ADDIS ABABA UNIVERSITY

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EFFECTS OF CRUDE EXTRACTS OF BIRBIRA (*MILLETTIA FERRUGINEA*) SEED POWDER IN SOLVENTS OF DIFFERENT POLARITY AGAINST PEA APHID, *ACYRTHOSIPHON PISUM* (HARRIS) (HOMOPTERA: APHIDIDAE)

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ABSTRACT

This study was conducted in order to test for the contact toxicity and settling deterrence of crude extracts of birbira, *Millettia ferruginea*, seeds powder in solvents of different polarity against pea aphid (*A. pisum*). It was evaluated as deionized water, acetic acid, acetone, chloroform, toluene and hexane extracts. Contact toxicity assay was carried out in the laboratory using these extracts at rates of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mg/ml whereas settling deterrence on excised leaves and contact toxicity on intact plants was based on the LC_{50} from the first assay. In ex-situ contact toxicity, all the extracts of birbira dissolved in water caused significantly ($p<0.05$) higher mortality to the *A. pisum* in 5 hrs time than the untreated control. However, the pea aphids responded differently to the birbira extracts and the deionized water extract was by far the most toxic that caused about 98.15% mortality followed by the acetic acid extract (88.61%) which brought significantly more death than the chloroform, toluene and hexane extracts. Birbira extract in hexane caused the least mortality than other extracts. The LC_{50} value was much lower for the deionized water extracted birbira than the rest of the extracts but, it was the highest for hexane extracted birbira. The contact toxic activity of birbira extracts decreased with decreasing polarity (from deionized water to hexane) of the extracting solvents used. For each extract of birbira significantly higher mortality was observed at the higher rates used. The LC_{50} birbira extracts of deionized water, acetic acid and hexane significantly ($p<0.05$) reduced the number of aphids settled on the treated leaves in the paired-choice settling deterrence activity assay, though these three extracts did not show significant settling deterrence of aphids at all time of the observations. Similarly, some settling deterrence effects of the extracts were observed in no-choice settling deterrence test. Significantly ($p<0.05$) fewer aphids settled only on acetone, chloroform and toluene extracted birbira treated than the control leaves at 24 hrs after treatment although the differences among all the extracts were not significant. In the contact toxicity on intact plants, all extracts except hexane extracted one caused significantly ($p<0.05$) higher mortality of aphids within three and four days time after treatments than the untreated control. Nevertheless, the differences among the extracts were not significant. Still the more polar solvents had the leading cumulative mortality records. Based on the results it can be concluded that polar solvent extracts of birbira is powerful contact toxicant against *A. pisum.*

1. INTRODUCTION

There are about 4700 species of Aphididae described in the world (Blackman and Eastop, 2007). Of these, about 450 species have been recorded from crop plants (Blackman and Eastop, 2000), but only about 100 have successfully exploited the agricultural environment to the extent that they are of significant economic importance. The agriculturally important species are mostly in the subfamily Aphidinae, because not only this is the largest subfamily, but also because it contains a very high proportion of the aphids that feed on herbaceous plants (Blackman and Eastop, 2006).

Aphids are a serious hindrance to world food production, although some crops are injured more than others. Most crops throughout the world are attacked by at least one species of aphid and they inflict injury *via* direct feeding and by vectoring plant pathogens (Katis *et al*., 2007; Quisenberry and Ni, 2007). As phloem feeders and major vectors of plant viruses, aphids are important pests of agricultural and horticultural crops worldwide (Powell *et al.,* 2006). The injury elicited by aphids and other sap-sucking arthropods can extract more energy per unit area than grazers and browsers, and they do so without consuming any of the plant structural tissues (Dixon, 1985a). For instance, Kemal Ali (1999) reported that estimated yield loss due to pea aphid on field pea might reach 48% depending on conditions and location.

