

ADDIS ABABA UNIVERSITY
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Studies on Susceptibility of Different Pulse grains to
***Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae)**
under Laboratory Condition

By
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ABSTRACT

A study was initiated with the objective to understand the susceptibility of different pulse grains to *Callosobruchus chinensis*, and then screen the resistance of different pulses to *C. chinensis* by evaluation of some biological parameters. All Laboratory Experiments were conducted at a controlled temperature of $27 \pm 2^{\circ}\text{c}$ and 40 ± 5 % R.H. In all the Laboratory Experiments 1-3 days old adults were used.

Six different pulses were screened for their relative resistance by exposing the seeds to the adults of the pest in controlled Laboratory conditions. The results indicated that preference of oviposition by *C. chinensis* varied among the treatments. The highest preference of oviposition was recorded on chick pea, faba bean and grass pea while on field pea and lentil least, and null on haricot bean. The treatments also differed in their suitability to the development of the pest since there were significant differences in the number of adult emergence and developmental period.

The treatments also varied in resistance and the variation was due to both non preference for oviposition and larval antibiosis. Field pea and faba bean were more resistant to the attack of *C. chinensis* and this was manifested in terms of a lowered fecundity of the females and number of adult emergence together with prolonged developmental period on field pea and lower number of adult emergence and prolonged developmental period on faba bean..

Germinations were inhibited in treatments with higher number of adult emergence where as 100% germination were recorded on the treatments with lower number of adult emergence and on which oviposition was deterred and also the oviposition deterrence of *C. chinensis* on haricot bean was due to the fact that this treatment may not a host for *C. chinensis*.

1. INTRODUCTION

Grain legumes are classified under the family Leguminacea (Glove, 1987). They are important components of agriculture and food systems in practically all over the world, and they complement the cereal crops in several aspects (Grahams and Vance, 2003).

Grain legumes had long been in Ethiopia and Ethiopia is considered as an important center of diversity for food legumes. Faba bean, chickpea, haricot bean and field pea are the major pulse crops that are produced in terms of area coverage and total production (CSA, 2002).

Faba bean or broad bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), chick pea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) are the four major high land pulses grown in Ethiopia while haricot bean (*Phaseolus vulgaris*) is a low land pulse which is produced all over the country although the major production area is the central rift valley (Gezahegn and Dawit, 2006 cited in Abraham Tadesse, *et al.*, 2008).

Legumes supply a high proportion of plant protein which are not only main but also the cheapest source of protein in areas where animal protein is scarce and too expensive for the large proportion of population. The protein content usually exceeds those of other food plants. Grain legumes are important sources of cash for the rural poor. Beside human food, their straw serves as feed for cattle mainly in rural areas, where grazing land is limited. They also help to improve soil fertility through nitrogen fixation and reduce the external fertilizer input (Okigbo, 1978).

It has been estimated that between 60% and 80% of all grain produced in the tropics is stored at farm level (Boxall, 1998). Delima (1987) demonstrated that between 25% and 40% of stored agricultural produce is lost annually in the tropics because of the activity of storage pests. Considerable amount of stored products, both in terms of quantity and quality are lost to insect and mite pests occurring in storage in Ethiopia (Tsedeke and Adhanom, 1985).

In Ethiopia, pulses are grown predominantly by private holdings (subsistence farmers) using primitive farm tools under rain fed condition with minimum inputs as compared to cereal crops. In such situations where production is seasonal food supply and food security is mainly dependant on good storages, it is estimated that about 60 % to 90% of the produces is by the farm house hold and stored for 6 to 12 months in Ethiopia (Abraham, 2003 cited in Abraham Tadesse, *et al.*, 2008).

Good quality of stored grains in general and grain legumes in specific is their ability to be stored long period for later consumption or good price. However, most of the grain legumes suffer major economic loss caused by grain infesting insects due to cumulative effects of feeding, breeding, transmission of toxic and saprophytic fungi and associated changes in the micro ecological conditions in the grain bulk, which hasten the deterioration process in the grain (Alloty, 1991).

Inspection of storage site to determine the source, type and importance of the infestation is the beginning of effective management which is normally called prevention. In general biological, cultural, and chemical methods are approaches for successful management of stored product pests (Gwinner *et al.*, 1990). Host plant resistance is a very good method of combating pest in storage. It is perhaps the easiest, most economical and effective means of controlling insect pests on stored grains as there is no special technology which has to be adopted by farmers. Screening of many seed varieties had led to the successful isolation of strains that are resistant to insect pests in some African countries (Ahmed and Yusuf, 2007). In spite of the effectiveness of host grain resistance in combating pests in storage, research works conducted on screening resistant host grains were not sufficient. Hence, the current investigation was initiated with the following objectives:

2. OBJECTIVES OF THE STUDY

2.1. General objective

- To generate baseline information for the management of *Callosobruchus chinensis*.

2.2 Specific Objectives

- To study the oviposition preferences of *Callosobruchus chinensis* on different grain hosts
- To study the number of adult progeny emergence of *C. chinensis* on different pulse grains
- To study the population growth of *Callosobruchus chinensis* on different pulse grains
- To study the developmental period of *Callosobruchus chinensis* on different grain hosts
- To screen different pulses for resistance towards *C. chinensis*

3. LITRATURE REVIEW

3.1. Storage Insect Pests

Stored product pests are very important in Ethiopian agriculture. Considerable amounts of stored products both in terms of quantity and quality are lost in this country. The main agents causing deterioration of stored products are microorganisms, rodents, birds, insects and mites. Among these, insects are the principal pests responsible for losses to food grains. During storage foods are currently destroyed by insects and other pests (Negamo *et al.*, 2007).

The most common insect pests to stored products belong to the order Coleoptera and Lepidoptera (Bekele *et al.*, 1997; Emanu and Assefa, 1998). The former is the largest order and contains the most important stored product pests (Girma Demissie, 2006; Emanu Getu, 1993).

Sitophilus zeamais, *S. oryze*, *Acanthoscelides obtectus*, *Callosobrochus chinensis*, *Zabrotes subfasciatus*, *Tribolium* species and *Crytolestes* species from the Coleoptera and *Sitotroga cerealella*, *Ephestia cautella*, *Plodia interpunctella*, *Phthorimaea operculella* from Lepidoptera were recorded as major pests of stored grains (Abraham,1991;1996; 1997).

Post harvest pests can be primary or secondary. Primary pests are those which possess strong mouth part and are able to attack intact grains, while secondary pests have weak mouth part and attack damaged grains or grain products (Addis Teshome, 2008).

