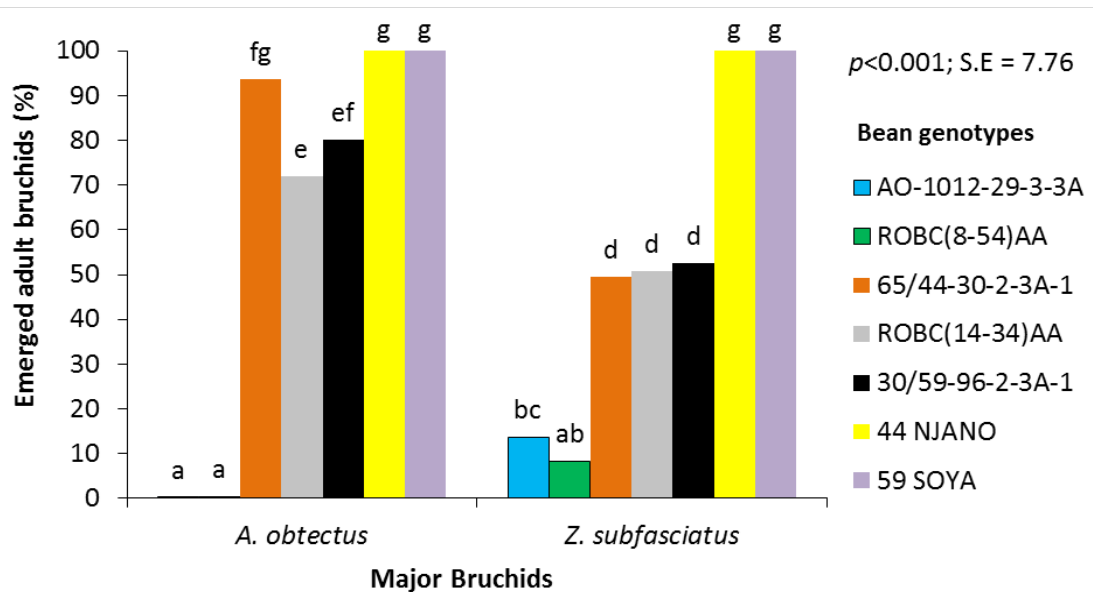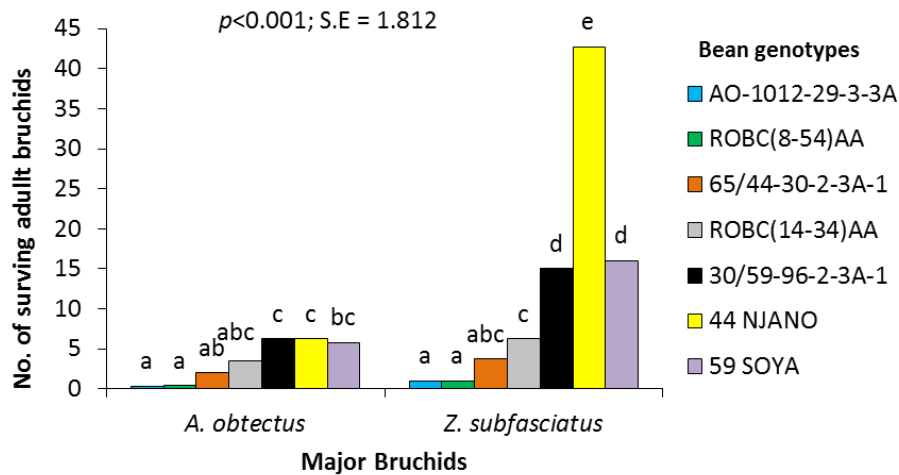
Figure 2.12: **Interaction effect of bean genotypes and bruchids species on the number of surviving adults**

There was a significant ($p<0.001$) difference on the surviving adult's percentage with respect to the interaction between bean genotypes and bruchids species (Fig. 2.13). The bean genotypes 44-NJANO and 59-SOYA recorded the highest surviving bruchids species *Z. subfasciatus* and *A. obtectus* adults' percentage (100%), respectively. While the lowest surviving bruchids species *Z. subfasciatus* and *A. obtectus* adults' percentage was recorded on genotypes AO-1012-29-3-3A (0.3%) and ROBC (8-54) AA (0.4%), respectively.

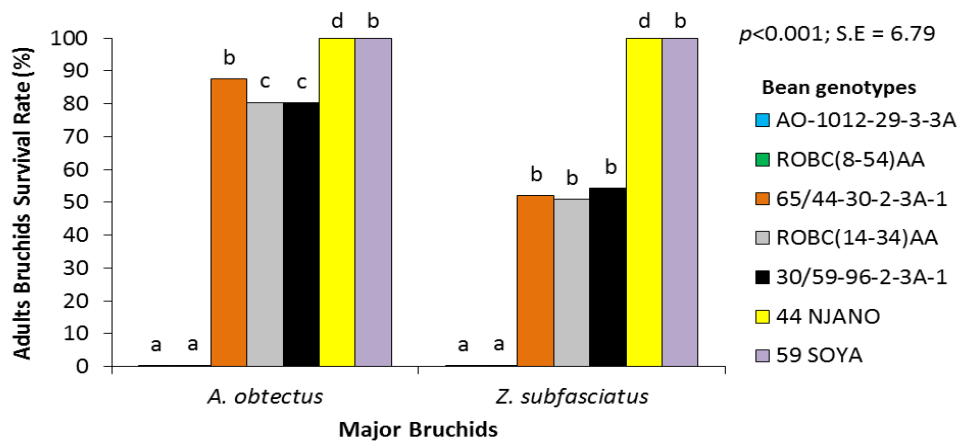**Figure 2.13**: **Interaction effect of bean genotypes and bruchid species on the surviving adult's percentage**

2.4.5 Common bean bruchids biological characteristics and population dynamics

2.4.5.1 Duration from egg laying to adult emergence

The results (Table 2.4) show that, *A. obtectus* recorded the highest mean number of days from egg laying to first adult emergence (56 days) on A0-1012-29-3-3A while, 59-Soya and 44-Njano scored the least mean number of days from egg laying to first adult emergence (36 days). *Z. subfasciatus* had the highest mean number of days from egg laying to first adult emergence (54 days) on ROBC (8-54) AA while, 59Soya and 44-NJANO recorded the least mean number of days from egg laying to first adult emergence (33 days). In this study, mean development period for *Z. subfasciatus* from laying eggs to adult emergence was shorter (38.3 days) than *A. obtectus* (43.6 days).

Table 2.4: **Developmental duration of major bruchids from egg laying to adult emergence**

	Number of days from laying eggs to adult emergence	
Bean genotypes	*Acanthoscelides obtectus*	*Zabrotes subfasciatus*
AO-1012-29-3-3A	56	46
65/44-30-2-3A-1	42	34
ROBC (8-54) AA	50	54
30/59-96-3-3A-1	42	34
ROBC (14-34) AA	43	34
44 NJANO	36	33
59 SOYA	36	33
Mean	** Expression is faulty **	** Expression is faulty **

2.2.5.2 Number of emerged adult bruchids with respect to storage duration

The results (Fig. 2.14) indicate that, following 90 days of storage the highest number of emerged *A. obtectus* adults occurred most quickly (at the 6th week ≈ 42 days) in bean genotypes 44-NJANO (64 adults) and 59-SOYA (48 adults). While the lowest number of emerged *A. obtectus* adults occurred more delayed (at the 8th week ≈ 56 days) in the bean genotype AO-1012-29-3-3A (2 adults) followed by ROBC (8-54) AA (4 adults) and 65/44-30-2-3A-1 (7 adults).
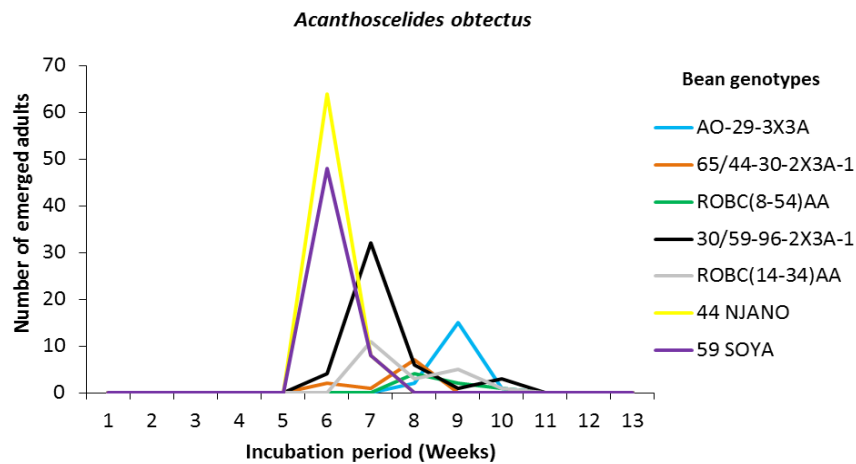
Figure 2.14: **Emergence of bruchid specie *Acanthoscelides obtectus* in seven common bean genotypes**