The pest status of these small insects is related to several features of their biology that enable them to locate and exploit their host plants. These biological features include their intricate life style associated with their host plants, their polymorphism and ability to reproduce both asexually and sexually (Kennedy and Stroyan, 1959; Dixon, 1985a; Powell *et al*., 2006). The evolution of parthenogenesis, enabling reproduction without males for most parts or all of the life cycle, conferred a clear reproductive advantage by doubling the intrinsic rate of population increase (r_m) relative to sexually reproducing individuals (Moran, 1992). Parthenogenesis has also conferred the ability to start development at ovulation, shortening the time between birth and reproductive maturity. This “telescoping of generations”, with ovarian development and embryo formation inside embryonic mothers, has led to an additional increase in r_m (Kindlmann *et al.,* 2007).

Prior to the advent of synthetic insecticides, humans have used natural insecticides to combat insect pests that compete for our food and fiber in the field and storages for centuries. Moreover, botanical insecticides held a prominent place in pest control (Isman, 1994). In the 20th century, synthetic insecticides have replaced these natural pesticides. Since at that time synthetic insecticides seemed cheaper, easier to apply and have long lasting effect, and as the effective means of controlling detrimental insects (Coats, 1994; Isman, 1994; Casida and Quistad, 1998). Since then the synthetic pesticides have been used to give economic and effective pest control, which significantly enhanced agricultural crop productions. However, the indiscriminate use of some pesticides for pests control has generated a lot of concerns relating to public health, environmental pollution, genetic resistance by insect species and pest resurgence (Casida and Quistad, 1998; Elhag, 2000; Dubey *et al.*, 2008; Isman, 2008). This has brought about rethinking of the use of synthetic insecticides for controlling pests in crops. The concept of integrated pest management emerged as a consequence of overcoming the problems created by totally over reliance on use of synthetic insecticides. This has paved the way for the popularity of botanical pesticides once again to increase and some plant parts are being used globally as botanical pesticides (Dubey *et al.*, 2008).

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 both in the field and in postharvest protection of food in developing countries (Isman, 2006). Botanical pesticides are often active against a limited number of species, biodegradable to non-toxic products and with minimal persistence in the environment in their nature. Moreover, they are potentially suitable for use in integrated pest management (Kim *et al.*, 2003). Therefore, the use of green pesticides in food crop pest protection globally seems to be the best resort of the day in organic food production (Dubey *et al.*, 2008).

2. LITERATURE REVIEW

2.1. Legume crop production in Ethiopia

Pulses are the second major crop in Ethiopia, grown mostly by subsistent farmers under rain-fed agriculture. Pulses cover 13.86% of the total cultivated land and provide 11.06% the total production of major crops in the country (CSA, 2008). The country produces cool-season food legumes that includes faba bean (*Vicia faba*), field pea (*Pisun sativum*), lentil (*Leins culinaris*), grass pea (*Lathyrus sativus*) and chickpea (*Cicer arietinum*) (Asfaw Telaye *et al.,* 1994). Cool season food legumes cover over 86% of the pulse crop area and provide 88% of the total pulse production. Faba bean ranks first in area of cultivated land, production and yield followed by field pea (Hailu Beyene *et al.*, 1994). Ethiopia is a major producer of these five cool season food legumes with production being distributed throughout nearly all its zones. However, the major producing zones include the central highlands of Shewa, the highlands of Gojam and Gondar, North Wolo and parts of Tigray, while Wolega, Arsi and Bale constitute the second major production zones (Asfaw Telaye *et al.*, 1994).

Humans in most parts of the world consume legumes and their products. They are excellent sources of proteins, which is approximately three times that of cereals. These plant proteins are important, particularly in developing countries because of the cost and scarcity of animal proteins (Senayit Yetneberk and Asrat Wondimu, 1994). Cool season food legumes are also of a major food crops in Ethiopia where they provide a considerable portion of the daily diet of the people. They are consumed in different forms in everyday meals of Ethiopians (Asfaw Telaye *et al.,* 1994). In addition to protein source to humans, the post harvest by-products such as straw, pod walls and other residues from threshing, and the hulls and brokens from industrial processing are used for animal feed (Senayit Yetneberk and Asrat Wondimu, 1994).