The pests of sound stored grains include rice, maize and granary weevils (*Sitophilus oryze*, *Sitophilus zeamaize* and *Sitophilus granaries*, the angoumois grain moth (*Sitotroga*

cerealella), the lesser grain borer (*Rythoperta dominica*), several species of pulse beetles (*Callosobruchus chinensis*, *Callosobruchus maculatus*, *Acanthoscelides obtectus*) and others. The pests of processed stored grains include Indian grain moth (*Plodia interpunctella*), Cabinet or ware house beetle (*Trogoderma granarium*), flour beetle (*Tribolium castaneum* and *Tribolium confusum*) and others (Sokoloff, 1972).

Stored product insect pests are capable of inflicting serious damage to stored commodities due to very rapid capacity to increase in number, migrate, infest and thus spreading the infestation (Addis Teshome, 2008).

3.1. 1.The Bruchidae

3.1.1.1. Biology and Behavior

Bruchids are primary pests of pulses. They pose serious post harvest problem to grain legumes. All are seed eaters and most attack seeds of legumes. Although legumes produce chemical substances that make them resistant to invasion by most insects, bruchids species have developed tolerance to many of these chemicals. This, in conjunction with the evolution of different behavioral strategies ensured different species of bruchids to feed on specific legume varieties (Golob *et al.*, 2002; Roger and Hamraoui, 1995).

Bruchids are a well studied family of seed eating beetles, relatively homogeneous in terms of external morphological traits. Adult bruchids do not feed and make damage themselves. According to Kemal (1988), flight is one of the most important bruchidae behavior and the adults are capable of making short as well as long flights.

Females deposit eggs on the seed and the newly hatched larvae bore in to the legume seeds. The first visible signs are the holes made in the seed by the emerging adults. This is perceived as damage (Koonan and Koonan, 2006).

Bruchids can mate and females produce their eggs to begin secondary infestation immediately after emergence and also when infested seeds are harvested and stored the bruchids continue to feed and eventually adults emerge and cause secondary infestation.

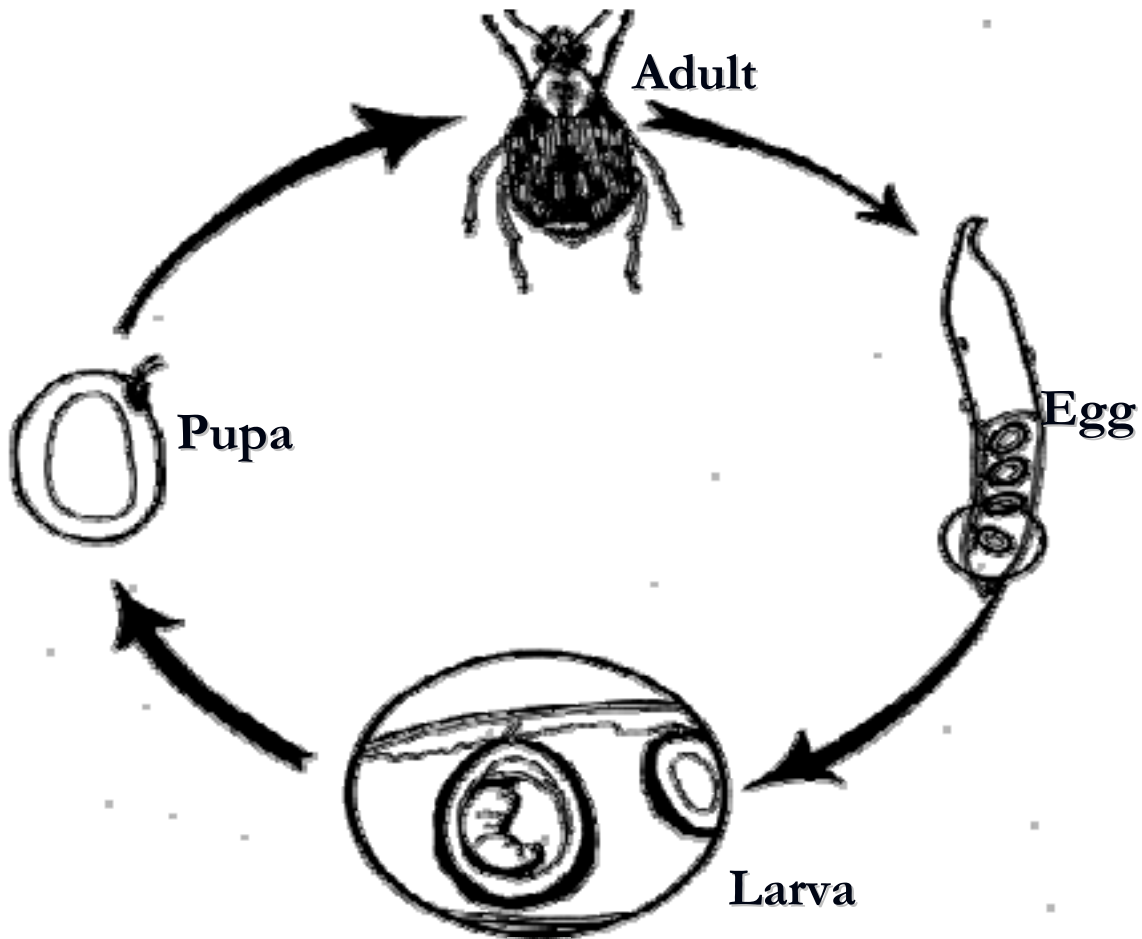


Figure1. Life cycle of bruchids.

Source [www.fao.org /docrep/066 /Q2585E /Q258E03.htm](http://www.fao.org/docrep/066/Q2585E/Q258E03.htm).

It may also begin when adults fly or crawl from other near by sources such as accumulation of waste grains. *Acanthoscelides obtectus* start to infest beans in the field and continue to infest the bean in storage while *Zabrotes subfasciatus* infest in the storage

only (Msolla and Misangu, 2002). Once infestation starts, bruchids have the capacity to increase in number very rapidly causing considerable damage. Even very small initial populations can build up to economically important numbers during the storage seasons. The long hot summer in tropics encourages the development of storage insects (Kemal Ali, 1988).

Bruchids associated to legumes come from six genera. These include *Bruchus*, *Bruchidius*, *Callosobruchus*, *Acanthoscelides*, *Zabrotes* and *Caryedon* (Emana *et al.*, 2003; Dal *et al.*, 2001).

