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EVALUATION OF *PARTHENIUM HYSTEROPHORUS* L. POWDER AGAINST *CALLOSOBRUCHUS CHINENSIS L.* (COLEOPTERA: BRUCHIDAE) ON CHICKPEA UNDER LABORATORY CONDITION

BY

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DEDICATION

I dedicated this thesis to my late:

Parents,

Turtie Gurara and Fekensa Tujuba;

Beloved Instructor,

Dr. Bekele Jembere and

Intimate friend,

Solomon Hailu!

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ABBREVIATIONS

AVRDC……Asian Vegetable Research and Development Center

CRD……..Completely Randomized Design

CSA……..Central statistical Authority

DZARC……....Debre Ziet Agricultural Research Center

HARC……..Holeta Agricultural Research Center

IPM……..Integrated Pest Management

RH……Relative Humidity

Sp...……..Species

ABSTRACT

The present study was undertaken to investigate the insecticidal properties of *Parthenium hysterophorus* against *C. chinensis* in the laboratory under ambient condition. Leaf, Inflorescence and stem powders were prepared and for each powder, four doses i.e. 0.5g, 1g, 1.5g and 2g per 50g chickpea seeds were separately mixed and tested for 24, 48, 72 and 96h. The test insects were reared in glass jar and tested on whole chickpea seeds. Pirimiphos-methyl and untreated check were used for comparison. The experiment was arranged in a completely randomized design in three replications**.**

Parthenium part's powders applied to chickpea seeds were toxic to *C. chinensis* that caused significant ($P<0.05$) mortality 24h after treatment application except stem powder at 0.5 and 1.0 g. The highest dose (2g/50g seed) inflorescence, leaf and stem powder caused 76.67, 73.33 and 56.67 per cent mortality. Similarly, all the powders induced significantly ($P<0.05$) lower number of F1 progeny emergence compared with the untreated chickpea seeds. However, inhibition of the F1 progeny emergence by all powders was significantly lower than Pirimiphos-methyl that resulted in 100% inhibition. The highest per cent inhibition in adult emergence was observed in case of leaf powder treated chickpea seeds (83.33) whereas the lowest in case of stem powder (52.78). The powder has also played a vital role in preventing seed weight loss. Based on the results obtained it can be concluded that the potential use of *Parthenium hysterophorus* parts for protection of legumes in storage.

1. INTRODUCTION

Grain legumes are grown on some 180 million ha which is 12 to 15% of the Earth's arable land and they account for 27% of the world's primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen (N) needs of humans (Vance, 2001). Out of this, Africa produces an estimated 8 million tones of grain legume seed from 17.7 million ha of the total area of production (FAO, 2000).

According to the Ethiopian Central Statistical Authority (CSA) agricultural sample survey 2009/2010 (2002 E.C.) report on area and production of crops revealed that pulses are the second major food crops both in terms of the area they are planted to and volume of production obtained next to cereals. Pulses grown by the year covered 12.95% (1.49 million ha) of the grain crop area and 10.5% (more than 1.99 million tones) of the grain production was drawn.

On the contrary, currently 840 million people are undernourished globally mainly on account of inadequate intake of proteins, vitamins and minerals in their diets (Bhalla *et al.*, 2008). Thus, pulses are excellent sources of proteins and minerals, showing two or more times the levels found in most cereals (Bhalla *et al.*, 2008). Furthermore, they play a vital economic role which is related with their capacity to fix atmospheric nitrogen, thereby reducing agricultural cost through a reduction of fertilizer use and decreasing environmental contamination and enrich the soil fertility (Omeozor, 2005; Hauggaard-Nielsen *et al.*, 2007; Kantar *et al.*, 2007).

Furthermore, according to UN's Food and Agriculture Organization, (FAO) (1985) estimation around 500 million people throughout the world suffer from mal-nutrition. Recently, following the 2007-2008 global food crisis, FAO (2010) estimated that in order to feed the world's projected population in 2050 – some nine billion people, up from six billion today – agricultural production must increase by a yearly average of at least 1 per cent and also added that storage pests and lack of proper storage methods destroy over 200 million tones of grains each year. This illustrates that food shortage in our world can be solved not only by increased food production, but also by reducing food losses in storage (http://www.africanagricultureblog.com/search/label/Ethiopia browsed on December 29, 2010; Koul and Walia, 2009).

Archeological evidence shows that chickpeas (*Cicer arietinum* L.) were domesticated in Middle East and were cultivated in India, Mediterranean area, the Middle East, and Ethiopia since antiquity. Ethiopia is the leading country in Africa for chickpea production, with a share of about 37% in area and 48% in production (http://www.investinethiopia.net browsed on 22, 2011). It is a highly nutritious pulse cultivated throughout the world and is placed third in the importance list of the food legumes and it contains 38-59% carbohydrates and 25.3-28.9% proteins (Shukla *et al*., 2007).

According to Lale (2002), grain storage has often resulted in quantitative and qualitative losses due to physical, chemical, and most important biological factors such as pests which may be birds, rodents, fungi and insects of which storage insect pests are the potent because apart from their direct losses by consumption of kernels, they accumulate frass, exuviae, webbing, and insect cadavers which may result in grain that is unfit for human consumption and/or induced changes in the storage

environment warm, moist ‘hotspots’ that are suitable for the development of storage fungi that cause further losses.

Callosobruchus chinensis L. is also known as pulse beetle attack all pulses, but beans and chickpea are significantly affected not only in terms of quantitative and qualitative, but also these grains lose their germinating capacity completely as well (Ahmed *et al*., 2003; Ahmed and Din, 2009; Kumar *et al*., 2009; Righi-Assia *et al*., 2010).

Reduction of insect damage in stored grains is mainly a serious problem in developing countries of the tropics due to favorable climatic conditions and poor storage structures. A warm and humid climate of the region is most conducive for losses of stored chickpeas by insects and storage moulds and the insect damage intensifies mould development (Kumar *et al.*, 2009).

Efficient control of stored grain pests has long been the aim of entomologists throughout the World and synthetic chemical pesticides have been used for many years to control stored grain pests (Salem *et al*., 2007). Even currently, pest control measures in storage rely on the use of synthetic insecticides and fumigants, which is the quickest and surest method of pest control (Shaheen and Khaliq, 2005). However, the persistent use of these insecticides in granaries of small-scale farmers has led to a number of problems, such as killing of non-target species, user hazards, toxic residues in food, development of genetic resistance in the treated pest, increased cost of application and the destruction of the balance of the ecosystem (Shaheen and Khaliq, 2005; Boateng and Kusi, 2008).