Highland pulses form an important rotation crop in the cereal production systems. Moreover, they also comprise one of the most important cash crops in cereal based farming systems as they are mainly produced under subsistence agriculture (Tesema Tanto and Eshetayehu Tefera, 2006). However, the availability of the major food legumes has never been in surplus in the subsistence farming communities in Ethiopia. In fact, it has often been said that internal markets

seriously compete with the external ones. Yet a substantial tonnage of these food legumes is exported from the country every year (CSA, 2008). According to CSA (2002) report, 352 million Ethiopian birr was earned in the production year 2001/ 2002 by exporting 123,000 tons of pulses.

2.2. Description and biology of *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)

Pea aphids belong to the family Aphididae, subfamily Aphidinae of Order Homoptera. The species is a Palaearctic /Old World in origin, having been introduced into the New World in the nineteenth century. Though originally a palaearctic species, *Acyrthosiphon pisum* now has an almost worldwide distribution and is virtually cosmopolitan in distribution (Hutchison and Hogg, 1985; Blackman and Eastop, 2007; Sullivan, 2008).

A. pisum is a rather large, green or pink aphid with long, slender appendages, forming colonies on young growing parts and developing pods of many leguminous plants (Blackman and Eastop, 2007). This large green aphid is pear shaped, about 3/16inch long and 1/16inch wide at maturity. Pea aphids have a dark band encircling the base of each antennal segment (Miyazaki, 1987). They also possess unique anatomical structures (paired "tails" near the end of the abdomen) called cornicles through which a defensive secretion containing alarm pheromone is often emitted when a predator attacks an aphid (Miyazaki, 1987; Mondor and Roitberg, 2002). The abdomens are long and slender in appearance (Miyazaki, 1987). The nymphs closely resemble the adults except that they are smaller in size.

2.2.1. Reproduction

There are two ways of reproduction in aphids. These are holocyclic, in which several parthenogenetic generations alternate with a single, mainly annual sexual generation and anholocyclic, with out sexual forms. In temperate regions, the sexual forms are produced in the autumn before the harsh winter condition in order to produce progenies mainly in the form of

over wintering eggs. They reproduce parthenogenetically during the rest seasons of the year. In the tropics and subtropics, however, the reproduction of aphids is mainly parthenogenesis (Dixon, 1987). This mode of reproduction evolved in aphids in the Permian, 200 million years ago, and has been of paramount importance in determining the population structure of the group. It led to the evolution of polymorphism and telescoping of generations, so characteristic of aphids. Parthenogenetic reproduction allows rapid increase in numbers and results in populations consisting of clones. In addition to the holocyclic species that reproduce both parthenogenetically and sexually, there are a few number of anholocyclic species that only reproduce parthenogenetically (Dixon, 1985b).

As hemimetabolous insects, pea aphids undergo incomplete metamorphosis. Many aphid species have a complex life cycle that alternates between asexual and sexual reproduction (Brisson and Stern, 2006). *A. pisum* passes through four nymphal instars before reaching adulthood. During the majority of growing seasons only parthenogenetic viviparous females, these can be either apterous (wingless) or alatae (winged). Males and sexual females are produced in late summer and fall (autumn). In cold temperate regions, the females lay eggs that overwinter and give rise to the following spring to fundatrix (parthenogenetic viviparous apterae) (Hutchison and Hogg, 1985; Bale *et al*., 2007). However, at warmer latitudes it may overwinter without a sexual phase (Blackman and Eastop, 2007).