Taylor (1981) pointed out that the family of Bruchidea consists of nearly 1400 species of bean and pea beetles and that they are major pests of leguminous plants throughout the tropics. The economic damage caused by bruchids is restricted to leguminous seeds, with a particular choice evidenced at the generic and specific levels (Howe and Currie, 1964).

The genus of bruchidae which contains the greatest pest species is *Callosobruchus*, although other genera such as *Acanthoscelides* and *Zabrotes* can also be serious pests (Southgate, 1978).

3.1.1.2. Adzuki bean beetle (*Callosobruchus chinensis* (L.))



Figure 2. Adult *Callosobruchus chinensis*

Source bruchitean.nhmus.hu/seed-beetles.html.

3.1.1.3. Distribution, Biology and Behavior of *C. chinensis*

C. chinensis was first described by Linnaeus. According to Kemal (1988) who thoroughly reviewed the life history, distribution, biology and control of the beetle, the species is one of the most widely spread of the genus *Callosobruchus*. It originated in Asia and spread to the tropical and sub tropical regions of the world. It is capable of breeding on the seeds of a number of legumes, but relatively few are considered suitable hosts. It is well recognized as a pest of chick pea (*Cicer arietinum* L.), field peas *Pisum sativum* L.), faba beans (*Vicia faba* L.), Adzuki beans (*Phaseolus angularis*) and lentil (*Lens culinaris* or *Lens esculenta* Medik.)

The females lay their egg which adheres to the seed coat of bean seeds in stores or in maturing pods in the field. This adhesion is effected by fluid secreted from the ovipositor immediately prior to oviposition (Arora and Singh, 1971 cited in Teshome, 1990). The eggs are elongate, oval in shape and translucent white in color, flattened on the side of attachment to the seed. Oviposition period ranges between 3 to 6 days. The duration of the egg stage has been given as 10 to 14 days and the total number of eggs laid per female is said to range from 32 to 91 (Arora and Singh, 1971).

The adult females in the majority of bruchids species attach their eggs directly to the seed or pod, therefore the larvae need only to bore through the shell and pod to reach the food. Most bruchid larvae leave the egg in characteristic manner; the larva bites a neat hole on the underside of the egg where it is in contact with substratum. Then, leaving the shelter of the egg shell, it proceeds to burrow into the seed pod or into the integument of the seed. (Arora and Singh, 1971).

The duration of a complete lifecycle may take only 4 weeks, or it may take several months depending largely on the environmental conditions. According to Arora and Singh (1971) the time taken from egg to adult ranged between 45 and 61 days in summer and 134 days in the winter. Various factors may influence the number of generation and the life span of the adults. Generally, the adults are short lived (less than two weeks) and don't feed. Davey (1965) reported that the minimum and maximum temperature required for the development of *C. chinensis* range between 20 to 35°C and the relative humidity ranges between 10-90 %. The adults are capable of making short as well as long flight (Kemal Ali, 1988).

3.1.2. Damage and Economic Loss Due to Bruchids

The term loss is often expressed in various ways and it is confusing whether it refers to the total amount of grain lost or damage. Similarly, the term loss has been synonymously used with the term damage. Nevertheless, in the context of stored food legumes, it is

usually expressed as loss of commodity weight in the period between harvest and consumption, loss of nutrients in stored legumes, qualitative deteriorations caused by contaminants or biochemical changes rendering legumes unfit for human consumption, loss of seed viability and loss as a result of physical damage. While damage refers to the superficial evidence of deterioration for example, holed or broken grains or bruised fruits or physical spoilage which may later result in loss (Salunkhe *et al.*, 1985).

Crop losses and deteriorations of produce during storage are likely to occur unless adequate precautions are taken. It is frequently reported that world wide a minimum of 10% of cereals and legumes are lost after harvest (Boxall *et al.*, 2002). Like wise, in Ethiopia loss of stored produce reached up to 20-30% (Abraham, 1996). However, it is widely agreed that food losses after harvest can be substantial and are important in terms of quantity, quality, nutritional and economic value (Golob *et al.*, 2002).

The degree of loss due to bruchid damage is quite variable and depends on the storage period, storage conditions, storage containers and varieties (Nchimbi-Msolla and Misangu, 2002; Mebeasilassie, 2004). In Ethiopia, the structural condition of the traditional granaries used for storage of pulses varies from one farm to another. The main problem associated with these storage structures are lack of repair or replacement of the old structures, poor hygiene store, and the distance at which they are located. Granaries that are not repaired permit easy access to rodents, insects and flooding. Moreover, poor store hygiene and storage structures located near by the farm land may cause the development, carry over and cross-infestation of insect pest from previous season harvest. As a result, they attribute to different amount of stored pulse loss and damage by bruchids. Study conducted in 1993 in south eastern Honduran communities indicated that post harvest weight losses associated with bruchids infestations in dry beans stored by subsistence farmers ranges from 5.5% to 8.5% (Espinal, 1993). In a related report, weight loss of pulses ranges from 4-29% in Eritrea (Adugna, 2006) and 3.2% in Ethiopia (Abate and Ampofo, 1996). Abate and Ampofo (1996) also showed that in Ethiopia stored bean damage by *A. obtectus* and *Z. subfaciatus* reached up to 38% and bean weight loss reach 3.2%.

In Nigeria, the dry weight loss of cowpea to bruchids infestation is over 29,000 tones each year (Casuell, 1981) with an estimated value of 30 million dollars. Koono and Koono (2006) indicated that *Acanthoscelides obtectus* (Say) on common bean (*Phaseolus vulgaris*) and *Callosobruchus maculatus* F. on cow pea (*Vigna unguiculata*) can cause seed loss of 20-100% at farm level if left untreated.

In Tanzania, bean loss up to 40% due to bruchids have been reported (Kiula and Karel, 1985).FAO (1985) estimated losses on pulses in the range of 25-50% due to infestation from bruchids, weevils and others.

Bruchids pose serious post harvest problem to chick pea and lentil in particular with the extent of damage sometimes exceeding 90% after three months of storage (DZARC, 1984 cited in Abrham Taddesse,*et al.*,2008).