Following 90 days of storage the highest number of emerged *Z. subfasciatus* adults occurred most quickly (at the 5th week ≈ 35 days) in bean genotypes 44-NJANO (133 adults) and 59-SOYA (108 adults). While the lowest number of emerged *Z. subfasciatus* adults occurred more delayed (at the 8th week ≈ 56 days) in the bean genotype ROBC(8-54)AA (4 adults) (Fig. 2.15).
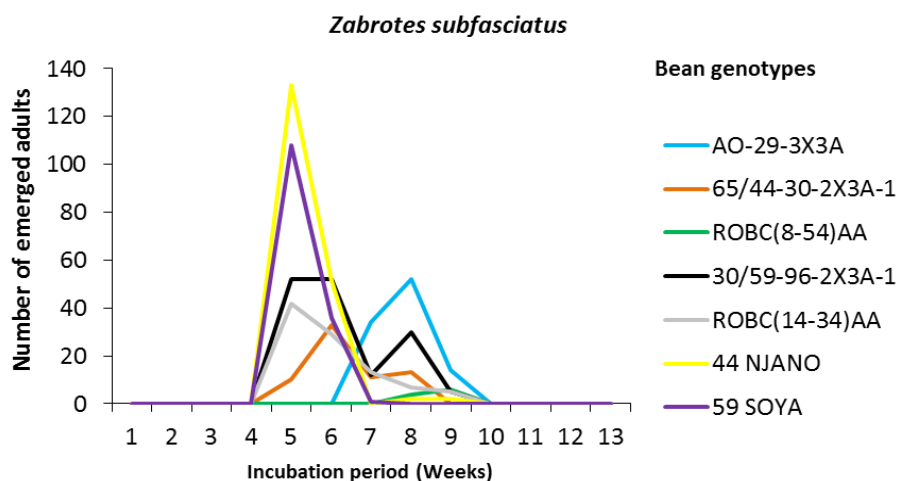
Figure 2.15: **Emergence of bruchid specie *Zabrotes subfasciatus* in seven common bean genotypes**

2.4.5.3 Effect of bean genotypes on life span of the major bean bruchids

In this study, the common bean bruchid (*A. obtectus*) had the highest mean life span i.e.,
from emergence of adult to death (20.23 days) on 59-SOYA, and least mean life span (9.2
days) on A0-1012-29-3-3A (Table 2.5). On the other hand, Mexican bean bruchid (*Z.
subfasciatus*) also had the highest mean life span (13.0 days) on 59-SOYA, and the least
mean life span (5.43 days) on AO-1012-29-3-3A. The mean adult life span of bruchid
specie *A. obtectus* was 13.69 days, and *Z. subfasciatus* (10.16 days) (Table 2.5).

Table 2.5: Effect of bean genotypes on the average adult life span (Longevity)

Bean genotypes	Average Life span (Number of days)	
	Acanthoscelides obtectus	*Zabrotes subfasciatus*
AO-1012-29-3-3A	9.2	5.43
65/44-30-2-3A-1	10.5	9.47
ROBC (8-54) AA	9.75	8.5
30/59-96-3-3A-1	14.05	11.9
ROBC (14-34) AA	13.43	10.16
44 NJANO	18.69	12.67
59 SOYA	20.23	13.0
Mean	**** Expression is faulty ****	**** Expression is faulty ****

2.4.5.4 Effect of bean genotypes on laying habit of adult females and eggs hatchability

This study indicates that the mean number of eggs laid by bruchid specie *A. obtectus* was
56 eggs per female with an incubation period of 9±1 days, at which 75.1% of the eggs
were hatched (Table 2.6). *Z. subfasciatus* had an average of 36 eggs laid per female, and
hatching occurred after 7.5±0.5 day at which 92% of the eggs were hatched.

Table 2.6: **Laying habit and eggs hatchability of females A.** *obtectus* **and** *Z.* *subfasciatus*

Bean genotypes	*Acanthoscelides obtectus*			*Zabrotes subfasciatus*		
	No. of Eggs laid/female	No. of Eggs hatched	Eggs hatched (%)	No. of Eggs laid/female	No. of Eggs hatched	Eggs hatched (%)
AO-1012-29-3-3A	66.00	49.00	74.24	32.00	30.00	93.75
65/44-30-2-3A-1	45.00	34.00	75.55	34.00	31.00	91.78
ROBC (8-54) AA	47.00	35.00	74.47	35.00	32.00	91.43
30/59-96-2-3A-1	54.00	41.00	75.93	39.00	35.00	89.74
ROBC (14-34) AA	56.00	42.00	75.00	35.00	33.00	94.29
44 NJANO	60.00	45.00	75.00	39.00	36.00	92.31
59 SOYA	66.00	50.00	75.76	41.00	38.00	92.68
Total eggs laid	**394.00**			**255.00**		
Average laying/female	**56.3.00**			**36.43**		
Total eggs hatched		**296.00**			**235.00**	
% of eggs hatched		**75.1.00**			**92.20**	

2.5 Discussion

2.5.1 Major bruchids female adults laying habit and eggs hatchability

Bruchid specie *A. obtectus,* had higher mean yield of eggs per female, hatching occurred within short period, and the hatching percentage was less compared to *Z. subfasciatus*. A recent study by Maro (2017) reported that, the mean number of eggs laid by female *A. obtectus* and *Z. subfasciatus* was 45-60, and 36-56 eggs, respectively. Nearly similar pattern of development of *Z. subfasciatus* infesting red kidney beans was reported (Golob and Kilminster, 1982). The mean eggs laid were 50.7±1.7 eggs per female and 91.3% of them hatched. The proportion of hatching was consistently high with a slight declining trend as the females aged (Golob and Kilminster, 1982). It was also reported by Schoonhoven (1976) that, generally, adults of *Z. subfasciatus* lay less (35.5 eggs per female) than those of *A. obtectus* (63.0 eggs per female).

Parsons and Credland (2003) reported that, for *A. obtectus* the presence or absence of a male through the lifetime of the female had no effect on total fecundity. Females kept alone after initial mating laid 67.95±2.32 eggs and those kept with the same male

throughout their life produced 68.16±3.09 eggs. Of the eggs laid, 79.6% hatched in the continued presence of a male, and 77.7% hatched when the female was isolated from the male after initial mating. Moreover, if the adults were only provided with one bean on which to oviposit, significantly higher numbers of eggs were laid (63.6±4.7 eggs) when a fresh bean was left in-situ for the lifetime of the female compared to when a fresh bean was provided daily (49.0 ± 5.4 eggs).

The presence of few numbers of laid eggs, hatched and the hatching percentage on bean genotype 65/44-30-2-3A-1 than on other bean genotypes with respect to bruchid specie *Z. subfasciatus* was likely not attributed to susceptibility or possession of arcelins and arcelin-like (ARL2) proteins among bean genotypes. According to Osborn *et al*. (1988) despite the presence of arcelin is correlated with bruchid resistance in common beans genotypes factors other than arcelin protein might confer the resistance property. Credland and Dendy (1992) reported that, eggs were more randomly distributed on 10 haricot bean seeds than on the other hosts on which were aggregated. However, aggregation does not imply that no eggs were laid on some seeds, merely, which the majority was laid on a few of those available. Nevertheless, it is clear that in many cases females concentrated their eggs onto only a few of the seeds available to them and laid no eggs on the remainder (Credland and Dendy, 1992).

2.5.2 Major bruchids adult's emergence and damage on bean genotypes

The lowest average number of holes, emerged adults, percentage emerged and survived adults on bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA with respect to bruchid specie *A. obtectus* may be attributed to the presence of the major 33kDa storage protein (Kusolwa, 2007; Mbogo *et al.,* 2009). This protein was incorporated into selected genotypes of common beans by interspecific hybridization which confers strong resistance

to the bruchids; *A. obtectus* and *Z. subfasciatus* (Kusolwa, 2007; Mbogo *et al*., 2009). The study by Kusolwa (2007) showed that, bean genotype G40199 possessing the arcelin-like proteins, was completely resistant with no emerging adults. This resistance was exhibited as delayed insect emergence with a mean of 63 days for 50% F_1 adults after inoculation due to reduced size and weight of adults, and reduced number of adults which was observed two months after bruchid infestation. The 33 kDa protein which is linked to co-expression of ARL-3^{pa}, ARL-4^{pa} and PHA protein subunits is a contributing factor to the observed resistance to *A. obtectus* among interspecific hybrids (Kusolwa, 2007).

The resistance of bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA to *A. obtectus* probably conferred by the presence of the APA proteins extracted from tepary bean, may lead to weakness, reduction in size and weight of the emerging adult bruchids (Kusolwa and Myers, 2011). A line of *Phaseolus vulgaris* (RAZ-2) containing arcelin-like proteins bred for resistance to *Z. subfasciatus*, as compared to local susceptible cultivars, significantly lower number of insects emerged from RAZ-2 as a result seed damage and weight losses in this line were negligible (Cardona *et al*., 1992).