Historical usage of nicotine and pyrethrum has encouraged scientists to focus their attention on alkaloids, flavonoids, terpenoids and other secondary compounds to be used as pest control agents (Rajapakse and Ratnasekera, 2008**)** and are working for the development and establishment of plant based pesticide, usually called as phytopesticide, botanical pesticide, biopesticide or natural pesticides (Verma *et al*., 2006; Yan-Zhang *et al*., 2007; Siddiqui *et al*., 2009; Tariq *et al*., 2010).

The search for alternative insect pest control methods and materials which are relatively cheaper and less harmful to the user and the environment has therefore become essential (Bekele *et al*., 1995; Bekele *et al.,* 1997*;* Emana, 1999; Rahman and Talukder, 2006; Ani, 2010). Sidewise, over 200 plant species have been reported to have insecticidal properties capable of controlling insects (Golob and Webley, 1980; Obeng-Ofori, 1997).

Hence, this investigation was initiated and conducted with the following objectives:

General Objective:

❖To look for potential of *Parthenium hysterophorus* as a component of integrated pest management options of *Callosobruchus chinensis*.

Specific Objectives

- To evaluate and compare the toxicity of different parts of *Parthenium hysterophorus* powder against *Callosobruchus chinensis*.
- To establish the effective dose of *Parthenuim hysterophorus* powder against *Callosobruchus chinensis*.

2. LITERATURE REVIEW

2.1 Adzuki bean beetle, *Callosobruchus chinensis* (L)

2.1.1 Description and Identification

Adzuki bean beetle, *Callosobruchus chinensis* (L), belongs to the order *Coleoptera*, family *Bruchidae* and subfamily *Bruchinae*, commonly known as Chinese weevil was first described and named by Linnaeus as *C. chinensis* in 1758 (Tuda, 2003). The species is one of the most widely spread of the genus *Callosobruchus (*Righi-Assia *et al*., 2010). It originated in Asia and especially common in the oriental region, but spread to the tropical and sub-tropical regions of the world (Anton, 2000).

The pest *C. chinensis* is associated with the legume tribe Phaseoleae and under storage conditions with diverse economic legumes of the subfamily Papilionoideae. The association of *C. chinensis* with cultivated legumes allowed its global geographical distribution (Tuda, 2003).

Adults are small about 3.5 mm. long, brownish in colour (Awasthi, 2007). They are robust beetles that appear square at the posterior and narrow at the front and elytra do not cover the tip of the abdomen (Robinson, 2005). The shape of the antennae in males, the fourth through apical segments are pectinate to strongly pectinate whereas in females these segments are serrate. The other common character to distinguishing *Callosobruchus spp* is the end femur hence, in *C. chinensis* it is ventrally bicarinate with a denticle situated on each carina near the apex. The outer tooth is blunt and the inner tooth is long and straight, and rounded at the tip (Talekar, 1988). Anton (2000) further identified it as the median lobe and lateral lobes usually strongly elongate, median lobe with ventral

valve more or less spear-shaped, internal sac with a single pair of denticulate plates usually sub-basally.

2.1.2 Biology and Behavior

The major host for the Adzuki bean beetle is Chickpea, *Cicer arietinum* (L.) and the biology of *C. chinensis* is similar to other Bruchids. The females lay their eggs in field or storage on the seed pods of legumes and the first-stage larvae bore the seed with holes then the adults often emerge after harvest in the storage where subsequent generations may develop (Deng *et al*., 2003). Adults mate within an hour after emergence from seed and mating lasts 5 to 8 minutes. Although the insects mate several times, only one mating is sufficient to ensure egg laying (Talekar, 1988). At the time of oviposition the female deposit a chemical 'oviposition marker' on the seed surface which has an ovicidal and arrestant action (Oshima *et al*., 1973; Yamamoto and Honda, 1977). This chemical, a mixture of fatty acids, triglycerides and hydrocarbons, prevents the hatching of more than one or two eggs per seed and helps regulate the pest population and maximize use of the food. Yamamoto (1976) suggested that this chemical can be used as a possible oviposition inhibitor to control the bruchids.

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 and lay an average of 78 eggs; and the duration for the 1^{st} to 4^{th} instar larva and pupal stage is 7-12, 12-16, 16-19, 19-22 and 22-27 day of oviposition, respectively (Ahmed *et al*., 2003). The optimal condition for development is temperature of 30^0c and relative humidity (RH) of 70% (Raina, 1970). Talekar (1988) pointed out that there is no difference in

developmental time and life span between male and female under similar environmental conditions and the sex ratio is 6:5 males to females.

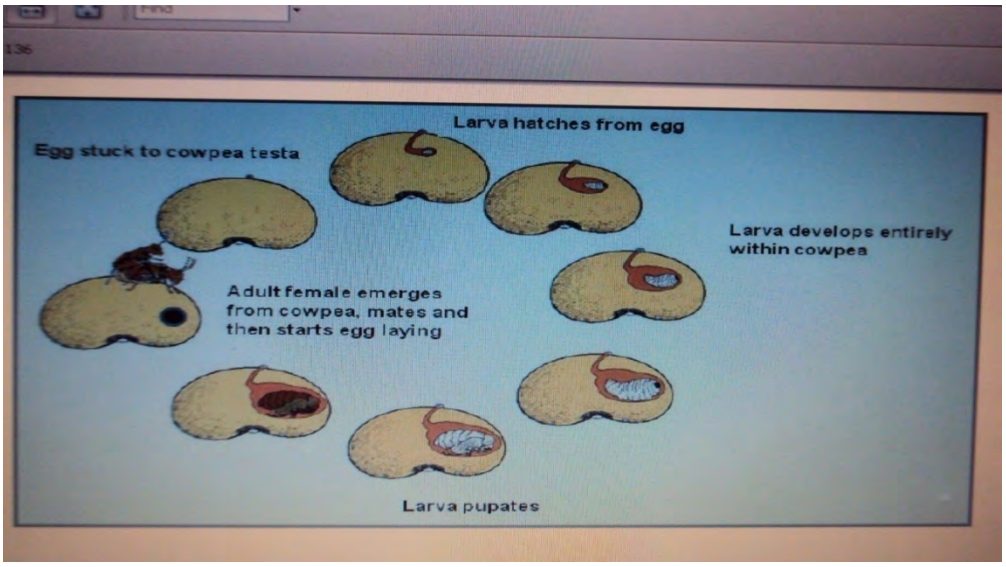

Figure 1**:** Typical life cycle of a bruchid infesting a pulse

Source: Cork *et al*., 2009

2.2. Post harvest loss of grain legumes

Haines (1982) described post harvest as a system which includes primary processing, storage, secondary processing, handling and transportation of natural products, that is a crucial task in meeting the overall goals of food security which is a major prominent problem for the third world as it rapidly deteriorates. Likewise, Salunkhe *et al.* (1985) noticed postharvest loss in food legumes as: loss of commodity weight in the period between harvest and consumption; loss of nutrients in stored legumes; qualitative deterioration caused by contaminants or biochemical changes rendering legumes unfit for human consumption; loss of seed viability; and loss as a result of physical damage.