Survey of faba bean in Chilalo (2050-2690m) and Yerer and Kereyu awraja (1900-2000m) indicated that more infestations were observed in warmer areas than cooler areas (Yemane and Yilma 1985 cited in Abrham Taddesse,*et al.*,2008)

Table1: Variability in degree of infestation depending on agro ecological area, storage time and crop varieties

Pulse	Agro ecological area	Altitude	Storage time	damage	Wt. loss
Faba bean	Chilalo awraja	2050-2690m	13 months	40.2%	4.8%
Faba bean	Yerer and Kereyu awraja	1900-2000m	6-7 months	41%	14.4%
Chick pea	Yerer and Kereyu awraja	1900-2000m lower altitude	6-7 months	27.5%	8.2%

Source (Yemane and Yilma 1985 cited in Abrham Taddesse *et al.*, 2008).

In addition to direct weight loss, bruchids also render qualitative loss, which is more frequently based up on subjective judgment and it perhaps identified via comparison with locally accepted quality standard. It may include the presence of contaminants, such as uric acid and other nitrogenous wastes, the presence of adult bruchids inside the seed, exit holes, glued eggs to the seeds, coastal larval skin, species of insect chitin and changes in appearance, texture and taste, making it unfit for human consumption, Commercial grain buyers usually reject or refuse to accept delivery of insect contaminated grain or may pay very low price for it (Hill, 1990; Espinal, 1993; Nchimbi-Mosolla and Miswangu, 2002).

According to FAO (1985) importers of bean and peas in the UK, objects to accept products infested with most bruchid species. This situation will lead to economic losses to the producer and quantitative losses to the consumer (Msolla and Miswangu, 2002). In addition to their probable negative impacts on the quality of products, these foreign particles have been reported to have serious abrasive effects on human alimentary canal (Southgate, 1978).

Bruchids damage can also cause nutrition loss which is represented by the reduction in the food value of the grain because of decreasing in its hydrocarbon, protein and vitamin content, (Sing, 1997) and it's also the product of both qualitative and quantitative losses. Mc Farlane *et al.* (1994) demonstrated that a reduction in a protein content of pulses due to the feeding activities of bruchids on cotyledons. In beans particular, loss of protein is very important where there is infestation, as up to 25% of dry matter may be crude protein (FAO, 1992).

Besides, contamination from uric acid by bruchids is correlated with negative changes in nutritive composition of pulses, causing an increased fat acidity and decreased levels of various vitamins in particular thiamin and essential amino acids (Salunkhe *et al.*, 1985).

A single larva of *Callosobruchus maculatus* infesting a seed of cowpea will remove about a quarter of the cotyledon of an average sized seed. When it infests the smaller seed of *Phaseolus radiatus*, it consumes virtually all the cotyledons, there by removing all possible chance of germination of the seed (Southgate, 1979). Venkatrao *et al.* (1960) reported considerable loss in viability and weight of field beans (*Dollchos lablab* L.) and black gram (*Phaseolus mungo*), an increased in acidity and non-protein nitrogen and a decrease in thiamine content. Finally, the damage of pulses by insect can provoke loss in qualitative and quantitative grain production enhancing more instability and poverty.

3.2. Storage Insect Pest Control

There is a continuous need to protect the stored products against deteriorations, especially loss of quality and weight during storage (Mohale, 2004; Negamo *et al.*, 2007). Effective management efforts always begin with a thorough inspection of storage site to determine the source, type, and importance of the infestation which is normally called prevention. In general, biotechnical, biological, cultural, and chemical methods are crucial approaches for successful control of the most dominant stored product pests in general (Gwinner *et al.*, 1990).

3.2.1. Cultural control

Traditional methods usually provide cheap and feasible ways of post harvest handling of the crops. During storage, some traditionally used materials are often added to the product, which contribute to the reduction of pests' activity. Inert dust, for example, is added in variable amounts to the stored product. Friction of the particles with insect's cuticle leads to desiccation and hampers the development of the pest (Golob *et al.*, 1997; Emanu and Assefa, 1998).

It is crucially important to limit the rate of insect migration into stored grain from infestation sites in bin bottoms; near by stored grain, or other places where grain, grain dust, or grain based materials accumulate before harvesting and bringing of new crops in to a store, it is very important to clean tracks, combines and the bins. Research conducted on control of the Coleopterans in stored agricultural products by non-chemical methods indicated that sanitation as preventive control measures of stored grains (Porca *et al.*, 2003).

Post harvest systems regulated by moisture content, temperature, aeration, pest access and time that the product is in a susceptible state. Within biological limits, the greater the temperature, moisture content, aeration and the time products are in a susceptible condition, the higher the resultant pest population. Thus, management systems built around these biological, management factors, and their influence on population dynamics. If planting and sowing is arranged so that harvest fall in dry season, there are no particular problem with drying the crop. Harvest time is also considered when infestation had started from the field. For instance, *Callosobruchus chinensis* (adzuki bean beetles) infest beans in the field only when the pods are almost dry. Timely harvesting can therefore ensure that the weevils not carried in to the store along with the beans (Stoll, 1988).

3.2.2 Biological Control

In biological control, natural enemies are used to keep pest populations at acceptable levels, usually in combination with other control methods. The natural enemies may be predators, parasitoids or pathogens (Flinn *et al.*, 2006). Predators such as spiders, ladybirds, lacewings or predatory mites, usually feed on a range of different insects. Parasitoids lay eggs on one host insect, and the larvae live and feed on the host, which dies (true parasites do not kill their hosts). The adult parasitoids are typically honey feeders. Pathogens may be bacteria, fungi, viruses, nematodes or protozoa. There are several ways that natural enemies can be used in biological control (Imamura *et al.*, 2008).

Management of the crop and its surrounding habitats can enhance the abundance of native parasitoids or predators. Parasitoids and pathogens can be mass reared in the laboratory, and inundative releases made in the field for biological control, or as bio-pesticides. Classical biological control is where natural enemies are introduced from overseas usually from the country of origin of the exotic pest (Schmale *et al.*, 2001).

3.2.3. Botanical Control

Botanical pesticides are an alternative to chemical pesticides i.e. insecticidal plants or plant compound and the use of natural compounds, such as essential oils that result from secondary metabolism in plants. Essential oil and their constituents have been shown to be a potent source of botanical pesticides. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of bruchids (Keita, *et al.*, 2000; Tripathi *et al.*, 2002). In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries, but can play a much greater role in the production and post harvest protection of food in developing countries (Isman, 2005).

The insecticidal activity of extracts derived from different plants and parts of the plant against stored product insects has been reported. Bekele *et al.* (1995) evaluated the bioactivity of plant materials from the leaves of *Ocimum kilimandscharicum* against *S. zeamais*; *R. dominica* and *Sitotroga cerealella* (Oliver). They reported 100% mortality after 48 hr exposure of adults of the three insects to dried leaves and essential oil extract of the plant. Bekele (2002) also evaluated toxicity of different plant extracts and seed powder of *Milletia ferruginea* (Hochest.) against *S. zeamais* in maize seeds.