Such kind of results might also be associated to the initially number of laid eggs, hatched, and survival of the immature stages (larvae and pupa) in the common bean grains (Credland and Dendy, 1992). Previous studies (Utida, 1967; Credland and Dendy, 1992) indicated that, many of the eggs are laid in close proximity to each other, resulting into the competition within a single seed. This may have a direct negative impact for larval survival and/or the fitness of the emerging adult bruchids. Schoonhoven and Cardona (1982) reported that, the resistant lines (G2450 and G5693) were due to low in adult bruchid emergence, slow in development and low in progeny weight of bruchids.

Furthermore, the lowest number of holes (damage) on bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA in the present study may be due to unpleasant surface of the bean genotypes to the bruchids species. Quentin *et al*. (1991) reported that, it takes over 24hrs for *A. obtectus* larvae to bore into a dry red kidney bean and boring can occur only at particular sites where a seed does not roll/tumble or firmly touches some other surface. Hence, larvae repeatedly forcing to initiate holes may die due to exhaustion.

2.5.3 Developmental duration and life span of major bruchids on bean genotypes

Mean development period for *Z. subfasciatus* from laying eggs to adult emergence was shorter than for *A. obtectus*. Previous research works revealed that mean development period from egg to adult emergence for *A. obtectus* was 44 to 54 days (Thakur and Renuka, 2014) and *Z. subfasciatus* was 35.9 ± 0.4 days (Golob and Kilminster, 1982).

Moreover, the present findings indicate that, the number of days from egg laying to adult emergence was extremely high for the bean genotypes (AO-1012-29-3-3A and ROBC(8-54)AA) which contain arcelins and arcelin-like (ARL2) proteins. Hence, bruchids species had relatively short life span compared to those emerged from susceptible bean cultivars (44-NJANO and 59-SOYA). *P. vulgaris* seeds contain a carbohydrate-binding lectin protein called Phytohemagglutinin (PHA) with ability to protect bean seeds from predation by some insects. Nevertheless, the PHA is ineffective against the two most important bruchid pests of bean as it does not affect their development, but arcelin-1 is associated with high level of resistance to major bruchid species (Osborn *et al*., 1988). A compound of the soluble carbohydrate fraction which inhibit oviposition and the development of insects on seeds were strongly metabolic to *A. obtectus* larvae (Van huis, 1991).

The treatments for AO-1012-29-3-3A × *A. obtectus* and (ROBC (8-54) AA × *Z. subfasciatus*) scored both the highest number of days from larva to pupa, and from pupa to adult emergence. The highest number of days from larva to pupa, and from pupa to adult emergence was also reported to be due to the presence of multiple arcelin-like proteins as the major seed storage proteins which confer resistance to bean bruchids (Kusolwa and Myers, 2012: Kusolwa *et al.,* 2016). Similar observations on biology, oviposition and larval-pupal development of bruchids have been reported by Thakur and Renuka (2014). In their study, the development of successive larval instars was completed inside the host seeds. All the larval instars were voracious feeders. The last larval instar prepares an emergence window before molting to pupal stage. Larval development was completed in 14-20 days. The pupal stage also completes development inside the host seeds and pupal development took 14-17 days. The time spent in the pupa is relatively short, from 7-28 days for the storage species of *Callosobruchus* and *Acanthoscelides* (Southgate, 1979).

High levels of resistance to each bean bruchid identified among non-cultivated wild beans; some wild accessions were resistant to both insects (Schoonhoven *et al.,* 1983). Resistance was expressed as reduced oviposition, a prolonged larval developmental period, and reduced progeny weight (Schoonhoven *et al.,* 1983), of which is similar to the present study findings. Similarly, to the present study findings, high mortality of the larva together with a markedly prolongation of the duration of the larva development of bruchids was associated to high levels of antibiosis resistance to the major bruchids in wild dry bean accessions (Cardona *et al.,* 1989). Antibiosis expressed with the adverse effect on larva of the storage pest feeding on the host plant may also involve morphological, physiological, and biochemical features of the host plant or their combination (Keneni *et al.,* 2011). As a result, antibiosis may definitely lead insect pest to death, similarly to what has be elaborated in the present study findings.

The mean adult life span of bruchid specie; *A. obtectus* was longer than *Z. subfasciatus* in the present study. Nearly similar results were reported by Golob and Kilminster (1982), whereby the life span of *Z. subfasciatus* adult females was 12.8 ± 0.1 days. It was also reported by Schoonhoven (1976) that, generally, adults of *Z. subfasciatus* live shorter (7.6 days) than those of *A. obtectus* (11.8 days). Previous research works revealed that, average life span for adults of *A. obtectus* and *Z. subfasciatus* were (14.0 days) and (10-13 days), respectively (Cardona 1989; Abate and Ampofo, 1996; Thakur and Renuka, 2014), of which comply with the present study findings. Moreover, previous research by Kusolwa *et al*. (2016) indicate that adults which emerged from bean genotypes that contain arcelins and arcelin-like (ARL2) proteins have relatively short life span compared to those emerged from susceptible bean cultivars. Probably that, the bean genotypes (AO-1012-29-3-3A and ROBC (8-54) AA) with short life span of bruchids in the present study confirms the effect of ARL2 proteins which are known to confer resistance.

2.6 Conclusion and Recommendations

From results obtained, it was possible to classify genotypes based on resistance, due to adverse effect on insect development, reproduction and survival. Resistant bean genotypes were AO-1012-29-3-3A and ROBC (8-54) AA. Moderately resistant bean genotypes were 65/44-30-2-3A-1, 30/59-96-2-3A-1, and ROBC (14-34) AA, and the rest bean genotypes 44-NJANO and 59-SOYA were susceptible. Genotypes (AO-1012-29-3-3A and ROBC (8-54) AA) presented prolonged development period for bruchids, lowest means for number of holes per grain, low number of emerged insects, lowest means for emergence percentage, low number of surviving adults, and low percentage adult survival indicating resistance of antibiosis type. This work demonstrates the superior resistance of the common bean backcross lines on the life stages and population dynamics of the major bruchids pests (*A. obtectus* and *Z. subfasciatus*) of common bean. Based on the

performance of bruchid resistant bean genotypes, it is recommended as an important strategy for reduction of postharvest losses of stored common beans in Tanzania and other bean growing regions where bruchid is a problem.

References

Abate, T., and Ampofo, J. K. O. (1996). Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* 41(1): 45 – 73.

Alvarez, N., Mckey, D., Hossaert Mckey, Martine., Born, C., Mercier, L. and Benrey, B. (2005). Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. *Molecular Ecology* 14(4): 1015 – 1024.

Baier, A. and Webster, H. B. D. (1992). Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. Seed stored on small farms II. Evaluation of damage. *Journal Stored Prod*uction 28: 289 – 293.

Blair, M. W., Prieto, S., Díaz, L. M., Buendía, H. F. and Cardona, C. (2010). Linkage disequilibrium at the APA insecticidal seed protein locus of common bean (*Phaseolus vulgaris* L.). *BioMed Central Plant Biology* 10(1): 79.

Cardona, C. (1989). Insects and other invertebrate bean pests in Latin America. In: *Bean Production Problems in the Tropics*. (Edited by Schwartz, H. F. and Pastor-Corrales, M. A.). Centro Internacional de Agricultural Tropical , Cali, Columbia. pp. 505 - 570.

Cardona, C., Dick, K., Posso, C. E., Ampofo, K. and SNadhy, S. M. (1992). Resistance of a common bean (*Phaseolus vulgaris* L.) cultivar to post-harvest infestation by *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae). Storage pest. *Tropical Pest Management* 38: 173 – 175.

Credland, P. F. and Dendy, J. (1992). Comparison of seed consumption and the practical use of insect weight in determining effects of host seed on the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.). *Journal of Stored Products Research* 28(4): 225 – 234.

Golob, P. and Kilminster, A. (1982). The biology and control of *Zabrotes subfasciatus* (Boheman)(Coleoptera: Bruchidae) infesting red kidney beans. *Journal of Stored Products Research* 18(3): 95 – 101.

Guzzo, E. C., Vendramim, J. D., Chiorato, A. F., Lourenção, A. L., Carbonell, S. A. M. and Corrêa, O. M. B. (2015). No correlation of morpho-agronomic traits of *Phaseolus vulgaris* (Fabaceae) genotypes and resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman)(Coleoptera: Chrysomelidae). *Neotropical Entomology* 44(6): 619 – 625.

Howe, R. W. and Currie, J. E. (1964). Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bulletin of Entomological Research* 55(3): 437 – 477.

Inra (2019). French National Institute for Agricultural research. [http://www. inra.fr/hyppz/RAVAGEUR/6acaobt.htm] site visited on 21/6/2019.

Jovanović, Z., Kostić, M. and Popović, Z. (2007). Grain-protective properties of herbal extracts against the bean weevil *Acanthoscelides obtectus* Say. *Industrial Crops and Products* 26(1)l 100 – 104.