All the following generations also reproduce parthenogenetically until autumn, when a new sexual generation emerges. This alternation of reproductive modes is called *cyclic parthenogenesis (*Kindlmann *et al.*, 2007). The sexually produced eggs are the cold hardy stage that persists through winter (Bale *et al*., 2007). In spring and summer, however, parthenogenetic females do not lay eggs but give birth to larvae. Moreover, the development of an aphid begins when its mother is still an embryo (Dixon, 1985a). Consequently, the embryos inside an adult parthenogenetic aphid carry embryos themselves. This so-called *telescoping* of generations is a major reason for the high intrinsic rate of increase in aphid populations and the agricultural harm that they have been causing. In addition to the differentiation into parthenogenetic and sexual morphs, development into winged or wingless forms is important to aphid. Because

winged forms can move longer distance so that they able to colonize and exploit new resources (Dixon, 1977; Hutchison and Hogg, 1985).

2.2.2. Dimorphism

Dimorphism is characteristic of aphids. Aphids develop an array of different morphs, which are associated with the passage of the seasons, movement from host to host, and overcrowding. Asexual aphids of some species can either possess wings (in which case they are termed alatae), or lack wings (these morphs are called apterae). Typically, there are several structurally different morphs in a species, including both sexual and asexual forms (Dixon, 1977).

Parthenogens typically develop without wings (Miyazaki, 1987). In the parthenogenic female pea aphid, *A. pisum*, the wing dimorphism is a polyphenism in which environmental factors determine whether wing development is initiated. In contrast, the wing dimorphism in the male *A. pisum* is a genetically determined polymorphism (Braendle *et al*. 2005; Caillaud *et al.*, 2002). A number of different environmental factors can cause the parthenogenetic females to produce winged daughters. First, in some of the aphids that have winged and wingless forms, the appearance of winged individuals is associated with crowding, the "group effect" (Miura *et al*., 2003). The component of crowding that is most important in inducing this change of morph is tactile stimulation (Dixon, 1977). Second, this switch in development is associated with changes in the quality of the host plant in other species (Miura *et al*., 2003). In these species crowding causes the switch to occur earlier, but it has not yet been determined whether this is due to direct effects of crowding or to aphid-induced changes in the quality of the plant (Dixon, 1977). Third, winged offspring can be induced as a defense mechanism: aphid colonies that have been exposed to natural enemies like parasitoids and predators produce more winged offspring in *A. pisum* and, perhaps, in other aphids (Sloggett and Weisser, 2002). Similarly, colonies that have encountered common predators such as ladybirds (Weisser *et al*., 1999), the larvae of hoverflies (Almohamad *et al*., 2007) or those that have been exposed to the pea aphid alarm pheromone, (E)-b-farnesene, produce winged individuals (Podjasek *et al*., 2005).

The components of an aphid's environment can also induce the change from the asexual to sexual reproduction. In many species, the change from asexual to sexual reproduction is induced by short day length. In other aphids, low temperature or quality of nutrition determines the change (Dixon, 1985a). However, the response of aphids to these conditions is governed by a biological clock, which blocks the appearance of sexuals in spring. As for other animals it is the length of the night rather than the day that is critical for the induction (Dixon, 1977).

2.2.3. Host plant selection

The majority of herbivore insect species are very selective feeders that choose their host plants based on visual, mechanical and chemical stimuli (Price, 1997). The efficient recognition of a host depends on its capacity for processing information about environmental cues. In aphids (Aphididae), host plant selection and acceptance involves a series of consecutive steps like pre-alighting behaviour, leaf surface exploration and probing of subepidermal tissues, deep probing of plant tissues, and evaluation of the phloem sap (Niemeyer, 1990; Caillaud, 1999; Caillaud and Via, 2000).

These successive phases of the host plant selection process of insects have been called host habitat finding, host finding within the habitat, host acceptance and host suitability. As in other animals recognition and orientation, a multitude of stimuli are available in the environment and may guide the behaviour of aphids. But, not all the stimulants are equally important (Klingauf, 1987).

The host or non-host nature of a plant can be recognized before alighting on the plant (Chapman *et al*., 1981; Nottingham and Hardie, 1993), on the leaf surface (Powell *et al*., 1999), at subepidermal levels (Bernays and Funk, 2000; Caillaud and Via, 2000; Powell and Hardie, 2000; Funk and Bernays, 2001; Gabrys and Tjallingii, 2002), and in the phloem itself. The relative importance of each of these stages may be affected by the degree of host specialization (Bernays and Funk, 1999; Funk and Bernays, 2001), and by the species studied (Tosh *et al*., 2003; Vargas *et al*., 2005). It appears, for example, that some aphid species land randomly, while others perhaps more specialized, species fly upwind to particular host odours (Pickett *et al*., 1992).