Moreover, Mebeasilassie (2004) reported the efficacy of *M.ferruginea* seed powder against *Zabrotes subfasciatus* and found that 100% mortality at the dose of 15g/250g of grain within 24 hr exposure time.

3.2.4. Pheromones

Semiochemicals determine insect life situations such as feeding, mating and egg laying (ovipositing). Semiochemicals are thus potential agents for selective control of pest insects. Biological control with pheromones or kairomones can be used for detection and monitoring of insect populations. Monitoring is important for the efficient use of conventional insecticides. Mating disruption by use of pheromones is a promising and, in many cases, a successful strategy for control (confusion strategy). The use of semiochemicals as feeding deterrents is another strategy. The most common strategy for control by the use of semiochemicals is to attract, trap and kill the pest insects (Norin, 2007).

The olfactory system of insects is very sensitive and limited amounts of semiochemicals are needed for control. This is demonstrated by the current application of pheromones for control (mating disruption by confusion strategy) of codling moth (*Cydia pomonella*) in apple orchards (Witzgall, *et al.*, 1999 as cited in Norin, 2007).

3.2.5. Varietal Resistance

Insect resistance in crop varieties refers to their inherent ability to combat specific insect pests and to achieve better performance over other varieties of the same crop at the same levels of insect populations. Crop varieties differ in their susceptibility to storage insect pests. Traditional varieties are more resistant than new varieties. Resistant varieties functions in insect control based on the mechanism of non-preference antixenosis) and antibiosis, in which biophysical or biochemical factors are involved (Pedigo, 2004 cited in Teshome Lemma, 1990).

3.2.5.1 Antixenosis

Oviposition may be affected by small differences in seed coat smoothness and convexity, by plumpness or wrinkling and perhaps by size and hardness of the seed as well as its odor. The cowpea beetle (*Callosobruchus maculates*) prefers smooth seeded to rough seeded cowpeas. More over, it doesn't oviposit on seed hilum, which is spongy in texture deep pit like and rich fibrils. Scanning electron microscopy revealed deep pit in rough coated but not in smooth coated seeds; seeds infested with eggs where less attractive for further oviposition (Nwanze *et al.*, 1975).

Research conducted in USA with 14 common chick pea varieties by using selective preference and no choice tests indicated that the variety G109-1 was least preferred for egg laying by *Callosobruchus analis*, *Callosobruchus maculates*, *C. chinensis*. G109-1 has a rough, almost spiny seed coat, a character deterrent to oviposition and absent in susceptible varieties (Raina, 1971 cited in Teshome, 1990).

3.2.5.2. Antibiosis

The mechanism where the pests feed but factors in the plant have an adverse effect on them usually expressed as reduced growth and thus rate of multiplication, or on survival. Level of resistance to pests varies among plant varieties (Hill, 1990).

The failure of *Callosobruchus chinensis* to develop in soy beans is attributed partly to the presence of saponins. Larvae of *Callosobruchus spp.* don't hydrolyze saponins in vitro. Saponins may therefore be regarded as specific metabolic defense mechanism of the Soybean against insects (Horber, 1978 cited in Teshome Lemma, 1990).

Host plant resistance is a very good method of combating pest in storage. It is perhaps the easiest, most economical and effective means of controlling insect pests on stored grains as there is no special technology which has to be adopted by farmers. Screening of many seed varieties had led to the successful isolation of strains that are resistant to insect pests in some African countries. Four varieties of groundnut (*Arachis hypogea* (L.) were found to be resistant to both Indian meal moth (*Plodia interpunctella* (Hubner) and rust red flour beetle (*Tribolium castaneum* (Herbest) (Ahmed and Yusuf, 2007).

3.2.6. Chemical control

Insect pest control in stored food products relies heavily on the use of synthetic insecticides and fumigants. The components of chemical insecticides can be classified in to four chemical types: Organo-chlorines, Organophosphates, Carbamates and Pyrethroids (Dent, 1991).

These groups of insecticides have been used for over five decades to control insect pests both at the field and in storage conditions. Many researchers have reported that the effective utilization of synthetic insecticides including fumigants, dusts, and sprays for the control of bruchids (Harberd, 2004).

Insecticidal application is one means of preventing some losses during storage. However, the choice of insecticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food and also the continuous use of chemical insecticides for control of storage insect pests has led to problems such as disturbance of the environment, pest resurgence, pest resistance and lethal effect on none target organisms in addition to toxicity to the users (Khan and Selman, 1987).

3.2.7. Integrated Pest Management

Integrated Pest Management (IPM) is the best option in pest control. This means, Pest control measures, in general, have to be integrated into an operational system, be it large or small scale, if they are to be effectively applied. This is a basic principle, not a novel concept, but it connects with the modern idea of integrated pest management (IPM). The use, in that term, of the word 'management' is appropriate, especially with regard to the need for the integration of pest control measures into management systems, but it should be remembered that the two words, 'management' and 'control,' are almost synonymous. The fundamentally important emphasis should be placed up on the word 'integrated' (Shaaya and Kostyukovsky, 2006).

Integrated pest management can be defined as the acceptable use of practicable measures to minimize the pest density/effect in cost effective manner and ecological safe situation the loss caused by pests in a particular management system. For the measures to be cost effective, they must be appropriate and acceptable in that system. They may be simple or complex but they must suit to systems' objectives and its technical capabilities. Furthermore, in this context, cost effectiveness requires that all costs and benefits, including sociological and environmental effects, should have been taken into account (Golob *et al.*, 2002; Mohale, 2004).

4. MATERIALS AND METHODS

4.1. Mass Rearing of Test Insects

Adult *Callosobruchus chinensis* were obtained from Holeta Agricultural Research Center and reared at Addis Ababa University, Insect Science Laboratory maintained at $27 \pm 2^\circ\text{c}$ and $40 \pm 5\%$ relative humidity following the method of Porca *et al.*, (2003). Seeds of pulses were brought from Holeta Agricultural Research Center and Debrezeit Agricultural Research Center (Table 2) and were disinfested in an oven at 40°c for 4hrs and kept in the air cooled condenser before use (Bekele *et al.*,1995). The seeds of pulses were used as food substrate for *C. chinensis*. Fifty pairs of unsexed adult *C. chinensis* were placed in 1- liter glass jars containing 200g of the disinfested seeds to start the culture.