Unfortunately, bruchids can impose al1 of these types of loss, principally through commodity consumption and contamination with frasses and uric acid.

Agricultural production and food distribution systems have evolved over many years to a complex system which allows for nearly year-round supply of most fresh commodities through long-term storage and long-distance shipments to consumers and thus, the expectation of year-round supplies has resulted in increased challenges for post-harvest handling systems (Mitcham, 1999).

Hall (1978) noticed that the general objective of storage is to make grains available or goods in satisfactory condition where and when they are needed. That is, grain storage is carried out: to retain a supply of food, to service a trading system, and to retain seed for planting in the following season. Storage also enables farmers and producers to sell their produce at the time when they can get the best prices (FAO, 1994). However, there are many factors, which may cause deterioration of products in the store which could be in the form of weight loss, chemical change in protein, carbohydrate, and oil content, and of contamination by chemical toxin, insects, rodent urine and frasses (Boxall *et al*., 2002; Lale, 2002).

The most economically important stored product insect orders belong to Coleoptera (beetles) and Lepidoptera (moths and butterflies) of which many of their species attack crops both in the field and in store. Crop damage by Lepidoptera is only done by the larvae, but in the case of Coleoptera, both larvae and adults often feed on the crop and the two stages are responsible for the damage (Bekele *et al*., 1997; Emana and Assefa, 1998; http://www.fao.org/inpho/ accessed on 10th March, 2011).

Losses of grain in storage due to insects are the final components of the struggle to limit insect losses in agricultural production and these losses can exceed those incurred while growing the crop. Hence, estimates of the worldwide post-harvest insect pests' loss of foodstuffs (especially stored grains) may amount to 5-10% in the temperate zone and 20-30% in the tropical zone (Moreira *et al.*, 2007). Likewise, in Ethiopia loss of stored produce reached up to 20-30 % (Abraham, 1996; Emana, 1999). Such damage may reach 10 to 40% in countries where modern storage technologies have not been introduced (Shaaya *et al*., 1997).

Bruchids, the seed beetles, are the primary pests of stored grain legumes (Southgate, 1979); the most destructive and econornically significant species belong to the genera *Acanthoscelides*, *Callosobruchus*, and *Zabrotes* (Credland, 1994; Kestenholz *et al*., 2007; Hill (1990) cited in Mulungu *et al*., 2007 and Shimizu and Hori, 2009). Among them the Chinese weevil, *Callosobruchus chinensis* (L.), the spotted cowpea bruchid, *C. maculatus* (F.), the bean beetle, *Acanthoscelides obtectus* (Say) and the Mexican bean beetle, *Zabrotes subfasciatus* (Boheman) are the most important insect pests of stored grain legumes (Schmale *et al*., 2002; Emana *et al*., 2003).

The genus *Callosobruchus* has the largest number of species, which cause great damage to many economically important legumes of the tropics (Righi-Assia *et al*., 2010). Those species attack cereals and pulses in store and cause a loss of 10-15% with a germination loss ranging from 50-92% (Adugna, 2006). Similarly, Gujar and Yadav (1978) reported 55-60% loss in seed weight and 45.50-66.30% loss in protein content due to its damage

and pulse seeds became unfit for human consumption as well as for planting. This is one of the reasons why farmers are often reluctant to grow legumes, as they have to dispose of their produce immediately after harvest, even though the market price may not be very remunerative at that time (Chauhan and Ghaffar, 2002).

2.3. Management options of storage insect pests

Bruchids, the seed beetles, are responsible for the greatest postharvest loss to stored grain legumes, directly by consuming the resource and secondarily by qualitative deteriorization of the commodity and reduced seed stock viability (Southgate, 1979; Salunkhe *et al*., 1985). Hence, according to Savidan (2002), control of stored pests will vary with the type of facility, the pest species and the type of host and the legal and economic methods of control available at a given time. Most often, however, effective management efforts begin with a thorough inspection of the site to determine the source, type, and importance of the infestation (Gwinner *et al.,* 1990). The following methods are crucial tasks for successful control of the most dominant stored produce pests in general and *Callosobruchus chinensis* in particular:

2.3.1. Cultural methods

Cultural control, the use of various agricultural practices that improve yield, but reduce pest populations directly, or by stimulating population buildup of a pest's predators or parasitoids, or by making plants or animals more tolerant of pest attack (Gillot, 2005). The most preventive factor in pest control in store grain is store hygiene. That is, removal of old grains and residue of organic matter present in storage structure including sub-

floor spaces, bins and old bags is important in the preparation of insect-free environments and prevention of carryover of pests to new grain. Preventing a new harvest produce from contact with remainders of previous harvest as well as used bags without washing (Manson and Obermeyer, 2004).

Emana Getu (1993) reported in Ethiopia that cultural measures are helpful in reducing pest infestation through prompt harvesting and threshing of the grains, elimination of other hosts of the pest from the vicinity of the main crop in the field or store, the use of materials (dolomite, wood ash, tobacco dust, sawdust and sand) that restrict movement of the pest when mixed with grains and complete drying of grains prior to storage. In the same manner, Adugna Haile *et al*., (2003) reported that farmers in Eritrea use different cultural storage management practices that include mixing of ash, and/or sand with grains to protect from storage pests. It has also been found that small seeded grains are mixed with large grained crops for control of stored pests. For example, teff, African finger millet and sand are mixed with pulses and other grains to control pests. They believe that in grain mixtures the space between grains are reduced, and hence the pest dies due to lack of aeration. In addition to this, farmers practice cleaning of the store before adding new harvested grains, sunning, winnowing, aeration of infested grains and use of pesticides. Drying seeds to moisture contents of below 9.5% before storage reduces bruchid infestation considerably (Talekar, 1988).