Keneni, G., Bekele, E., Getu, E., Imtiaz, M., Damte, T., Mulatu, B. and Dagne, K. (2011). Breeding food legumes for resistance to storage insect pests: Potential and limitations. *Sustainability* 3(9): 1399 – 1415

Kifle, F. (2017). College of Natural and Computational Sciences Department of Zoological Sciences Insect Sciences Stream. (Doctoral dissertation, Addis Ababa University.

Kiula B. A. and Karel A. K. (1985). *Effectiveness of Vegetable Oils And Other Plant Products in Protecting Beans Against Mexican Bean Weevil (Zabrotes Subfasciatus) (Boheman)*. Annual Report No. 28. Bean Improvement Cooperation, New York. 5pp.

Kornegay, J. and Cardona, C. (1991). Breeding for insect resistance in beans. In: *Common Beans: Research for Crop Improvement.* (Edited by Schoonhoven and O. Voysest, O.), Commonwealth for Agriculture Bureau International, Wallingford, UK. 619648pp.

Kusolwa, P. M. (2007). Breeding for bruchid resistance in common bean proteins from tepary bean (*P. acutifolius* A. Gray), Genetic Control and Bruchid Resistance Characterization. Thesis for Award of PhD Degree at Oregon State University, 185pp.

Kusolwa, P. M. and Myers, J. R. (2011). Seed storage proteins ARL2 and its variants from the APA locus of wild tepary bean G40199 confers resistance to *Acanthoscelides obtectus* when expressed in common beans. *African Crop Science Journal* 19: 255–265.

Kusolwa, P. M. and Myers, J. R. (2012). Peptide sequences from seed storage proteins of tepary bean (*Phaseolus acutifolius*) accession G40199 demonstrate the presence of multiple variants of APA proteins. *International Journal of Biochemistry and Biotechnology* 1(1): 12 – 18.

Kusolwa, P. M., Myers, J. R., Porch, T. G., Trukhina, Y., González-Vélez, A. and Beaver, J. S. (2016). Registration of AO-1012-29-3-3A Red Kidney Bean Germplasm Line with Bean Weevil, BCMV, and BCMNV Resistance. *Journal of Plant Registrations* 1-5.

Maro, C. (2017). Genetics and mechanisms of Bruchid resistance in selected common bean (*Phaseolus vulgaris* L.) landraces from Tanzania and Malawi (Masters dissertation, Sokoine University of Agriculture).

Mbogo, P. K., Myers, J. R. and Davis, J. (2009). Transfer of the arcelin phytohemagglutinin– alpha amylase inhibitors seed storage proteins locus from tepary bean (*P. acutifolius* A. Gray) to common bean (*P. vulgaris* L.). *Biotechnology* 8: 285 – 295.

Mutungi, C., Affognon, H. D., Njoroge, A. W., Manono, J., Baributsa, D. and Murdock, L. L. (2015). Triple-layer plastic bags protect dry common beans (*Phaseolus vulgaris*) against damage by *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) during storage. *Journal of Economic Entomology* 108(5): 2479 – 2488.

Osborn, T.C., Alexander, D., Sun, S.S.M., Cardona, C. and Bliss, F.A. (1988). Insecticidal activity and lectin homology of arcelin seed protein. *Science* 240: 207 – 210

Parsons, D. M. and Credland, P. F. (2003). Determinants of oviposition in Acanthoscelides obtectus: a nonconformist bruchid. *Physiological Entomology* 28(3): 221 – 231.

Paul, U. V., Lossini, J. S., Edwards, P. J. and Hilbeck, A. (2009). Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. *Journal of Stored Products Research* 45: 97–107.

Quentin, M. E., Spencer, J. L. and Miller, J. R. (1991). Bean tumbling as a control measure for the common bean weevil, *Acanthoscelides obtectus*. *Entomologia Experimentalis Et Applicata* 60(2): 105 – 109.

Randome, I., Segwabe, M. and Allotey, J. (2016). Damage caused, loss assessment and emergence pattern of *Acanthoscelides obtectus* (Say) on the beans, *Phaseolus vulgaris* L. in Gaborone

Ribeiro-Costa, C. S. and Almeida, L. M. (2012). Seed-Chewing Beetles (Coleoptera: Chrysomelidae: Bruchinae). Insect Bioecology and Nutritional for Integrated Pest Management. CRC Press, Boca Raton, Florida p 325-352.

Schoonhoven, A. V. (1976). Pests of stored beans and their economic importance in Latin America. In: *Proceedings of the 15th International Congress of Entomology*, August 19 – 27, Washington DC., USA. 26pp.

Schoonhoven, A. V. and Cardona, C. V. (1982). Low levels of resistance to the Mexican bean weevil in dry beans. *Journal of Economic Entomology* 75(4): 567 – 569.

Schoonhoven, A. V., Cardona, C. V. and Valor, J. (1983). Resistance to the bean weevil and the Mexican bean weevil (Coleoptera: Bruchidae) in non-cultivated common bean accessions. *Journal of Economic Entomology* 76(6): 1255 – 1259.

Southgate, B. J. (1979). Biology of the Bruchidae. *Annual Review of Entomology* 24(1): 449 – 473.

SUA, (2016). Sua Main Campus Masterplan Which Was Approved by Council at its 143rd Held on 30th June 2016.Sokoine University of Agriculture, Morogoro, Tanzania. 36pp.

Syombua, M. E. (2015). Effects of essential oils of lantana camara and two ocimum species on bean weevil (*Acanthoscelides Obtectus*) and their chemical compositions. Dissertation for Award of MSc Degree at School of Pure and Applied Sciences, Kenyatta University, 82pp.

Thakur, D. R. (2012). Taxonomy, distribution and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae)-A New Record. *Pakistan Journal of Zoology* 44(1).

Thakur, D. R. and Renuka (2014). Biology and biointensive management of *Acanthoscelides obtectus* (Say)(Coleoptera: Chrysomelidae)–a pest of kidney beans worldwide. In: *Proceedings of the 11th International Working Conference on Stored Product Protection* pp. 1 – 13.

Utida, S. (1967). Collective oviposition and larval aggregation in *Zabrotes subfasciatus* (Boh.)(Coleoptera, Bruchidae). *Journal of Stored Products Research 2*(4): 315 – 322.

Van Huis, A. (1991). Biological methods of bruchid control in the tropics: a review. *International Journal of Tropical Insect Scienc*e 12(3): 87 – 102.

CHAPTER THREE

3.0 EVALUATION OF SELECTED COMMON BEAN LINES FOR RESISTANCE TO MAJOR BEAN BRUCHIDS UNDER FARMERS' STORAGE CONDITION

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3.1 Abstract

Proper storage and ability of Common bean (*Phaseolus vulgaris* L.) to inherently resist storage pests plays an important role in preventing postharvest losses (PHLs) inflicted by the Common bean bruchid (*Acanthoscelides obtectus*) and Mexican bean bruchid (*Zabrotes subfasciatus*). The study aimed to reduce PHLs by resistant bean genotypes. The lab promising bean lines AO-1012-29-3-3A and ROBC (8-54) AA which were found to have lowest number of holes per grain and emerged adults were evaluated through on-farm storage for the period of 90 days. The study was laid out as a 2x4 factorial experiment arranged in randomized complete block design (RCBD) with two factors and five replications (randomly selected farmers per agroecological zone). Factor A entailed the common bean genotypes at four levels i.e., two resistant lines; AO-1012-29-3-3A, ROBC (8-54) AA, and two susceptible lines; 44-NJANO and 59-SOYA as control. While Factor B consisted the Agroecological zones at two levels namely; Southern Highlands Zone (Nambala village in Mbozi district, Songwe region) and Northern Zone (Mungushi village in Hai district, Kilimanjaro region). Each bean genotype weighing 0.75 kg was placed in a 1 kg brown paper bag and stored in farmers' warehouse under natural infestation of bruchids. The quantitative data for number of holes per grain, number of emerged adults, percentage grain damage and percentage weight loss were subjected to

analysis of variance. Mean separation test was conducted using Duncan's Multiple Range Test ($\alpha = 0.05$). The results showed that, regardless of the agroecological zones, the bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA had significantly lowest weight loss (0.23%; 0.65%), seed damage (4.1%; 4.25%), number of emerged adult bruchids (2.4; 2.9) and number of holes per grain (0.42; 0.3), respectively than 44-NJANO and 59-SOYA. While the bean genotype 59-SOYA had significantly highest weight loss (11.5%), seed damage (45.0%), number of emerged adult bruchids (111.9) and number of holes per grain (8.9). Hence on-farm storage results confirmed those from laboratory, implying that the bean genotypes AO-1012-29-3-3A and ROBC (8-54) AA can be stored for longer period at both agroecological zones and would possibly give farmers food security and surplus grain for sale at favorable price. It is therefore recommended that, further studies to be conducted to determine the efficacy of the promising resistant bean genotypes in combination with other storage management practices to prevent yield losses.