2.2.4. Host plant specificity

Aphid life cycles are mainly of two types: autoecious (host specific) and heteroecious (host alternating). Autoecious species of aphids are monophagous, living on one or a few species of a particular genus of plants. Heteroecious aphids spend autumn, winter, and spring on a primary woody host and the summer usually on secondary herbaceous plants. The primary and secondary hosts belong to different families of plants, and the aphids are classified as polyphagous. Over 90% of aphids are autoecious (Dixon, 1985b). In cold temperate regions, *A. pisum* is holocyclic, producing oviparae and males on various leguminous hosts. As in other members of the genus *Acyrthosiphon*, there is no true host alternation (Blackman and Eastop, 2007).

The host range of *A. pisum* includes many species of the Leguminosae; on some of these hosts including alfalfa, this aphid is considered a pest. The pest status of *A. pisum* results from the effect of direct feeding damage plus the effect that the aphid is a vector of many plant pathogenic viruses (Hutchison and Hogg, 1985).

2.2.5. Dispersal

Pea aphids, *A. pisum*, are herbivores of leguminous plants. As with many aphid species, they can reach high densities where intraspecific competition can be intense (Hutchison and Hogg, 1985). They are cyclically parthenogenetic and individual females can give birth to both winged and wingless morphs. Apterous pea aphids disperse from low quality hosts (Hodgson, 1991) and increased numbers of winged offspring are produced in response to crowding or poor quality host plants (Sutherland, 1969; Hazell *et al.*, 2005).

As plants eventually die or are killed, aphids need to disperse to ensure their long-term survival (Dixon, 1985b). Individuals of most morphs are capable of moving to alternative parts of their host plants or to adjacent plants. Pea aphids can move to new patches by either flying (longer distance dispersal) or walking (local dispersal) from plant to plant (Hazell *et al.*, 2005). Movements within and between adjacent plants are commonly undertaken by apterae, but alatae

disperse over greater distances. Dispersal is most noticeable when aphids are abundant, especially in summer (Dixon, 1985b).

2.3. Economic damage inflicted by aphids

Aphids are serious pests causing damage either directly by feeding or indirectly by the transmission of viruses, in many areas all over the world (Fiebig and Poehling, 1998; Katis *et al.*, 2007). Aphids may also cause damage by injecting toxic salivary secretions while feeding. They tap photosynthate, an energy source of the plants, which may result in aphid feeding effect that include yellowing and premature death of leaves, stunting of the stems and finally reduction in grain size (Quisenberry and Ni, 2007). Aphid feeding on plant sap causes significant reduction in grain protein (Ba-Angood and Stewart, 1980).

The pea aphis, *A. pisum*, has a worldwide importance as a pest of leguminous crops. It causes direct damage through phloem feeding, and indirect economic loss through acting as a vector of economically important plant viruses (Lane and Walters, 1991). Both the adults and nymphs suck plant juices from the stems, leaves and flower buds. It also transmits several important virus diseases (Weber and Hampton, 1980) such as bean leaf roll (BLR), legume yellows virus (LYV) and pea enation mosaic (PEM) (Sylvester, 1980).