2.3.2. Biological control

Biological control is described as the regulation of pest populations by natural enemies (parasitoids, predators, and pathogens at acceptable levels (Gillot, 2005). In stored

products, biological control is being drawing increasing interest since they are nontoxic and do not damage human health or the environmet (Flinn *et al.,* 2006; Schmale *et al.,* 2006). It is increasingly recognized as the foundation of many successful integrated pest management programs. It is environmentally benign and once established can give long-term control; After initial costs it is relatively inexpensive and ensures control over a large area compared to other control measures such as chemical control which only gives relief in the treated area . Hence, can contribute to healthy food, cleaner environment and increased profits for farmers (Lenne, 2000). For instance, *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) is a parasitoid of several stored product insects attacking especially *Callosobruchus* species. The *D. basalis* larva is an external parasitoid of mature larvae and pupae of the bruchid host (Verma, 1991). Thus, use of *D. basalis* as a biological control agent in stored product has been studied and recommended by several researchers (Huis, 1991; Alebeek, 1996 and Ketoh *et al*., 2002).

2.3.3. Varietal resistance

Plant resistance to insects consists of inherited genetic qualities that result in plants being less damaged than another (susceptible one) that is subjected to the same conditions, but lacks these qualities. Hence, the production of plant resistant to particular insect pests is accomplished by selective breeding for resistance traits (Gullan and Cranston, 2005).

In similar manner, Painter (1958) defined plant resistance as the relative amount of heritable equalities possessed by a plant, which influence the ultimate degree of damage done by the insects and further divided the mechanism into different functional categories

as: *Tolerance*, where the plant is able to withstand or recover from insect damage, so that no reduction in final yield.

Antixenosis, in which the plant is a poor host, deterring any insect feeding (resistance is a property of the plant, not the resultant behavior of the insect). Raina (1971) reviewed that research conducted in USA with fourteen common chickpea 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 maculatus* and *Callosobruchus chinensis.* Since it possesses a rough, almost spiny seed coat, a character deterrent to oviposition and absent in susceptible varieties.

The other functional category is *Antibiosis*, 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. For instance, *Callosobruchus chinensis* fail to develop in soy-beans which are partly attributed to the presence of saponins that cannot be hydrolyzed by *Callosobruchus spp* larvae *in vitro.* Saponins may therefore be regarded as specific metabolic defense mechanisms of the soy-bean against insects **(**Horber, 1978). Correspondingly, Arcelin, a lectin-like protein, present in wild bean accessions is also the factor responsible for resistance to the Mexican bean weevil and it is inherited as a monogenic dominant trait that provides the highest level of resistance to bruchids when in the homogenous state with heterozygous Arc+/Arc^{-} individual seed less resistance than Arc+/Arc+ (Teshale Assefa, 2010). Moreover, Rajapakse *et al.* (1983) screened 11 mung bean cultivars for resistance to *C. chinensis*. Cultivars Uthong 1, H101 and CES 87 were

relatively resistant as the number of insects emerging from the seeds of these cultivars were the least and *C .chinensis* required a longer period to develop from egg to adult in these cultivars.

In general, host-plant resistance is a very good method of combating pest depredation 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 (Ahmed and Yusuf, 2007). Schmale *et al*. (2003) and Velten *et al*. (2008) further noticed that the potential of combining plant resistance factors together with biological control agents, especially parasitoids, has been shown to be a powerful method to control storage pests like bruchids.

2.3.4. Use of Pheromones

In pest management, survey and monitoring with pheromones and other attractants are practiced worldwide against a broad array of insect pests, and these techniques are the integral parts of a growing number of control options (Silverstein, 1990).

Pheromones are volatile compounds released by insects for communication between the sexes and the sex attractants allow males to find and recognize females of the same species (Cox and Collins, 2002). Since they determine all pest life situations such as feeding, mating, and egg-laying, it is considered to be potential agents for selective control of pest insects (Norin, 2007). They have been used in several different ways: for

pest detection, surveillance, monitoring, control by mass trapping and mating disruption (Matthews and Matthews, 2010).

In general, the use of semiochemicals in stored-product pest management has been an important option for many years (Phillips, 1997; Jones, 1998). Cox (2004) further emphasized as the pheromones, especially sex attractants and aggregation pheromones, potentially might be used in the following pest management situations: (1) for monitoring pest population density; (2) as lures to attract pests into traps; and (3) for permeating the environment, so that individuals are unable to locate mates. For these possibilities, pheromones have been an outstanding success and are now an integral component of many pest management programs, with commercial preparations available for more than 250 species. For instance, in a laboratory study at AVRDC 1976 cited in Talekar, (1988) which utilized virgin females and unmated males, blowing of air over virgin females placed in an olfactometer attracted large number of males towards the virgin females. One to two day-old females attracted large number of males than the older ones. The male showed characteristic excitatory behavioral response including rapid antennal movement and extension of wings. The chemical properties of *C. chinensis* sex pheromone have been isolated and described and it consists of a mixture of callosobruchusic acid [(E)-3, 7-dimethyl-2-octenedioic acid] and several hydrocarbons (Yamamoto and Honda, 1977 and Tanaka *et al*., 1982).

2.3.5. Chemical Control

Controlling pests in stored products using synthetic chemicals has been a common strategy for minimizing post harvest loss. Many researchers have reported that the effective utilization of synthetic insecticides including fumigants, dusts or admixture of seeds and sprays for the control of bruchids (Hill, 1990 and Gwinner *et al*., 1996).

Insecticides most commonly used to protect stored grains from insect pests include Aluminium phosphide, Lindane, Methyl bromide, Ethylene dibromide, Iodefenphos, Pirimiphos methyl, Permethrin, Malathion, Sumithion, Chlorpyriphos methyl, Propoxur, Fenithrothion, Dichlorvos, Bromophos, Fenvalerate, Bioresmethrin, Phenothrin, and Delta methrin (Lale, 2002).

Fumigation and dusting of grains are the most commonly of used methods chemicals control (Gwinner *et al*., 1990). Hill (1990) reported that for small amounts of grains, dust can be mixed with grains thoroughly and distributed evenly all over the product. The most commonly used insecticide dusts include Malathion, Delta methrin, Permethrin and Pirimiphos-methyl. On the other hand, fumigants are widely used for large scale storages and the most widely spread fumigants in use are Methyl bromide and Phosphine (Manson and Obermeyer, 2004).

Synthetic chemical pesticides have been used for many years to control stored grain pests (Salem *et al*., 2007). Even currently, pest control measures in storage rely on the use of synthetic insecticides and fumigants, which is the quickest and surest method of pest

control (Shaheen and Khaliq, 2005). However, the persistent use of these insecticides in granaries of small-scale farmers has led to a number of problems, such as killing of non-target species, user hazards, toxic residues in food, development of genetic resistance in the treated pest, increasing cost of application and the destruction of the balance of the ecosystem (Shaheen and Khaliq, 2005; Boateng and Kusi, 2008).

2.3.6. Botanical Control

Plant parts and extracts have been, and still are, used in many parts of the world to control insects. Plants are known to produce a range of secondary metabolites which can possess multiple modes of action, including acute toxicity, repellency, antifeedant or antioviposition effects and inhibition of growth, development or reproduction (Coats *et al.*, 1991).