Keywords: Common bean, Major bean bruchids, Postharvest losses, Agroecological zones, Kilimanjaro, Songwe

3.2 Introduction

Grain storage plays an important role in preventing losses which are caused mainly due to insect pests (Prakash *et al*., 2016). Moreover, pre-harvest and post-harvest damages by insect pests are the major limiting factors of bean production in resource poor smallholder farming conditions. In most places where beans are grown, they also suffer heavy losses in terms of both quality and quantity when stored while unprotected (Abate and Ampofo, 1996).

Bruchids such as the common bean or dry bean bruchid (*Acanthoscelides obtectus*), and the Mexican bean bruchid (*Zabrotes subfasciatus*) are storage pests attacking dried beans

in Africa (Jones *et al.,* 2011). These bruchids are pests of Neotropical origin/region (i.e., Central America, the Caribbean and South America) (Thakur, 2012). The bruchids have crossed the geographical boundaries and distributed over different countries (became cosmopolitan) through human-arbitrated migrations and import/export of food grains/feeds. The pest species are highly adaptive, hence distributed from temperate to tropical climates such as in Sub-Saharan African countries including Tanzania (Thakur, 2012). These bruchids can attack pods in the field laying eggs on ripening pods and in storage (Schoonhoven, 1976; Abate and Ampofo, 1996).

The major bruchids are small bruchids (3-5 mm) grey, brown to reddish-brown in colour. Females of the Mexican bean bruchid lay eggs glued to the bean seeds, while females of the dry bean bruchid lay eggs scattered between the bean seeds. Both common and Mexican bean bruchids' development takes place inside the bean and takes about one month before the adult emerges (Southgate, 1979; Credland and Dendy, 1992).

The larvae of these major bruchids feed on the seeds which lead the grains to be unfit for human consumption and also causing unpleasant smell. They also reduce the seed and/or grain quality and marketability, furthermore destroying the seed and reducing germination capacity (Blair *et al.,* 2010). The adult emerges from the seeds leaving small round holes on the bean seeds. However, heavy infestations of stored grains can result in a large number of holed seeds, so far infested seeds loose mass, viability, quality and nutritional value, the latter two due to the presence of frass, eggs and dead insects in the stored beans (Luz *et al.,* 2017).

On-farm storage is of short term however, it is subsequently inflicted by severe losses due to the major bruchid species in inadequately secured storage. Moreover, the importance of

storage protection to a market-oriented farmer is a result of price seasonality, value loss prevention, and their respective opportunity costs of capital (Jones *et al.*, 2011). The average dry weight losses in unprotected stores range from 10-40% and up to 70% grain damage (Kiula and Karel, 1985; Paul *et al*., 2009).

The resource poor farmers use mostly traditional methods on the storage insect pest control and the more readily available low-cost items are ash, plastering of storage bins with clay and cow dungs, sand, salt, botanicals and other plants extracts (Prakash *et al*., 2016). On the other hand, botanical extracts can affect even beneficial organisms to some extent, and the use of dust, wood ashes in spaces between seeds provides some control of bruchids, although not highly effective and is too expensive and laborious for resource-poor farmers (Tripathy, 2016).

The cultural control techniques such as timely and frequent harvesting, legumes planted away from granaries, crop hygiene and storage in pods, are within the technical and financial means of the small-scale farmer. This means, in order to effectively implement control measures at farmer's level, the socio-economic and cultural aspects should be considered (Van Huis, 1991). Like any other leguminous crops, insect management of common bean pests mainly depends on the use of chemical insecticides, however a wide use of these chemicals has resulted to more pest problem due to upset of balance between insect and natural enemies, increase in insecticide resistance, and have tremendous effect on non-target beneficial organisms. Thus, IPM strategy which includes cultural, botanicals and resistant genotypes for common bean insect pests may offer sustainable and environmentally friendly pest management options (Thomas and Waage, 1996; Mendesil, 2014).

The storage structures under small-scale production conditions of the Tropics and Sub-tropics are built within the same houses that families dwell-in, and makes the use of chemical insecticides more hazardous to human health (Keneni *et al*., 2011; Okwute, 2012). In the case of storage aspects, there is no economic threshold level for storage insect pests, unlike field insects. The insecticides are applied as prophylactic measure which aggravates the problem by destroying the population of natural enemies (predators and parasitoids) of the target insect pests (Keneni *et al*., 2011).

The industrial made insecticides like methyl bromide, are pollutants with a long period persistence in food stuffs, environment and its ecosystems, they are expensive and unreliable in supply when required especially in rural remote area where many farmers lives, but also they are naturally toxic and harmful to human health especially when used at the situation where storage compartments or structures are built within a family living houses (Cork *et a*l., 2009; Keneni *et al*., 2011; War *et al*., 2017).

Plant resistance (Varietal resistance) is a fundamental method for insect pest control which is effective, practical, and low cost to farmers (Keneni *et al*., 2011; Kifle, 2017). The genes for complete resistance to storage insects are of rare occurrence in nature for cultivated species. They have often been reported in cultivated species or germplasm collections of wild relatives for a number of legume crops such as haricot bean, field pea, cowpea, black gram and chickpea (Keneni *et al*., 2011). Nevertheless, complete resistance to major bruchids of common bean has not yet been reported. Therefore, the present study aimed to evaluate the selected common bean lines (AO-1012-29-3-3A and ROBC (8-54) AA) for resistance to major bean bruchids under the farmer's storage conditions from different agroecological zones.

3.3 Materials and methods

3.3.1 Description of the study area

The study was conducted in two agroecological zones namely; Southern Highlands Zone (SHZ) and Northern Zone (NZ). The first site was at Nambala village in Mbozi district, Songwe region, 09º07' S, 33º09' E with an altitude of 1500 meters above sea level. The second site was at Mungushi village in Hai district, Kilimanjaro region, 03º 44' S, 37º 43' E with an altitude of 893 meters above sea level. Mbozi district, Songwe region has tropical type of climate experiences the temperatures range from about 16°C in the highlands to 30°C in the lowland areas (URT, 1997; Batho *et al.*, 2019). The weather condition is moderately hot during the months of August to December, while cold weather in June and July and the remaining period which covers the months of January to May is on average warm. It is characterized with clearly distinguished rainy and dry seasons. The rainy season usually starts in October and ends in May while dry season starts from June to September. It receives adequate and reliable rains which range between 650 mm and 2600 mm per annum (URT, 1997; Batho *et al.*, 2019). Whereas, Hai District, Kilimanjaro region receives a mean annual rainfall of 521±188 mm, and the mean annual temperature of 23.3±0.66°C (Munishi *et al.*, 2015). The area experiences a bimodal rainfall pattern mainly the long rainy season which starts in March and ends in June, and the short rainy season usually between the months of November and December (Munishi *et al.*, 2015).

3.3.2 Description of experimental materials (bean genotypes)

AO-1012-29-3-3A is a multiple virus (possesses *I* and *bc-1***2** genes that confer resistance to BCMV and BCMNV), and bean bruchid-resistance germplasm. It is a dark red kidney seed type from the cross of the BC_3F_4 lines 'Rojo'*3///SMARC-2-PN-1// 'ICA Pijao'*2/G40199. ROBC (ROBC (8-54) AA) is a Rojo backcross line with genes for APA resistance to bruchids developed from crosses between Rojo and RAZ lines with arcelins

2, and introgressed with APA proteins from tepary beans G40199. 44-Njano and 59-Soya are the landraces and common market and farmers preferred beans susceptible to bean bruchids (Kusolwa *et al.* 2016).

3.3.3 Experimental design

The study was laid out as a 2x4 factorial experiment arranged in randomized complete block design (RCBD) with two (2) factors and five (5) replications (randomly selected farmers per agroecological zone). Factor A entailed the common bean genotypes at four (4) levels i.e., two (2) resistant lines; AO-1012-29-3-3A, ROBC (8-54) AA, and two (2) susceptible lines; 44-NJANO and 59-SOYA as control. While Factor B consisted the Agroecological zones at two (2) levels namely; Southern Highlands Zone (Nambala village in Mbozi district, Songwe region) and Northern Zone (Mungushi village in Hai district, Kilimanjaro region). Each bean genotype weighing 0.75 kg was placed in a 1 kg brown paper bag and stored in farmers' warehouse under natural infestation of bruchids as illustrated in (Plate 3.1).

Plate 3.1: **Experiment set under farmers' storage conditions. (a) Bean genotypes placed in a brown paper bag (25 x 35 cm) and stored in farmers' warehouse under natural infestation of bruchids (b) Farmers inspecting the performance of bean genotypes for resistance to bruchids**

3.3.4 Data collection and processing

3.3.4.1 Grain damage

The data was collected at the 90th day from confinement/storage period as described by Schoonhoven *et al.* (1983) whereby, grain damage was expressed as the percentage perforated grains in the sample. The number of damaged grains (with characteristic holes/perforations) were counted and used to establish the Percentage Grain Damage (PGD) based on equation 3.1.

$$PGD(\%) = \frac{Total\ number\ of\ perforated\ grains}{Total\ number\ of\ sampled\ grains} \times 100 \dots\dots\dots\dots\dots\dots\dots\dots (3.1)$$

3.3.4.2 Number of holes per seed

The number of holes from the damaged grains (with characteristic holes or perforations) was counted and recorded at the 90th day from confinement/storage period.