2.3.1. Economic importance of pea aphid on pulses in Ethiopia

Production constraints of cool season food legumes are many in the country (Hailu Beyene *et al*., 1994). Insect pests are among the production constraints of these crops. Though a number of insect pests have been causing varying degree of damage to field pea, pea aphid, *A. pisum*, is one of the most destructive ones reducing the performance of field pea in most field pea growing areas of the country (Tsedeke Abate *et al*., 1982; Melaku Wale *et al.*, 2000). In Ethiopia, the pea aphid, *A. pisum*, has become the major insect pest of food legumes such as lentil, peas and grass pea. For the last 20 years, lentils and peas have been seriously attacked. Beginning from the early 1990s, grass pea has been extensively attacked and this may force farmers to abandon the crop (Melaku Wale *et al.*, 2000). Though its attack is preferentially

pronounced on peas, lentils and grass peas, it has also been reported infesting faba bean as well (Kemal Ali and Tibebu Habtewold, 1994). However, faba bean is less suitable for the pea aphid survival and reproduction (Melaku Wale *et al.,* 2003).

Pea aphid attack is more severe in mid-altitude (1800-2200m.a.s.l.) areas of Shoa, Arsi, Bale and Gojam (Kemal Ali and Tibebu Habtewold, 1994; Kemal Ali, 2006). Its attack is widespread, especially in the warmer areas of the country and entire crop loss is a common experience in such places (Melaku Wale *et al.,* 2000). The pest may cause entire crop loss during bad years, however, where the amount and distribution of rainfall is optimum; there may be little damage to the crop. Dry, cool conditions favor the development of dense populations. Infestations in the mid-altitude may occur at any time under the above conditions but are most likely to occur during late summer (August) or spring (September to November) under Ethiopian condition (Kemal Ali, 1999). Estimated yield losses due to pea aphid include 22%, 29% and 48%, which were recorded at Holeta, Denbi and Kulumsa, respectively (Kemal Ali and Tibebu Habtewold, 1994).

2.4. Management of pea aphid

Prevention of damage to the plant is especially important when an agricultural crop would be destroyed by aphids at greater financial loss to the producer. Control involves using methods to protect the crop from aphid attack by various means some of such methods are biological (natural enemies), cultural, plant resistance, chemical, pheromones, botanical, and finally IPM (Sullivan, 2008).

2.4.1. Biological control

Biological control refers to the intentional use of an insect pest's natural enemies such as predators, parasitoids, and disease causing organisms in order to lower the population of insect pest below the economic threshold (Sullivan, 2008). In some families of predatory insects (e.g. Coccinellidae - ladybirds), both the larvae and the adults are predaceous on aphids, whereas in other families (e.g. Syrphidae - hoverflies, Chrysopoidae - lacewings, and Itonididae - midges),

only the larvae are predaceous (Volkl *et al.,* 2007). For instance, aphid predators like *Sphaerophoria rupelli* (Diptera; Syrphidae), *Adonia variegata,* Spp. *tredecinsignata,* and *Cheilomenes lunata* (Coleoptera; Coccinellidae) have been recorded preying on wheat aphids and have a significant role in reducing the population of this pest (Mulugeta Negeri *et al*., 1999). Similarly, coccinellids (*Cheilomenes lunata,* and *Cheilomenes sulphurea*) and syrphids were reported as natural enemies of aphid, *A. pisun,* in field peas by Kemal Ali (1999). However, the efficacy of predators in reducing pest populations depends on the synchronization of natural enemies populations with the build up of pest populations (Kemal Ali, 1999).

Aphid parasitoids are important in the biological control of aphid pests (Schmidt *et al*., 2003; Brewer and Elliott, 2004). Among insect parasitoids, all species in the Braconid subfamily Aphidiinae and some genera in the family Aphelinidae develop as endoparasitoids of aphids, with one larva completing development in each host (Volkl *et al.,* 2007; Rakhshani *et al.,* 2008). Pea aphids have been parasitized by Braconids (Hymenoptera) such as *Aphidius* spp. and *Praon* spp. in clover, alfalfa and pea fields (Fox *et al.,* 1967, Sullivan, 2008). For example, *Aphidius ervi* and *Aphidius smithi* have been successfully controlling *A. pisum* in the United States (Sullivan, 2008). The study by Kemal Ali (1999) on population dynamics of *A. pisum,* also indicated that the possible potential of *Aphidius* spp. as bio-control agent in Ethiopia at some locations. The result revealed the parasitoids caused up to 42% mortality of aphids at Kulumsa while it was only about 1.2% at Dembi during the same year.