Thus, use of plant products or insecticides is one of the most important approaches of insect pest management and it has many advantages over conventional insecticides. Plant materials with insecticidal properties provide small-scale farmers with locally available, biodegradable, safer to human and the environment and inexpensive methods of pest control for storage (Bamaiyi, 2006). Tadele Tefera (2006) noted that farmers in Ethiopia use local herbs by mixing with grain to reduce infestation in stored grains.

In sight of the adverse effects of insecticides on the environment, peasant farmers and researchers often claim successful use of material of plant origin in insect pest control including spices and powders of plant parts (Akinneye *et al*., 2006). Previous research

indicated that some plant powders, oils and extracts have strong effects on stored grain insects such as toxicity and the inhibition of reproduction (Emeasor *et al*., 2005; Nadra, 2006). The simplest way to apply plants to a stock of seeds is harvesting the plant and adding it to the seeds. The modes of action of powders vary, but with low to moderate dosages (Rajapakse, 2006).

Emana Getu and Araya G/Silassie (2009) have proved that essential oils from *C. ambrosioides, R. officinalis, E. globulus, T. ammi* and *C. citrates* essential oil extracts provided 100% mortality toward *Z. subfasciatus* at the dose of 750 mg/10 ml of acetone and application rates of 1, 2 and 3 ml per filter paper in 24-hr exposure time. The selective efficiency of Parthenium leaf extracts against *A. aegypti*, as diethyl ether extracts proved to be the most effective oviposition deterrent and ovicidal agent, while the least effective as irritant extract (Kumar *et al*., 2011).

2.3.6.1. Description of the Test Plant

Parthenium hysterophorus L. (here after referred as *Parthenium*) bears a number of other common names as *P. hysterophorus* in Australia; Congress grass, carrot weed, bitter weed, broom bush, and star weed in India; false ragweed and ragweed in USA; 'Biyabasa', 'Kinche arem', 'Feremsisa' in Ethiopia; white top and fever few in the Caribbean (Carlos *et al*., 2000; Taye Tessema, 2002). *Parthenium* is an annual herb with a deep taproot and an erect herbaceous shoot of up to 2 m in height and with a multi-branched stem that is covered with trichomes; leaves are pale green, lobed, hairy, initially forming a basal rosette of strongly dissected leaves that are up to 30 cm in length, having

small hairs on both sides, resembling the leaves of carrot; flower heads are creamy white (http://www.fao.org/forestry/13378-1-0.pdf accessed on Sep 01 2010; Evans, 1997).

During the last century *Parthenium* weed has spread from its endemic habitat, mainly the region around the Gulf of Mexico including West Indies and presumably central Argentina (Picman and Towers, 1982), throughout the tropics and has become a serious problem in many parts of the world like Pakistan, India, China, Australia, Kenya and Ethiopia (Navie *et al*., 1996; Javaid, 2009; Riaz and Javaid, 2009). It spreads easily through trade as contaminants of grain and other crop products and by means of farm machineries (Mack and Lansdale, 2001). *Parthenium* was introduced into Asia, Africa and Oceania with cereal and grass seed shipment from America during the 1950s (Bhowmik and Sarkar, 2005).

According to Datta and Saxena (2001) the chemical analysis of *Parthenium* has indicated that all the plant parts including trichomes and pollen contain toxins called sesquiterpene lactones. The major component of these toxins being parthenin and other phenolic acids of caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid and p-anisic acid (Oudhia, 2001; Kumar *et al.*, 2010).

2.3.7. Integrated Pest Management

Kogan (1998) defined integrated pest management (IPM) as a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost-benefit analyses that take into account the interests of and impacts on producers, society, and the environment. In the same manner, Ebesu

(2003) reviewed as the way of eliminating or reducing potentially harmful pesticide use by using a combination of control methods that will reduce the pest to an acceptable level and the control methods should be socially acceptable, environmentally safe, and economically practical.

According to Katsoyannos *et al.* (1992) it is a pest management strategy employing all methods consistent with economic, ecological and toxicological requirements to maintain pests below economic threshold while giving priority to natural limiting factors and its essence is to maintain natural biodiversity whilst controlling the pest species.

3. MATERIALS AND METHODS

3.1. Insect Culture

Adults of *Callosobruchus chinensis* (L) brought from Holeta Agricultural Research Center (HARC) were cultured at Addis Ababa University, Faculty of Life Science, Insect Science Insectary at the temperature of 30±2 ^{o}C and relative humidity of 65±5 % (Ahmed and Din, 2009). Chickpea seeds brought from Debre Ziet Agricultural Research Center (DZARC) were kept in an oven at 60 ^{0}C for 4 h to disinfest the seeds from any prior infestation before using them as a substrate for insect rearing (Bekele, 2002). To obtain newly emerged pulse beetles of the same generation, 25 pairs of unsexed adult of *C. chinensis* were placed in three 1-litre volume glass jars containing 250g of chickpea seeds each. The jars were covered with nylon mesh to allow ventilation and were held in place with rubber bands to prevent the escape of bruchids. The parent bruchids were allowed 6 days in the jars for mating and oviposition and were removed from the jar. Seeds with eggs were kept under laboratory condition until the emergence of F1 progeny. The insects emerged after four weeks were used in the entire experiments.

3.3. Plant Material Collection

The experimental plant, *Parthenium* parts (leaves, succulent stem and inflorescence) used for the study was harvested from the road-side around Debre Ziet (Bishoftu) town and the identity of the plant was confirmed at the herbarium of Life Science Faculty, Addis Ababa University.

3.3.1. Preparation and application of plant powder

To obtain the fine powder, a significant amount of each plant parts were dried in the open air for as long as one month. After being dried well, the plant parts were crushed to fine powder using mortar and pestle. The resulting powder was passed through a 25-mesh diameter sieve to obtain a fine and uniform dust. The test materials were admixed thoroughly and gently in plastic containers by manual agitation until the materials were evenly distributed among the grains and ensure a homogeneous admixture. The powders were applied at the rates of 0.5g (1%), 1g (2%), 1.5g (3%) and 2g (4%)/ 50 g of grains following the procedure by Ahmed and Din (2009).