3.3.4.3 Number of emerged adult bruchids

The number of emerged adult bruchids from the damaged grains (with characteristic holes/perforations) was counted and recorded at the 90th day from confinement/storage period.

3.3.4.4 Percentage weight loss

The initial and final weight of the bean grains was measured using a digital kitchen scale (Ozeri, ZK 14-S) and percentage physiological grain weight loss (PGWL) was established based on equation 3.2 as described by Kananji (2007).

$$PGWL(\%) = \frac{(IGW - FGW)}{IGW} \times 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots$$

(3.2)

Where; FGW = final grain weight; IGW = initial grain weight for the sample

3.3.5 Data analysis

The data collected were subjected to analysis of variance (ANOVA) using Genstat Statistical Software 16th Edition (VSN International, UK). Mean separation test was conducted using Duncan's Multiple Range Test (DMRT) at 5% significance level.

3.4 Results

3.4.1 Weather condition during the study period in the year 2019

The weather condition during the study period from February to May in the year 2019 was as follows; In Songwe region, the mean air temperature was lowest in April to May and highest in February to March. The Mean monthly maximum air temperature ranged from 23.2°C to 24.6°C while the average monthly minimum air temperature ranged from 12.2°C to 15.6°C. The mean monthly maximum relative humidity ranged from 77% to 87% (Fig. 3.1a). In Kilimanjaro region, the lowest and highest mean air temperature was recorded in February. The Mean monthly maximum air temperature ranged from 27.2°C to 32.8°C while the average monthly minimum air temperature ranged from 19.4°C to 20.6°C. The mean monthly maximum relative humidity ranged from 24% to 67% (Fig. 3.1b).
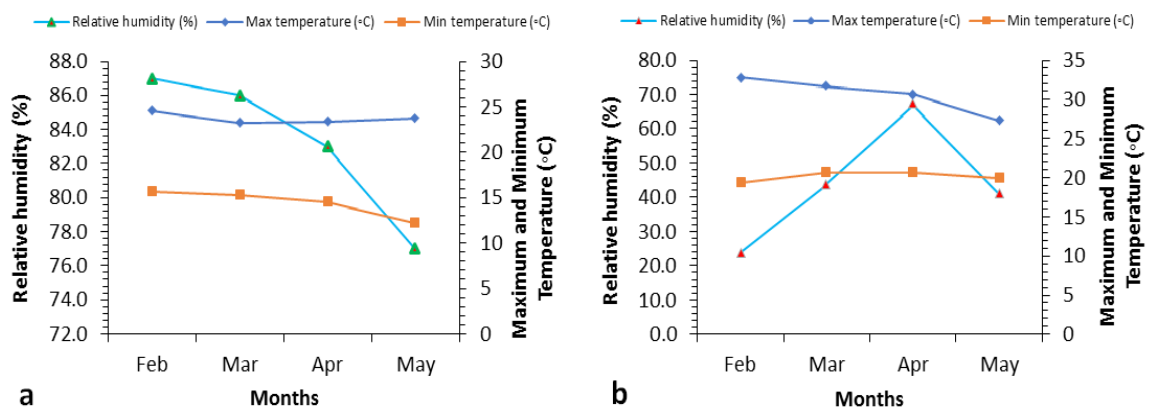
Figure 3.1: **Weather condition during the study period in 2019. (a) Songwe region (b) Kilimanjaro region**

3.4.2 Effect of bean genotypes and major bruchids on grain damage and adult's emergence
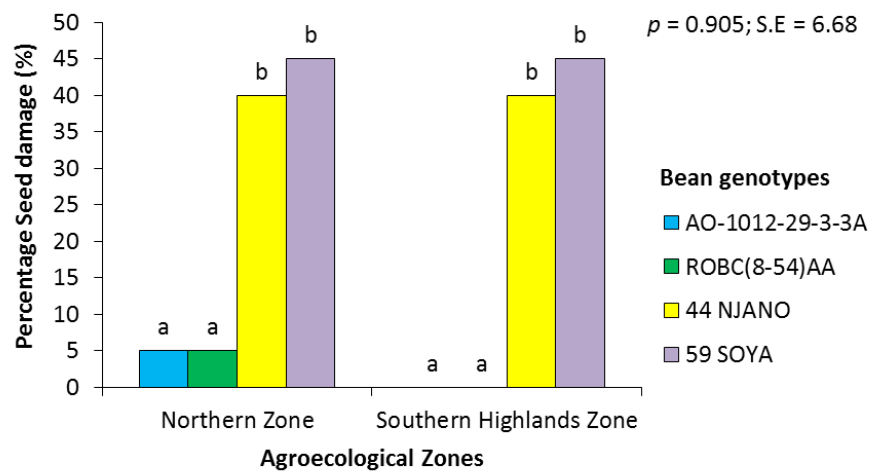
The average number of holes per grain was significantly different ($p<0.001$) among the bean genotypes (Table 3.1). Bean genotype 59-SOYA had the highest average number of holes per grain (11.5) while the lowest average number of holes per grain was recorded on genotypes ROBC (8-54) AA (0.3) and AO-1012-29-3-3A (0.42). Nevertheless, the average number of holes per grain of bean genotypes was not significantly different among the agroecological zones ($p = 0.23$) (Table 3.1) and the interaction between bean genotypes and agroecological zones ($p = 0.536$) (Fig. 3.2). The holes/perforations and damage per grain as inflicted by major bruchids in the study area 90 days after storage are illustrated in (Plate 3.2).
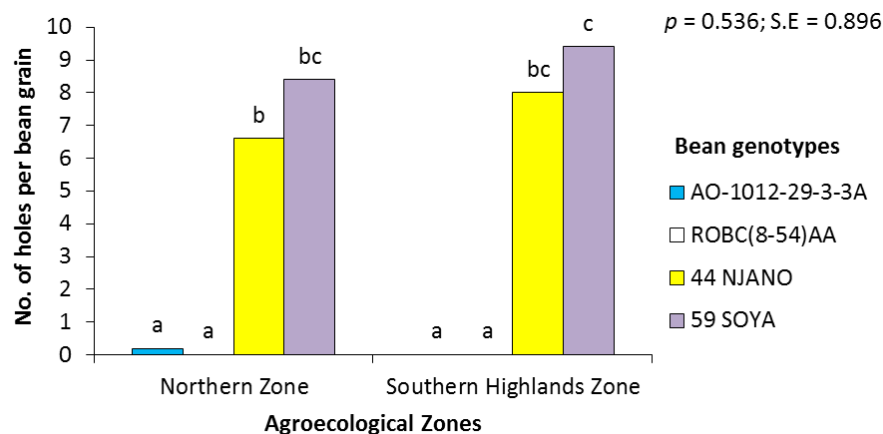
Figure 3.2: **Interaction effect of bean genotypes and agroecological zones on number of holes per grain**

The percentage seed damage by the major bruchids was significantly different ($p<0.001$) among the bean genotypes (Table 3.1). The bean genotype 59-SOYA had the highest seed damage (45%) while the lowest seed damage was recorded on bean genotypes AO-1012-29-3-3A (4.1%) and ROBC (8-54) AA (4.25%). Furthermore, seed damage was not significantly different between the agro ecological zones ($p = 0.461$) (Table 3.1) and the interaction between bean genotypes and agro ecological zones ($p = 0.905$) (Fig. 3.3).

Figure 3.3: **Interaction effect of bean genotypes and agroecological zones on percentage grain damage**

Plate 3.2: **Number of holes and damage per grain as inflicted by major bruchids in the study area 90 days after storage. Bean genotypes (a) 44-NJANO (b) 59-SOYA (c) AO-1012-29-3-3A (d) ROBC (8-54) AA**

The number of adult bruchids emerged from the grain was significantly different ($p<0.001$) among the bean genotypes (Table 3.1). The bean genotype 59-SOYA had the highest mean number of adult bruchids emerged from the grain (111.9) while, the lowest

mean number of adult bruchids emerged from the grain was recorded on bean genotypes AO-1012-29-3-3A (2.4) and ROBC (8-54) AA (2.9). However, number of adult bruchids emerged from the grain was not significantly affected by agroecological zones ($p = 0.572$) (Table 3.1) and the interaction between bean genotypes and agroecological zones ($p = 0.99$) (Fig. 3.4).