2.4.2. Cultural methods

Cultural practices control insect pests by manipulating the field environment making it unfavorable for the pests. This affects the pest population by increasing the mortality or adversely affecting the natural reproduction; or promoting the natural enemies of the pest species (Hill, 1993).

Polyculture or mixed cropping is one of the cultural practices that have received much emphasis in recent agro-ecological studies. These cropping systems influence the abundance, diversity and relative importance of pests and their natural enemies and the yield performance of

component crops. Commonly, pest populations are lower in mixed-crop as compared to monocrop system (Ogenga-Latigo *et al*., 1993). The lower incidences have previously been attributed to 1) patchy distribution and low concentration of food resources for pests (the resource concentration hypothesis) (Risch, 1981 cited in Ogenga-Latigo *et al*., 1993) 2) adverse modification of microclimate (the microclimate hypothesis) 3) increased abundance and/or effectiveness of natural enemies (natural enemy hypothesis). The intercrop systems, thus, vary in the structural and spatial relationships between component crops, micro-climatic conditions, their influence on pest incidence and activity of natural enemies (Ogenga-Latigo *et al*., 1993).

Nampala *et al*. (2002) reported that mixed cropping of cowpea with sorghum reduced infestation by aphids. The reasons for this reduction of aphids' population in the intercrop may be due to the micro-environmental effect of the associated crop which may attract predators and/or disruption of insect visual search for preferred host. Another report by Ogenga-Latigo *et al.* (1992) showed that intercropping cowpea with maize reduced the incidence of *Aphis fabae* on cowpea than on sole cropped cowpea. Furthermore, various studies have shown that variations in herbivore load in crop mixtures compared to monocultures increase the abundance of natural enemies that could reduce their host populations on a given crop (Ogenga- Latigo *et al*., 1992; Kyamanywa *et al.*, 1993). For instance, according to Kemal Ali (2002) field pea intercropped with Ethiopian mustard sustained less incidence of *A. pisum* compared with the pea field planted in a monoculture system at Holeta, Dembi and Kulumsa. Recently, Hassan (2009) also reported that intercropping has the potential to reduce aphid populations on cowpeas.

2.4.3. Host plant resistance based control

Host plant resistance is defined as including those characteristics, which enable a plant to avoid, tolerate, or recover from the attacks of insects under conditions that would cause greater injury to other plants of the same species (Beck, 1965). Many plant species have defenses against herbivores including insects that is genetically heritable and hence controlled by one or more genes. Resistance of plants to insect attack is related to these heritable quantities of the genes of the plant that may reduce the damage (Sullivan, 2008). This heritable resistance to insect attack could be through physico-chemical characteristics of the plant that kill the insect (antibiosis),

pest insect may be repelled by the plant or at least has no preference for it (antixenosis) and the plant can recover even after some feeding damage by the insect (tolerance) (van Emden, 2007a; Sullivan, 2008).

One of the best methods to reduce the impact of pea aphids is to select varieties with high levels of pea aphid resistance. Because the use of resistance pea varieties may reduce economic loss that could be incurred by pea aphid, and it might also reduce the economic and environmental costs resulting from insecticide use (van Emden, 2007a). Some varieties of pea are known to have varying degrees of resistance to pea aphids (Beck, 1965). Thus, variety selection can be an important means of reducing pea aphid damage and establishing a vigorous plant stand.

Screening for resistance to pea aphid has been carried out at various research centers in Ethiopia. Accordingly, Kemal Ali and Tibebu Habtewold (1994) reviewed that out of 140 genotypes tested at Kulumsa 15 entries showed an acceptable degree of resistance to *A. pisum*. Kemal Ali (2006) also reported that from more than 600 germplasm accessions and breeding lines evaluated at Sinana Agricultural Research Center under natural pressure of the pest, 16 lines were found to be better in their resistance to pea aphid. Based on antibiosis, antixenosis and tolerance effects, lines 061K-2P2/9/2, JI-896 and 304WA1101937T performed better against *A. pisum* (Kemal Ali *et al.*, 2005).