3.4 Toxicity Assessment bioassay

About 50 g of fresh, intact and disinfested chickpea seeds were weighed and placed in 1L- volume glass jars and were treated with 0.5, 1.0, 1.5 and 2.0g of dried and ground leaf, inflorescence and stem powder of *Parthenium*. Pirimiphos methyl at the rate of 0.125 g/ 50 g grain dust was also applied as a standard check. In addition, untreated grains were included as a control. After treatment, 10, 0-2 days-old *Callosobruchus chinensis* of unsexed adults were introduced to the treated and untreated seeds in the glass jars. The jars were covered with nylon mesh and held in place with rubber bands. Insects in each jar were sieved and counted after the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of introduction and dead bruchids were discarded while alive insects were introduced back to their respective jars. The experiment was designed in a completely randomized design (CRD) in three replications.

Percentage insect mortality was calculated using Abbott formula (Abbott, 1925) cited in Bekele Jembere *et al*. (1996) as follows:

$$\textbf{Corrected \% mortality} = [1 - \frac{Nt}{Nc}] * 100$$

Where: Nt= number of insects in treated jars, Nc= number of insects in control jars.

3.5 F1 Progeny Assessment bioassay

The treated jars were kept for additional five days of oviposition time after mortality assessment. All alive and dead insects were sieved and discarded after ten days of introduction. Insects were counted as dead when they failed to move any part of their body after prodding with fine brush bristle. The treated and control grains were then kept until emergence of F_1 progeny. Then, the number of F1 progeny produced by the *C. chinensis* was counted. Counting was stopped after 31 days from the days of introduction to avoid overlapping of generation. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated using Tapondjou *et al*. (2002) method as follows:

$$\textbf{\%IR} = \frac{(Cn - Tn)}{Cn} * 100$$

Where Cn is the number of newly emerged insects in the untreated (control) jar and Tn is the number of insects in the treated jar.

3.6 Weight loss Assessment Assay

Damage assessments were carried out by counting treated and untreated grains. Samples of 100 seeds were taken from treated and untreated grains and the number of damaged (grains with characteristics hole) and undamaged grains were counted and weighed.

Percent weight loss of the seeds were calculated using the method adapted by FAO (1985b) cited by Dawit Kidane and Bekele Jambere (2010).

$$\% \text{ Weight Loss} = \frac{[(Ua*N) - (U+D)]}{Ua + N} * 100$$

Where, U-weight of undamaged fraction in the sample,

N- Total number of grains in the sample

Ua- average weight of one undamaged grain and

D-weight of damaged fraction in the sample.

The assessment was carried three times for each treatment.

3.7 Germination Test Assay

For seed germination test, 100-seed samples were taken at random from each replication of all the treatments and pirimiphos-methyl treated seeds. The seeds were placed in Petri dishes containing moistened filter paper (Whatman No. 1) and arranged in a CRD in three replications. Healthy untreated seeds were used as a control. The number of emerged seedlings from each Petri dish were counted and recorded after 7 days. The percent germination was computed according to Ogendo *et al*. (2004) as follows:

$$\text{Viability Index (\%)} = \frac{NG}{TG} * 100$$

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

3.8 Data Analysis

Data entry and analysis were done using Microsoft Excel and SPSS Version 15, respectively. Data were transformed using Arcsine transformation when necessary. To observe the effects of the treatment on % mortality, % number of F1 progeny reduction, % weight loss and effect of treatments on germination of Chickpea seeds one-way analysis of variance (ANOVA) was run. In cases where significant results were obtained, mean separation was conducted using Tukey's studentized range (HSD) test at 5% level of significance.

4. RESULTS

4.1. The effect of powders of *Parthenium* parts on mortality of parent bruchids.

Cumulative percent mortality of young adult *C. chinensis* in Chickpea seeds treated with different parts of *Parthenium* with different dosages ranging, from 24 - 96h exposure time is indicated in **Table 1**. The one-way ANOVAs are also annexed in Annexes 1, 2, 3 and 4, respectively.

No significant mortality was recorded on seeds treated with stem powder at lower doses (0.5 and 1g/ 50g seeds) 24h after treatment application, whereas all the powders caused significantly ($P<0.05$) higher mortality to *C. chinensis* starting from 24h after treatment.

Mortality was significantly high ($P<0.05$) on seeds treated with Pirimiphos-methyl at the rate of 0.125g/50g grain within 24h. The highest doses of inflorescence, leaf and stem powder at 2g/50g of Chickpea gave 76.67, 73.33 and 56.67 % mortality 96h after treatment application, respectively (**Table 1**). It was observed that toxicity of the powders increased with increase in dosage and exposure time. Compared with leaf and inflorescence powders at all doses, stem powder was less toxic.

Table 1: Mean percent cumulative mortality ± SE of adult *C. chinensis* exposed to different parts powders, after different exposure time.

Treatments	Dose (g/50g grain)	Mean % adult mortality, hr after treatment application			
		24	48	72	96
P. hysterophorus Leaf Powder	0.5	6.67±3.33bc	16.67±33def	30.00±5.77cd	40.00±0.00f
	1.0	6.67±3.33bc	20.00±0.00def	40.00±0.00bcd	50.00±def
	1.5	10.00±0.00bc	30.00±0.00bcd	50.00±5.77bc	66.67±3.33bcde
	2.0	16.67±3.33b	40.00±0.00b	60.00±0.00b	73.33±3.33bc
P. hysterophorus Inflorescence Powder	0.5	10.00±5.77bc	23.33±3.33cdef	30.00±0.00cd	43.33±3.33f
	1.0	6.67±3.33bc	16.67±3.33def	33.33±6.67cd	53.33±8.82cdef
	1.5	10.00±5.77bc	26.67±3.33bcde	50.00±0.00bc	70.00±5.77bcd
	2.0	16.67±3.33b	36.67±8.82bc	56.67±6.67b	76.67±3.33b
P. hysterophorus Stem Powder	0.5	0.00±0.00c	10.00±0.00fg	23.33±3.33d	36.67±3.33f
	1.0	0.00±0.00c	13.33±3.33efg	26.67±6.67d	46.67±6.67ef
	1.5	3.33±3.33bc	20.00±0.00def	26.67±6.67d	50.00±5.77def
	2.0	6.67±3.33bc	16.67±3.33def	30.00±0.00cd	56.67±3.33bcdef
Pirimiphos-methyl	0.125	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control (untreated)	0	0.00±0.00c	0.00±0.00g	0.00±0.00e	0.00±0.00g

Means within a column followed by different letters are significantly different at the 5% level of probability using Tukey Studentized Range Test (HSD).

4.2. The effect of the *Parthenium* parts powder on the F1 progeny of *C. chinensis*.

The number of F1 progeny produced by *C. chinensis* in untreated and treated Chickpea seeds with different plant parts powders is presented in **Table 2**. All the powders induced significantly ($P<0.05$) number of F1 progeny emergence compared with the untreated Chickpea seeds. However, inhibition of the F1 progeny emergence by all powders is significantly ($P<0.05$) lower than Pirimiphos-methyl, that resulted in 100% inhibition. The highest per cent inhibition in adult emergence was observed in case of leaf powder treated Chickpea seeds (83.33%), where as the lowest was observed in stem powder (52.78%).