The jars were covered with nylon mesh and held in place with rubber bands. The parent beetles were sieved out after 10 days of oviposition and the seeds were kept under laboratory condition until the emergence of F_1 progeny.

4.2. Experiments on Biology of *C. chinensis* on different Pulse grains

About 200g of disinfested legume seeds (*Pisum sativum* (L.), *Cicer arietinum* (L.), *Lathyrus sativus*, *Lens culinaries* (Medik.), *Vicia faba* (L.), *Phaseolus vulgaris* (L.) were placed in each of the six 1-litre glass jars and 10 pairs of 1-3 days old adult *C. chinensis* of mixed sex were introduced to each jar and each of the treatment was replicated three times. Observation and data recording were done every 72 hrs on: number of eggs laid, number of adults emerged, days of adult emergence and number of seeds with damaged hole. When the insects were needed for experiments, the culture medium was sieved through a 3 mm-mesh sieve and the procedure was repeated until the final experiment.

Sources and varieties of pulses used in Laboratory Screening were listed as follows: varieties of pulses collected from Holleta Agricultural Research Center were: field pea Adi variety, chick pea (Worku), lentil (Alemaya), faba bean (Moti), Haricot bean (Awash) and grass pea (Wassie) collected from (Debrezeit).

4.2.1. Bioassay on Oviposition Preference of *Callosobruchus chinensis*

Females

For the assessment of oviposition preference, 30 seeds of pulses were randomly taken from each jar containing 200g of seeds and the number of eggs oviposited on each treatment were counted. Simple and compound microscopes as well as light bulb were regularly used to carry out the experiment and the observations were recorded every 3 days and the eggs were left intact on the seeds. Each of the treatments was replicated three times. Counting was proceeded until the completion of egg stage.

4.2.2. Evaluation of Progeny Production of *C. chinensis* on different Pulse grains

The eggs oviposited on 200g of seeds in each jar were further kept under laboratory condition and when emergence began, the flasks were checked daily for adult emergence. The adults that emerged were counted and recorded every 3 days. Dishes, 3-mm mesh size sieve light bulb as well as simple and compound microscopes were used in conducting the experiments. The same procedure was repeated until third generation (F₃).

4.2.3. Developmental Period Assay

The mean developmental period of *C. chinensis* on each grain host was determined by subtracting the first day of egg laying from first day of adult emergence.

4.3. Weight Loss Assessment on different Pulse grains

Weight loss assessment was conducted on 200gm of seeds in each jar. The grains were separated into damaged (grains with characteristic holes) and undamaged portions. The grains in each portion were then counted and weighed using the appropriate balance used to measure weight. Dishes, light bulb and simple microscopes were used to conduct the experiment. Percent weight loss was calculated using the formula given by Adams (1976) as follows:

$$\% \text{ weight loss} = \frac{(UNd) - (DNu)}{U (Nd + Nu)} \times 100$$

Where U- the weight of undamaged grains

Nu - the number of undamaged grains

Nd - the number of damaged grains

D- the weight of damaged grains

4.4. Assessment of Percent Infestation of Pulse grains

The damaged seeds which were separately counted and weighed were divided by the total number of seeds and then multiplied by 100 according to the following formula:

$$\% \text{ infestation of pulses} = \frac{\text{damaged no.}}{\text{Total no.}} \times 100$$

4.5. Susceptibility Indices

Index of susceptibility was calculated using the procedure proposed by Dobie (1974) where by:

$$\text{Index of susceptibility} = \frac{\text{Log } y \times 100}{T}$$

Where Y is the number of insects reaching adult stage and T is the average developmental period in days.

4.6. Germination Test Assay

Germination test was carried out on 50 seed samples randomly taken from each replication of all the treatments. The seeds were placed in Petri dishes containing moistened filter paper (Whatman No.1) and arranged in a CRD in three replications. The number of emerged seedlings from each Petri dish was counted and recorded after 7 days. The percent germination was computed according to Ogendo *et al.* (2004) as follows:

$$\text{Viability index (\%)} = (\text{NG} \times 100) / \text{TG}$$

Where NG= number of seeds germinated and TG = total number of seeds tested in each Petri dish.

4.7. Experimental Design and Data Analysis

Experiment on host preference test was laid on Completely Randomized Design (CRD). Data entry and analysis were done using Microsoft Excel and SPSS. Data were transformed using Log and Arcsine transformation when necessary. To determine the effect of inherited character of different hosts on: oviposition preference, number of progeny production, developmental period, % weight loss and % germination of pulses one - way analysis of variance (ANOVA) was run. In cases where significant results were obtained means separation were conducted using Tukeys studentized (HSD) test at 5% level of significance.

5. RESULTS

5.1. Oviposition Preference of *C. chinensis*

The suitability of treatments for oviposition of *C. chinensis* was highly significant ($P \leq 0.01$) (Annex1). Highest number of eggs were laid on chick pea, faba bean and grass pea, and lowest number on field pea and lentil, where as no eggs were laid on haricot bean (Table 2).

Table 2: Number of eggs laid /30 seeds by *C. chinensis* (Mean + Se)

Treatments	Mean number of eggs /30seeds *
Field pea	1.68±.029b
Chick pea	2.36±.028a
Grass pea	2.30±.031a
Lentil	1.69±.022b
Faba bean	2.35±.036a
Haricot bean	0.00±.00

Means within the same column followed by the same letters are not significantly different ($P > 0.05\%$). Tukeys Studentized Range Test (HSD).

*-log transformed

5.2. Mean Number of Progeny Emergence of *C. chinensis* on different Pulses

The number of progeny emerged in F_1 generation were not significantly ($P > 0.05$) different. On the other hand, the number of progeny emerged on the treatments in F_2 and F_3 generations were highly significant ($P \leq 0.01$). The highest numbers of adult progeny

were recorded on chick pea, grass pea and lentil where as the lowest number on field pea and faba bean and no progeny emergence on haricot bean (Table 3).

Table 3: Number of progeny emergence of *C. chinensis* on different grain pulses (Mean + Se)

Treatment	Mean number of progeny *		
	F ₁	F ₂	F ₃
Field pea	1.41 ±.053a	1.16 ±.062b	1.29 ±.051b
Chick pea	1.53 ±.066a	2.27 ±.10a	2.44 ±.11a
Grass pea	1.41 ±.11a	2.032 ±.12a	2.38 ±.11a
Lentil	1.28 ±.12a	2.041 ±.12a	2.30 ±.12a
Faba bean	1.27 ±1.28a	0.89 ±.15b	1.083 ± .07b

Means within the same column followed by the same letters are not significantly different (P>0.05%), Tukeys Studentized Range Test (HSD).