Figure 3.4: **Interaction effect of bean genotypes and agroecological zones on number of emerged adult bruchids**

The weight loss due to the major bruchids was significantly different ($p<0.001$) among the bean genotypes (Table 3.1). Bean genotype 59-SOYA had the highest weight loss (11.5%) while the lowest weight loss was recorded on AO-1012-29-3-3A (0.23%) and ROBC (8-54) AA (0.65%). However, the weight of bean genotypes was not significantly different between the two agroecological zones ($p = 0.536$) (Table 3.1) and the interaction between bean genotypes and agroecological zones ($p = 0.892$) (Fig. 3.5).
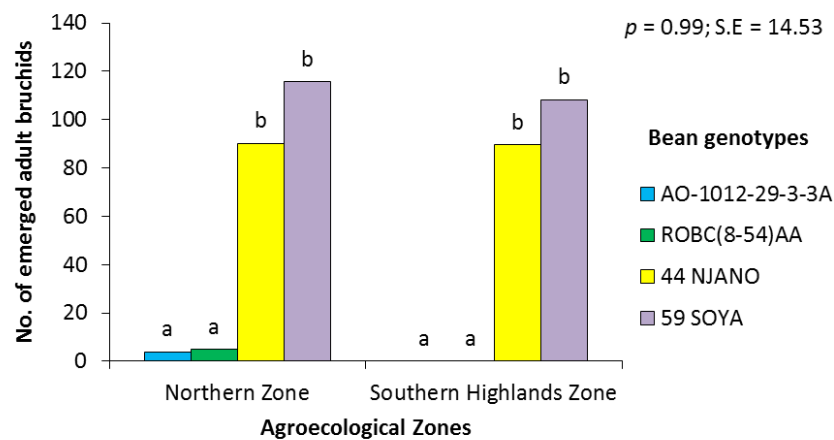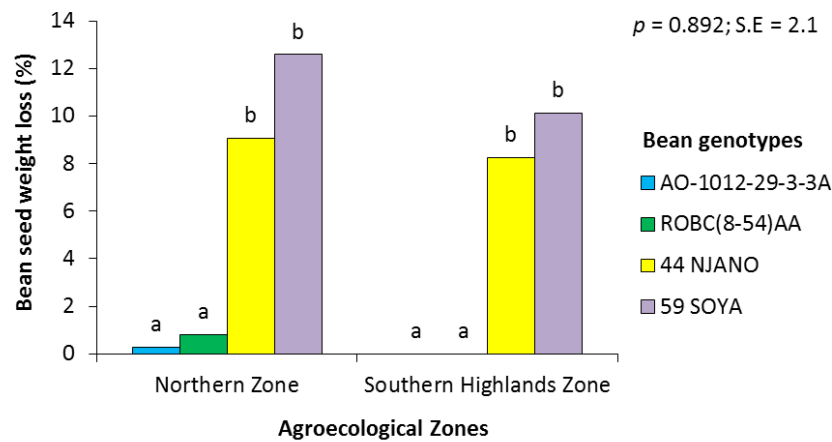
Figure 3.5: **Interaction effect bean genotypes agroecological zones on grain percentage weight loss**

Tables 3.1: **Effect of bean genotypes and major bruchids on grain damage and adults emergence**

	No. of holes per grain	No. of emerged adults	Grain damage (%)	Weight loss (%)
Factor A: Bean genotype				
AO-1012-29-3-3A	0.42a	2.40a	4.10a	0.23a
ROBC (8-54) AA	0.30a	2.90a	4.25a	0.65a
44-NJANO	7.30b	89.90b	40.00b	8.70b
59-SOYA	8.90c	111.90b	45.00b	11.50b
Mean	4.08	52.5	22.5	5.1
S. E	0.634	10.27	4.74	1.48
CV%	34.8	41.8	47.0	64.6
P. value	<0.001	<0.001	<0.001	<0.001
Factor B: Agroecological zone				
Northern Zone	3.80	53.60	23.80	5.70
Southern Highlands Zone	4.35	49.40	21.20	4.60
Mean	4.08	52.5	22.5	5.1
S. E	0.448	7.27	3.34	1.05
CV%	34.8	41.8	47.0	64.6
P. value	0.23	0.572	0.461	0.536

Means bearing the same letter(s) within the column are insignificantly ($p<0.05$) different according to Duncan's Multiple Range Test (DMRT)

3.5 Discussion

The lowest weight loss, seed damage, average number of holes per grain, number of adult bruchids emerged from the grain was recorded in resistant bean genotypes namely AO-1012-29-3-3A and ROBC (8-54) AA in both locations. Probably these results are due to the effect of replacement of phaseolin by arcelin-2 and presence of phytohemagglutinin as

a storage protein in order to enhance resistance to common bean bruchids *A. obtectus*, and *Z. subfasciatus* (Kusolwa and Myers 2011, 2012).

Bruchids larvae developing on resistant lines suffers higher levels of mortality than those on other test varieties, and individuals which survive to adult emergence take longer to complete their development. Consequently, the larvae may decline and colonies may disappear, resulting into very low damage levels. This situation occurred during the first laboratory experiment with the seven bean genotypes (five resistant lines; AO-1012-29-3-3A, 65/44-30-2-3A-1, ROBC (8-54) AA, 30/59-96-2-3A-1, ROBC (14-34) AACIAT and two susceptible control lines: 44-NJANO and 59-SOYA). Among these genotypes, AO-1012-29-3-3A and ROBC(8-54)AA had the lowest number of holes per grain and emerged adults, thus the lab promising bean lines. Therefore, what happened in the farmers' storage/on-farm turns out to be a validation of the laboratory experiment. Similar findings were also reported by Cardona *et al*. (1990) and Padgham *et al*. (1992) during their laboratory experiments.

However, Cardona *et al*. (1992); Padgham *et al*. (1992) also reported that, significantly lower number of insects emerged from a line of Phaseolus vulgaris (RAZ-2) bred for resistance to *Z. subfasciatus*. Hence, seed damage and weight losses in this line (RAZ-2) were negligible as compared to local susceptible cultivars. The RAZ-2 was a double inbred back-cross line obtained by selection for the presence of arcelin-2 in early segregating populations similar to AO-1012-29-3-3A and ROBC(8-54)AA in the present study.

Furthermore, Cardona *et al*. (1992) also stated that, the combined analysis for EMP 175 and RAZ-2, the two varieties tested in both Uganda and Colombia, revealed no significant genotype x strain interactions. This means that RAZ-2 was equally resistant in both

locations. This confirms laboratory results indicating that RAZ-2 was highly resistant to two strains of *Z. subfasciatus* from widely different geographical locations. This was similar to AO-1012-29-3-3A and ROBC (8-54) AA in the present study.

There are also reasons for such performance in AO-1012-29-3-3A and ROBC (8-54) AA that, possibly due presence of Arcelin (ARL2) protein from a wild tepary bean accession G40199. This was transferred into common bean cultivars ICA Pijao and Rojo which are backcross lines with homozygous expression of arcelin and ARL2 proteins of tepary bean. ICA Pijao and Rojo bean lines have clearly shown delay to *A. obtectus* and *Z. subfasciatus* adult emergence, reduction in size, weight and number of emerged adults (Kusolwa and Myers, 2011) like AO-1012-29-3-3A and ROBC (8-54) AA in this study.

The interspecific backcross progenies and wild accession (G40199) of *P. acutifolius* which resulted to AO-1012-29-3-3A and ROBC (8-54) AA contain arcelins and arcelin-like (ARL2) proteins. These proteins are co-expressed with other proteins of the arcelin, α-amylase inhibitor and Phytohemagglutinin (APA) locus which express resistance against the major bean bruchids (Kusolwa and Myers, 2012; Kusolwa *et al.,* 2016).

The presence of seed storage proteins (ARL2, α-AI, and PHA in the complex APA locus) inside the bean cotyledons where larva feeds, they pose inhibition of growth and development of bruchids while feeding in the seed contents (Kusolwa *et al.,* 2016). Moreover, these progenies being a result of backcross between G40199 and cultivated common bean (Phaseolus vulgaris) cultivars ICA Pijao and//Rojo, they do possess the multiple arcelin-like proteins as the major seed storage proteins and occurrence of multiple variants of the APA seed storage proteins in a single accession and can be a result for high resistance to major bean bruchids (Kusolwa and Myers, 2012). The development of major

bruchids resistant common bean lines like ROBC(8-54)AA and that of AO-1012-29-3-3A which combine resistance to BCMV, BCMNV, and bean bruchids can help to reduce yield loss during the growing season and avoid seed damage and loss during storage (Kusolwa *et al.*, 2016).

3.6 Conclusion and Recommendations

From results obtained, genotypes AO-1012-29-3-3A and ROBC(8-54)AA presented lowest means for number of holes per grain, minor means of emerged bruchids, lowest seed damage, and percentage weight loss, indicating resistance of antibiosis type. With these high qualifications, the results of the recent trials under farmers' storage conditions both at Nambala village in Mbozi district, Songwe region (Southern Highlands Zone) and Mungushi village in Hai district, Kilimanjaro region (Northern Zone) indicate that major bruchids-resistant common bean lines developed at SUA are likely to bring significant benefits (towards reduction of postharvest losses) to bean farmers in Tanzania where these bruchids species are major damaging pests. Hence, further efforts may be of importance to ensure a significant access of these common bean lines to farmers in Tanzania and other bean growing regions facing similar constraints linked to the major bruchid species globally.

References

Abate, T., and Ampofo, J. K. O. (1996). Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* 41(1): 45 – 73.