Table 2. Mean F1 progeny production of *C. chinensis* in chickpea seeds treated with powders of *Parthenium* parts at different doses.

Treatments	Dose (g/50gm grain)	Mean number of F1 progeny± SE	% Inhibition rate ±S.E
P. hysterophorus Leaf Powder	0.5	12.67±0.88bc	64.81±2.45bc
	1.0	11.00±2.65bc	69.44±7.35bc
	1.5	10.67±1.86bc	70.37±5.15bc
	2.0	6.00±0.58cd	83.33±1.60ab
P. hysterophorus Inflorescence Powder	0.5	11.68±0.33bc	67.59±0.92b
	1.0	8.00±1.53c	77.78±4.24b
	1.5	8.33±1.76c	76.85±4.90b
	2.0	6.33±0.88cd	82.41±2.45ab
P. hysterophorus Stem Powder	0.5	17.00±1.15b	52.78±3.21c
	1.0	13.00±1.15bc	63.89±3.21bc
	1.5	13.33±2.33bc	62.96±6.48bc
	2.0	9.67±1.45bc	73.15±4.04b
Pirimiphos- methyl	0.125	0.00±0.00d	100.00±0.00a
Control (untreated)	0	36.00±0.00a	0.00±0.00d

Means within a column followed by different letters are significantly different at the 5% level of probability using Tukey Studentized Range Test (HSD).

4.3. The effect of the plant's parts powder on weight loss of Chickpea due to infestation by *C. chinensis*.

Results on assessment of percent weight loss caused by infestation of *C. chinensis* to treated and untreated Chickpea seeds are given in **Table 3**. All the plant parts powders admixture significantly ($P<0.05$) reduced weight loss of the Chickpea seed compared with the untreated check, where the highest per cent of grain weight loss was recorded. The highest weight loss was observed in stem powder at 0.5g next to the control whereas, the lowest weight loss was observed in leaf powder at 2.0g next the standard check. There was no weight loss recorded on grains treated with Pirimiphos-methyl (**Table 3**).

Table 3. Mean percent weight loss caused by *C. chinensis* on seeds treated with powders of the plant's parts at different doses.

Treatments	Dose (g/50gm grain)	% weight loss
P. hysterophorus Leaf Powder	0.5	0.42±0.00bc
	1.0	0.46±0.25bc
	1.5	0.46±0.25bc
	2.0	0.08±0.00bc
P. hysterophorus Inflorescence Powder	0.5	0.40±0.01bc
	1.0	0.28±0.10bc
	1.5	0.28±0.10bc
	2.0	0.18±0.10bc
P. hysterophorus Stem Powder	0.5	0.76±0.17b
	1.0	0.40±0.10bc
	1.5	0.46±0.24bc
	2.0	0.28±0.10bc
Pirimiphos- methyl	0.125	0.00±0.00c
Control (untreated)	0	2.81±0.08a

Means within a column followed by different letters are significantly different at the 5% level of probability using Tukey Studentized Range Test (HSD).

4.4. The effect of *Parthenium* parts powder on germination of Chickpea seeds.

The percent germination of chickpea seeds treated with different parts of *Parthenium* at different doses and untreated check are presented in **Table 4**. The effect of plant parts powders on seed germination of Chickpea seeds revealed that no significant (P>0.05) harmful effect was observed. The highest germination (96.67%) was recorded on Chickpea seeds treated with *P. hysterophorus* stem powder at the rate of 1.0 g/ 50 g of grain and the least (86.67%) on inflorescence powder at 2.0g/50g of grain. It indicated that there was no adverse effect any dose of the powder on the germination capacity of seed.

5. DISCUSSION

Different studies demonstrated that sesquiterpene lactone derivatives of parthenin obtained from *Partherium hysterophorus* were proved for their antifeedant action against sixth instar larvae of *Spodoptera litura* and *Tribolum castneum* and for insecticidal activity against the adults of stored grain pest *Callosobruchus maculatus* and Cabbage leaf webber (*Crocidolomia binotalis* Zell). Among the eleven derivatives of parthenin, the saturated lactone was found to be about 2.25 times more active than parthenin. The pyrazoline adduct was found to be the most effective as an insecticide, with LC50 values after 24, 48 and 72h of 96, 43 and 32mg per litre, respectively, which are comparable with neem extract (Datta and Saxena, 2001).

Thus, the results of the present laboratory study demonstrated that different parts of *Parthenium* showed different potencies against *C. chinensis*. Among the treatments, inflorescence powder caused the highest mortality followed by leaf powder and the least was stem powder. Varying activity by different parts powders of the plant indicated that the pest controlling factors are not uniformly present in every part of a plant. Further, it was observed that mortality of adult bruchids due to treatment of plant parts powder was directly related to application dosages and the time of exposure. It indicated that higher dosage and longer exposure periods are required to achieve appreciable management of *C. chinensis*. Like wise, different scholars reported as parthenin inhibited a dose-dependent toxicity effects on a range of test species (Patil, and Hedge, 1988 cited in Paudel *et al.*, 2009; Pandey, 1994 and Kraus, 2003).

Kumar *et al*. (2011) has also illustrated that diethyl ether *parthenium* leaf extract was found to be the most effective resulting in maximum effective repellency (99.7%) leading to the highest levels of reduced fecundity and 100% egg mortality followed by benzene extracts causing 93.8% reduced oviposition and 100% ovicidal effect on *Aedes aegypti*, the primary carrier for viruses that cause dengue fever.

Furthermore, Wabale and Kharde (2010) noticed that an extract of *Parthenium* has a tendency of (81.87%) in damaging the life cycle of sugar cane woolly aphid (*Ceratovacuna Lanigera* Zehntner.). Similarly, Roth *et al.* (2008) confirmed that the water extract of *Parthenium* exhibited a tremendous reduction (down to 29% of the initial infestation) in the number of *Lipaphis erysimi*, one of the most important pests of *Brassica juncea*, may be due to the effect of phenolic acids.

The findings of the present investigation is in accordance with those and other workers who have previously reported that plant powders causing mortality to bruchids *Lantana camara* (Koona and Njoya, 2004); *Murraya koenigii* and *Eupatorium cannabinum* (Shukla *et al*., 2007) and Dried leaves powders of *Guirea senegalensis*, *Piliostigma reticulatum* and dried fruit powder of *Piper guineense* (Abdullahi and Muhammad, 2004).