*-log transformed

The density of *C. chinensis* on different pulses varied among generations as shown in the above chart. The density increased continuously from F₁-F₃ on chick pea, grass pea and lentil. On the other hand, no pronounced increment on field pea and faba bean among the generations and null on haricot bean since oviposition was null.

5.3. Developmental Periods of *C. chinensis* on different Pulses

Developmental periods recorded on the treatments were significantly ($P < 0.05$) different (Annex 4) the shortest mean developmental period was recorded on chick pea while the longest on field pea and faba bean and developmental period was not recorded on haricot bean since oviposition was deterred on this treatment (Table 4).

5.4. Percent Grain Weight Loss and Percent infestation of different Pulse grains due to *C. chinensis*

Percent grain weight loss recorded in F_1 generation was highly significant ($P \leq 0.01$) though there was no significant difference on the number of F_1 progeny emerged on the above treatments (Annex 5). The highest percent of grain weight loss was recorded on faba bean and the lowest on field pea; no grain weight loss recorded on haricot bean moreover their results are shown in (Table 4).

Percent infestation recorded on different pulse grains was significantly ($P \leq 0.05$) different. Highest percent of infestation was recorded on faba bean and the lowest on field pea, where as no infestation recorded on haricot bean (Table 4).

Table 4: Developmental periods of *C. chinensis* on different pulses, percent infestation and percent weight loss due to *C. chinensis* (Mean \pm Se)

Treatment	Mean developmental period (days)	Percent infestation	Percent weight loss
Field pea	25.67 \pm .33c	2.90 \pm .034e	0.65 \pm .03d
Chick pea	20.67 \pm .33a	12.97 \pm 0.096c	2.16 \pm .045c
Grass pea	22.00 \pm .58b	21.69 \pm .04b	2.17 \pm .009c
Lentil	22.67 \pm .33b	6.43 \pm .15d	2.85 \pm .078b
Faba bean	25.33 \pm .67c	34.12 \pm 1.25a	4.89 \pm .37a

Means with in a column followed by the same letters are not significantly different. (P>0.05 %). Tukeys studentized test (HSD).

5.5. Susceptibility Indices

The susceptibility index was significantly ($P \leq 0.05$) different among the treatments (Table 5). Field pea and faba bean were least preferred whereas chick pea was highly preferred.

Table 5: Susceptibility of pulse grains to infestation by *C. chinensis*

Treatment	Percent weight loss (+Se)	No. of emerged adults(+Se)	Developmental period(days)(+Se)	Index of susceptibility
Field pea	0.65±.03 d	1.16±.062b	25.67 ± .33c	0.25
Chick pea	2.16±.045c	2.27±.10a	20.67±.33a	1.72
Grass pea	2.17±.009c	2.032±.12a	22.00±.58b	1.39
Lentil	2.85±.078b	2.041±.12a	22.67±.33b	1.39
Faba bean	4.89±.37a	0.89±.15b	25.33±.67c	-0.22

Means within the same column followed by the same letters are not significantly different. ($P > 0.05$ %). Tukeys Studentized Range Test (HSD).

Log transformed

5.6. Effect of *C. chinensis* on Percent Germination of different Pulses

Percent germinations of pulse grains are shown in (Table 6). Significantly ($P < 0.05$) different capacities of germinations were recorded on the treatments (Annex 6). 100 % germinations were recorded on field pea, faba bean and haricot bean and germinations were inhibited on chick pea, grass pea and lentil.

Table 6: Mean percent germinations of host grains

Treatment	% germination
Field pea	90 ± .00a
Chick pea	.00 ± .00b
Grass pea	.00 ± .00b
Lentil	4.31 ± 4.31b
Faba bean	90 ± .00a
Haricot bean	90 ± .00a

Means within the same column followed by the same letters are not significantly different. ($P > 0.05$ %). Tukeys Studentized Range Test (HSD).

6. DISCUSSION

Results of the present laboratory study indicated that some of the pulse grains tasted was resistant to *C.chinensis* while others were susceptible. The number of eggs laid on different treatments was significantly different. Relatively higher number of eggs was laid on chick pea, faba bean and grass pea where as lower number recorded on field pea and lentil which might be due to the considerable variations in seed size among the treatments since seed size is among the features that have been reported to influence the performance of bruchids on different varieties. The lower number of eggs recorded on lentil which are small sized seeds might be due to the smaller surface area provided for egg lying and not attractive for further oviposition. This condition was confirmed the works of (Nwanze *et al.*, 1975; Schoonhoven, 1983) who associated small grain size with resistance, The highest number of eggs were recorded on faba bean (large sized seed) in the present investigation, although reports regarding the relationship between seed size and susceptibility to bruchids are diverse as Kemal (1988) found non significant negative correlation between seed size and ovipositon on faba bean.

.Chick pea, one of the treatments on which higher number of eggs was recorded had a rough seed coat, although rough seed coat was a character deterrent for oviposition and absent in susceptible vareieties according to (Raina, 1971cited in Teshome, 1990). Research conducted in USA also indicated that variety G109-1 which has a rough, almost spiny seed coat, was least preferred for egg lying by *Callosobruchus analis*,

Callosobruchus maculatus, *C. chinensis*. Oviposition was deterred on haricot bean and this indicated that it may not be a host for *C. chinensis*.

Developmental periods recorded on all the treatments were significantly different (Table 5). Developmental period was prolonged in field pea; this together with the reduced oviposition and adult emergence on this treatment indicates that its resistance is based on non-preference for oviposition as well as larval antibiosis. The shortest developmental period was recorded on chick pea which in addition to the highest preference for oviposition and the highest number of adult emergence indicates the susceptibility of this treatment was based on higher preference for oviposition and suitability of the seeds for larval development. This is for the reason that suitability of the treatments to the development of the beetles would be reflected by the number of adults that completed their development.

The highest preference for oviposition and the reduced number of progeny (F_1 - F_3) in faba bean and the least preference for oviposition and highest number of emerged adults (F_1 - F_3) in lentil confirmed the works of (Singh *et al.*, 1980a and Messina and Renwick, 1985 cited in Teshome Lemma, 1990) that the preference for oviposition is independent of development.