Batho, P., Shaban, N. and Mwakaje, A. (2019). Impacts of rainfall and temperature variation on maize (*Zea mays* L.) yields: A case study of Mbeya Region, Tanzania. *Archives of Agriculture and Environmental Science* 4(2): 177 – 184.

Blair, M. W., Prieto, S., Díaz, L. M., Buendía, H. F. and Cardona, C. (2010). Linkage disequilibrium at the APA insecticidal seed protein locus of common bean (*Phaseolus vulgaris* L.). *BioMed Central Plant Biology* 10(1): 79.

Cardona, C., K. Dick, C. E. Posso, K. Ampofo, K. and Nadhy, S. M. (1992). Resistance of a common bean (*Phaseolus vulgaris* L.) cultivar to post-harvest infestation by *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae). Storage pest. *Tropical Pest Management* 38: 173-175.

Cardona, C., Kornegay, J., Posso, C. E., Morales, F. and Ramirez, H. (1990). Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. *Entomologia Experimentalis et Applicata* 56: 197 – 206.

Cork, A., Dobson, H., Grzywacz, D., Hodges, R., Orr, A. and Stevenson, P. (2009). Review of pre and postharvest pest management for pulses with special reference to East and Southern Africa. Natural Resources Institute, University of Greenwich, London. 136pp.

Credland, P. F., and Dendy, J. (1992). Comparison of seed consumption and the practical use of insect weight in determining effects of host seed on the Mexican bean weevil, *Zabrotes subfasciatus* (Boh.). *Journal of stored products research*, 28(4), 225-234.

Jones, M., Alexander, C. and Lowenberg-DeBoer, J. (2011). *Profitability of Hermetic Purdue Improved Crop Storage Bags for African Common Bean Producers*. Department of Agricultural Economics, Purdue University. 29pp.

Kananji, G. A. D. (2007). A study of bruchid resistance and its inheritance in Malawian dry bean germplasm. Thesis for Award of PhD Degree at University of Kwazulu Natal, South Africa, 187pp.

Keneni, G., Bekele, E., Getu, E., Imtiaz, M., Damte, T., Mulatu, B. and Dagne, K. (2011). Breeding food legumes for resistance to storage insect pests: potential and limitations. *Sustainability* 3(9): 1399 – 1415.

Kifle, F. (2017). College of Natural and Computational Sciences Department of Zoological Sciences Insect Sciences Stream (Doctoral dissertation, Addis Ababa University

Kiula, B. A. and Karel, A. K. (1985). Effectiveness of Vegetable Oils and Other Plant Products. In: Protecting Beans Against Mexican Bean Weevil *(Zabrotes Subfasciatus) (Boheman*. Annual Report, vol. 28. Bean Improvement Cooperation, New York. 5pp.

Kusolwa, P. M. and Myers, J. R. (2011). Seed storage proteins ARL2 and its variants from the APA locus of wild tepary bean G40199 confers resistance to *Acanthoscelides obtectus* when expressed in common beans. *African Crop Science Journal* 19: 255–265.

Kusolwa, P. M. and Myers, J. R. (2012). Peptide sequences from seed storage proteins of tepary bean (*Phaseolus acutifolius*) accession G40199 demonstrate the presence of multiple variants of APA proteins. *International Journal of Biochemistry and Biotechnology* 1(1): 12 – 18.

Kusolwa, P. M., Myers, J. R., Porch, T. G., Trukhina, Y., González-Vélez, A. and Beaver, J.S. (2016). Registration of AO-1012-29-3-3A Red Kidney Bean Germplasm Line with Bean Weevil, BCMV, and BCMNV Resistance. *Journal of Plant Registrations* 1-5.

Luz, C. E., Araujo, T. A., Ribeiro, A. V., Bastos, C. S., Torres, J. B. and Krieger, Y. S. (2017). Resistance of important bean genotypes to the Mexican bean beetle ['*Zabrotes subfasciatus'*(Bohemann)] during storage and its control with chemical synthetic and botanical insecticides. *Australian Journal of Crop Science* 11(9): 11 – 68.

Mendesil, E. (2014). *Plant-resistance to Insect Herbivores and Semiochemicals. Implication for Field Pea Pest Management*. Swedish University of Agricultural Sciences Alnarp, Sweden. 37pp.

Munishi, L. K., Lema, A. A., Ndakidemi, P. A. (2015) Decline in maize and beans production in the face of climate change at Hai District in Kilimanjaro Region, Tanzania. International Journal of Climate Change Strategies and Management 7(1) 17 – 26.

Okwute, S. K. (2012). Plants as Potential Sources of Pesticidal Agents: A Review. *In: Pesticides-Advances in Chemical and Botanical Pesticides*. University of Abuja, Nigeria. 26pp.

Padgham, J., Pike, V., Dick, K. and Cardona, C. (1992). Resistance of a common bean (*Phaseolus vulgaris* L.) cultivar to post harvest infestation by *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae). I. Laboratory tests. *International Journal of Pest Management* 38(2): 167 – 172.

Paul U. V., Lossini J. S., Edwards P. J. and Hilbeck A. (2009). Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. *Journal of Stored Products Research* 45: 97–107

Prakash, B. G., Raghavendra, K. V., Gowthami, R. and Shashank, R. (2016). Indigenous practices for eco-friendly storage of food grains and seeds. *Advance Plants Agriculture Research* 3(4:, 100 – 101

Schoonhoven, A. V. (1976). Pests of stored beans and their economic importance in Latin America. In: *Proceedings of the 15th International Congress of Entomology*, August 19 – 27, Washington DC., USA. 26pp.

Southgate, B. J. (1979). Biology of the Bruchidae. *Annual Review of Entomology* 24(1): 449 – 473.

Thakur, D. R. (2012). Taxonomy, distribution and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae)-A New Record. *Pakistan Journal of Zoology* 44(1): 189 – 195.

Thomas, M., and Waage, J. (1996). Integration of biological control and host plant resistance breeding: a scientific and literature review. CTA.

Tripathy, S. K. (2016). Bruchid resistance in food legumes-an overview. *Research Journal of Biotechnology* 7: 98–105.

URT (1997). Mbozi District Socio-Economic Profile. The United Republic of Tanzania (URT); The Planning Commission Dar Es Salaam and Mbozi District Council Mbeya, Tanzania. 146pp.

Van Huis, A. (1991). Biological methods of bruchid control in the tropics: A review. *International Journal of Tropical Insect Science* 12(3): 87 – 102.

War, A. R., Murugesan, S., Boddepalli, V. N., Srinivasan, R. and Nair, R. M. (2017). Mechanism of Resistance in Mungbean [*Vigna radiata* (L.) R. Wilczek var. radiata] to bruchids, *Callosobruchus* spp. (Coleoptera: Bruchidae). *Frontiers in plant science* 8: 10-31.

CHAPTER FOUR

4.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

i. The study aimed at reducing postharvest losses by evaluation of resistant bean genotypes against *Acanthoscelides obtectus* and *Zabrotes subfasciatus*. Out of seven bean genotypes evaluated for bruchids resistance under laboratory conditions, genotype AO-1012-29-3×3A and ROBC(8-54)AA were observed to have highest resistance to *A obtectus and   Z subfasciatus* by delayed lava development, reduced number of hole and number of survived adults as compared to the control variety 44-NJANO and 59-SOYA.

ii. Under farmer's storage conditions, resistant bean lines AO-1012-29-3×3A, ROBC (8-54) AA presented lowest means for number of holes per grain, minor means of emerged bruchids, lowest seed damage and percentage weight loss, indicating antibiosis type of resistance as compared to susceptible checks 44-NJANO and 59-SOYA in a period of three months of storage.

4.2 Recommendations

This work demonstrates the superior resistance of the common bean backcross lines AO-1012-29-3×3A, ROBC(8-54)AA on the life stages and levels of resistance against the major bruchids pests (*A. obtectus* and *Z. subfasciatus*) of common bean.

i. The results from this study on the performance of bruchid resistant beans is important strategy for reduction of postharvest losses of stored common beans in Tanzania and other bean growing regions where bruchid is a problem.

ii. With these high qualifications, the results of the recent trials under farmers' storage conditions both at Nambala village in Mbozi district, Songwe region (Southern

Highland Zone) and Mungushi village in Hai district, Kilimanjaro region (Northern Zone) indicate that major bruchids-resistant common bean lines developed at SUA can be stored for long at different agro-ecological zones and give farmers food security and excess for sale at favorable price. Thus, likely to bring significant benefits (towards reduction of postharvest losses) to bean farmers in Tanzania where these bruchid species are major damaging pests.

iii. Further efforts may be of importance to ensure a significant access of these common bean lines to farmers in Tanzania and other bean growing regions facing similar constraints linked to the major bruchid species globally.

APPENDICES

Appendix 1: Common Bean Lines used in the present study

ROBC(14-34)AA   **44-NJANO**   **ROBC(8-54)AA**

30-59-96-3-3A-1   **59-SOYA**   **65-44-30-2-3A-1**

AO-1012-29-3-3A