Rajapakse (2006) noticed that the modes of action of powders vary, but with low to moderate dosages and Law-Ogbomo and Enobakhare (2007) also further added that the way major active ingredients of botanical powders exert their bioactivities against insect pests. The insecticidal effect of plant powders may attribute to one or more of the following properties including, repellency, stomach poisoning effect where insects feed

on admixed grains and pick up lethal doses of treatment particles, and these powders might reduce insect movement and also cause death through occlusion of their spiracles, thereby, preventing respiration via trachea.

Hence, in the present finding, all the powders induced significantly lower number of F1 progeny emergence compared with the untreated Chickpea seeds. The reduction in adult emergence could either be due to increased adult mortality, ovicidal, repellency property of the tested plant, larval mortality or even reduction in the hatching of the eggs. It has been reported that the larvae which hatch from the eggs of *Callosobruchus* species must penetrate the seeds to survive (FAO, 1999). The larvae are unable to do so unless the eggs are firmly attached to the seed surface. In the present study, the eggs were found to be loosely attached to the Chickpea seed surface. The leaf powders might thus have inhibited the larval penetration into the seed and thus showed maximum inhibition rate (90.64%), in leaf powder followed by inflorescence powder (88.30%). The current finding has showed that *Parthenium* part's powder has an effect in reducing F1 adult emergence even though not effective as Pirimiphos-methyl. This result is in agreement with those obtained by many other workers. Araya and Emana (2009) recorded no adult emergence of *Zabrotes subfasciatus* (Boheman) on beans treated with 10 and 15g/150g of *Chenopodium. ambrosioides* and *Azadrachta indica* powders. Righi-Assia *et al*, (2010) also reported that *Thymus vulgarus*, *Santolina chamaecyparissus* and *Anagyris foetida* significantly reduced progeny emergence of *C. chinensis* on chickpea at dosage of 1g.

All the plant parts powder admixture significantly reduced weight loss of the Chickpea seed compared with the untreated check, even though not as effect as Pirimiphos-methyl treated seed where no weight loss was recorded.

The effect of plant parts powder on seed germination of Chickpea seeds revealed that no significant harmful effect was observed. During the germination test, all the treatments including Pirimirhos-methyl favor the seed germination. As the result, per cent of germination ranged from 88.33% - 96.67% that was not significantly different from the untreated check.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The present laboratory investigation of *Parthenium hysterophorus* parts showed some insecticidal property against *Callosobruchus chinensis*. However, the distributions of the secondary chemical metabolite vary on its different parts. Inflorescence powder was found to be the more toxic followed by leaf and the least was stem powder at their highest doses and longer exposure time. Thus, after 96h post treatment inflorescence, leaf and stem powders at 2g/50g grain caused 76.67, 73.33 and 56.67% mortality which show the more the exposure period, the more effect on the target insect. Based on the result obtained, it was possible to conclude that all parts of *Pathenium* contain toxic secondary metabolite that distributed unevenly and act against *C. chinensis*. Thus the use of *Parthenium* part's powder needs to be encouraged for use at household level and further work need to be done on other parameters.

6.2. Recommendations

- This laboratory study indicated the potential use of *Parthenium hysterophorus* parts for protection of legumes in storage. However, much further works in practical storage situations should be conducted for related pests and legumes.
- Even though the powders were effective in controlling the pest, further investigation should be done on the quality of agricultural products treated with them (For example, Colour, flavor and odor etc.).
- Much work needs to be done to develop effective formulations, by isolating parthenin and its derivates which can be commercialized as biopesticide.
- Furthermore, studies should be conducted on other parameters of *Parthenium*, like mammalian toxicity, shelf life etc to combine with other pest management techniques.

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8. APPENDICES

Annex 1: Summary table for analysis of variance (ANOVA) for mean percent mortality of *C. chinensis* due to *P. hysterophorus* parts powder after 24h on treated chickpea seeds.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	15346.19	13	1180.48	17.11	0.00*
Within Groups	1931.49	28	68.98		
Total	17277.68	41			

* = Significant at P<0.05

Annex 2: Summary table for analysis of variance (ANOVA) for mean percent mortality of *C. chinensis* due to *P. hysterophorus* parts powder after 48h on treated chickpea seeds.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	11793.18	13	907.17	61.49	0.00*
Within Groups	413.11	28	14.75		
Total	12206.29	41			

* = Significant at P<0.05

Annex 3: Summary table for analysis of variance (ANOVA) for mean percent mortality of *C. chinensis* due to *P. hysterophorus* parts powder after 72h on treated chickpea seeds.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	11590.94	13	891.61	48.76	0.00*
Within Groups	511.94	28	18.28		
Total	12102.88	41			

* = Significant at P<0.05

Annex 4: Summary table for analysis of variance (ANOVA) for mean percent mortality of *C. chinensis* due to *P. hysterophorus* parts powder after 96h on treated Chickpea seeds.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	11994.03	13	922.62	47.18	0.00*
Within Groups	547.59	28	19.56		
Total	12541.62	41			

* = Significant at P<0.05

Annex 5: Summary table for analysis of variance (ANOVA) for F1 progeny inhibition Of *C. chinensis* on chickpea seeds treated with *P. hyesterophorus* parts powders.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	19740.04	13	1518.47	32.67	0.00*
Within Groups	1301.44	28	46.48		
Total	21041.48	41			

* = Significant at P<0.05

Annex 6: Summary table for analysis of variance (ANOVA) for F1 progeny emergence Of *C. chinensis* on chickpea seeds treated with *P. hyesterophorus* parts powders.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	2558.31	13	196.79	32.68	0.00*
Within Groups	168.67	28	6.04		
Total	2726.98	41			

* = Significant at P<0.05

Annex 7: Summary table for analysis of variance (ANOVA) for weight loss produced By *C. chinensis* on seeds treated by *P. hysterophorus* parts powders.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	18.35	13	1.41	25.33	0.00*
Within Groups	1.56	28	0.06		
Total	19.91	41			

* = Significant at P<0.05

Annex 8: Summary table for analysis of variance (ANOVA) for mean germination Test of Chickpea treated with *P. hysterophorus* parts powders.

Source of error	Sum of Squares	df	Mean Square	F ratio	P-value
Between Groups	443.45	13	34.11	2.05	0.06*
Within Groups	466.67	28	16.67		
Total	910.12	41			

* = Significant at P<0.05

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

Name: Tesfu Fekensa

Signature: _____

Submission date: _____

Place: Zoological Sciences Program Units, Addis Ababa University

This thesis has been submitted for examination with my approval as a university advisor.

_____

Emana Getu (Ph.D)