The grain weight loss was highly significantly different with the highest loss recorded on faba bean although there were no significant differences in F_1 progeny emergence of the entire treatments. This condition may be due to antibiosis which is a mechanism where

the pests feed but factors in the seeds have an adverse effect on them usually expressed as reduced growth and thus rate of multiplication, or survival. This confirmed the work of (Horber, 1978 cited in Teshome Lemma, 1990) who reported that the failure of *Callosobruchus chinensis* to develop in soy beans is attributed partly to the presence of saponins. Larvae of *Callosobruchus spp.* don't hydrolyze saponins. Saponins may therefore be regarded as specific metabolic defense mechanism of the soybean against insects.

Besides this, it may also be due to the strong seed coat of faba bean through which the newly emerged adults may failed to penetrate and emerge and thus resulted in reduced number of progeny emergence although highest number of larvae fed inside the seeds and cause highest percent of grain weight loss and no grain weight loss was recorded on haricot bean due to oviposition deterrence.

There were highly significant variations in susceptibility index which is a value derived from both adult progeny number and developmental period among the treatments (Table 6).It also showed that field pea and faba bean were the least preferred ones exhibiting a considerable resistance to *C. chinensis*. Susceptibility index was highest in chick pea which showed that it is the most susceptible of all the treatments.

100% germinations were recorded on field pea, faba bean and haricot bean. In haricot bean, oviposition was deterred therefore no emergence of adults and the grains were fully germinated this again realized that it might not a host of *C. chinensis*. Field pea and faba

bean were fully germinated due to lowest number of adults that emerged in F₁-F₃ and there was no pronounced increment in progeny emergence in the consecutive generations of this treatments though inclination may be reduced when the food supply will exhaust.

The number of progeny emerged in chick pea; grass pea and lentil were higher so that the endosperms were totally lost therefore germination was inhibited. This confirmed the works of Southgate (1979) and Venkatrao *et al.* (1960) who reported that a single larva of *Callosobruchus maculatus* infesting a seed of cowpea will remove about a quarter of the cotyledon of an average sized seed. When it infests the smaller seed of *Phaseolus radiatus*, it consumes virtually all the cotyledons, there by removing all possible chance of germination of the seed and also showed the considerable loss in viability and weight of field beans (*Dollchos lablab* L.).

Field pea and faba bean were generally more resistant to attack by *C. chinensis*. Variations in resistance among the treatments were due to both non preference for oviposition and unsuitability for larval development while chick pea was among the most susceptible hosts due to high preference for oviposition, high number of adult emergence and short developmental time.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

The study was initiated with the objective to understand the susceptibility of different pulses to *C. chinensis* by evaluation of some biological parameters and then to screen different pulses for their resistant to *C. chinensis*. The results of evaluations of some biological parameters of *C. chinensis* on different pulses indicated that preference of oviposition by *C. chinensis* varied among the treatments and oviposition was deterred on haricot bean. The treatment also differed in their suitability to the development of the pest since there were significant differences in the number of adult emergence and developmental period.

The treatments varied in resistance and the variation was due to both non preference for oviposition and larval antibiosis. Field pea and faba bean were more resistant to the attack of *C. chinensis* and this was manifested in terms of a lowered fecundity of the females and number of adult emergence together with prolonged developmental period on field pea and lower number of adult emergence and prolonged developmental period on faba bean. The relatively higher susceptibility of chick pea could be attributed to the fact that there were high fecundity of the females and the number of adult emergence and short developmental time.

The result of weight loss assessment conducted to screen resistance of different pulses to *C. chinensis* revealed that there were significant differences among the treatments. Relatively higher grain weight loss was recorded on faba bean although the situation couldn't affect the capacity of germination due to large grain size and the least grain weight loss was recorded on field pea since this treatment was least preferred for oviposition and lower number of adult emergence due to antibiosis.

Percent germinations of pulses were significantly different in that germinations were inhibited in treatments with higher number of adult emergence (chick pea, grass pea and lentil) while 100% germinations were recorded on the treatments with lower number of adult emergence (field pea and faba bean) as well as on the treatment which deterred oviposition (haricot bean)

The oviposition deterrence of *C. chinensis* on haricot bean which resulted in non-emergence of progeny, no grain weight loss and 100% germination indicated that haricot bean may not be a host for *C. chinensis*.

7.2. Recommendations

- ✓ The present Laboratory evaluation on screening of pulses for resistance to *C. chinensis* illustrated the potential of resistant variety as management of storage insect pests.

- ✓ Most plant resistance is a very good method of combating pest in storage. It is perhaps the easiest, most economical and effective means of controlling insect pests on stored grains as there is no special technology which has to be adopted by farmers. Screening of many seed varieties has led to successful isolation of strains that are resistant to insect pest.

- ✓ Since Ethiopia is a center of diversity for pulses, it is necessary to conduct an extensive selection and screening work on amore representative samples of germplasm for resistance to bruchids.

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9. APPENDICES

Annex 1: Summary table for analysis of variances (ANOVA) for mean egg production of *C. chinensis* on different pulses

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.581	4	1.895	130.393	.000
Within Groups	1.046	72	.015		
Total	8.627	76			

The mean difference is significant at the 0.05 level

Annex 2: Summary table for analysis of variances (ANOVA) for mean F2 progeny emergence of *C. chinensis* on different pulse

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.203	4	7.551	27.046	.000
Within Groups	25.406	91	.279		
Total	55.609	95			

The mean difference is significant at the 0.05 level

Annex 3: Summary table for analysis of variances (ANOVA) for mean F3 progeny emergence of *C. chinensis* on different pulse

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.099	4	7.775	47.366	.000
Within Groups	13.952	85	.164		
Total	45.051	89			

The mean difference is significant at the 0.05 level

Annex 4: Summary table for analysis of variances (ANOVA) for mean developmental period (days) of *C. chinensis* on different pulses

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.267	4	14.067	21.100	.000
Within Groups	6.667	10	.667		
Total	62.933	14			

The mean difference is significant at the 0.05 level

Annex 5: Summary table for analysis of variances (ANOVA) for percent weight loss due to *C. chinensis* on different hosts

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.407	4	7.102	1207.770	.000
Within Groups	.059	10	.006		
Total	28.466	14			

The mean difference is significant at the 0.05 level

Annex 6 summary tables for analysis of variances (ANOVA) for percent germination of different pulses due to the effect of *C. chinensis*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35736.660	5	7147.332	1946.415	.000
Within Groups	44.065	12	3.672		
Total	35780.725	17			

The mean is significant at the 0.05 level differences