2.4.4. Pheromone based control

Pheromones are part of many successful IPM programs. They are used to monitor moths, flies, and beetles in stored products, agriculture, and forestry. They can also help control pest insects, primarily through mass trapping and mating confusion. Generally, pheromone mating confusion techniques especially provide non-toxic, nonpolluting methods of controlling pests and reducing pesticide applications (Quarles, 2007). Aphid pheromones to control aphid pests could also reduce the problems, such as resistance and environmental contamination, seen with toxic pesticides (Powell and Pickett 2003; Quarles, 2007).

2.4.5. Chemical control

Use of insecticides is one of the control options for aphids. Application of a single spray of pirimicarb against pea aphid on pea at either the bud stage or at first flower, when the proportion of plants infested was over 50%, could give economic yield responses. However, later sprays at first pod did not produce significant responses (Dewar, 2007). Kemal Ali (1997) also reviewed that applications of insecticides like Dimethoate, pirimicarb, and primiphose-methyl for their effective control of pea aphid. The same author also reported that more than two sprays were not economically justifiable to control pea aphid with these chemicals.

Though chemical insecticides are powerful agents, some aphids mutate over time to develop resistance. This renders the pesticide inefficient, resulting in attempts to restore success by repeating applications, then increasing the dosage. In addition, the beneficial natural enemies are negatively affected. There are reports of 20 or more aphid species (e.g. green peach aphid, *Myzus persicae*) that have developed resistance to various chemical insecticides (Sullivan, 2008).

2.4.6. Botanical control

Many synthetic insecticides have been reported as effective in controlling field and storage insect pests (Hill and Waller, 1990; Ogunleye, 2001; Anyim, 2003; Ogunleye, 2006) in spite of their serious environmental consequences. In addition to their environmental concerns, adoption and use of insecticides by most tropical farmers are hindered by several factors including prohibitive costs, inconsistent supplies of the chemical, quality of the products, and safety of workers and consumers (Saxena *et al*., 1990; Wolfson *et al*., 1991; Isman, 2007). Insecticides can also lead to pest resurgence and resistance (Casida and Quistad, 1998).

Current research focus in crop protection includes the development of alternative non-synthetic chemical pesticide control technologies, which may minimize the use of synthetic pesticides and have economic and health benefits for the applicators, consumers and the environment (Isman, 1994; Elhag, 2000; Talukder and Howse, 2000; Isman, 2006). The use of more natural and sustainable methods of protecting crops from insect damage is most favored (Isman, 2006;

Dubey, *et al*., 2008). Botanicals /plant extracts/ are among natural pesticides. Many plants are being investigated for their insecticidal attributes and possible application to control insect pests on food crops. In this regard, some works that showed botanical pesticide properties of some plant species on storage and field crop pests have been reported in Ethiopia (Bayeh Mulatu and Tadesse Gebremedhin, 2000; Bekele Jembere, 2002; Melaku Wale, 2004; Bekele Jembere *et al.*, 2006; Bayeh Mulatu, 2007; Gemechis Legesse, 2008; Bayeh Mulatu and Biruk Wubshet, unpublished data). Higher plants contain a wide range of secondary metabolites like flavonoids, essential oils, phenols, tannin, steroids and alkaloids. Such plant-derived chemicals may be exploited for their different biological properties (Dubey *et al*., 2008).

2.4.7. IPM

Public concern over perceived hazards of pesticides, increasing costs of conventional pest control, and the effect on non-target organisms all serve in their own way to advance the prospects for implementation of Integrated Pest Management (IPM) programs (Koehler, 1989). IPM is an approach to keeping pest populations below a level causing economic loss, through the judicious and compatible use of two or more of several possible control measures described in the above sections.

IPM projects are being developed throughout the world for the control of serious pests of agricultural importance. Selection of the control measures adopted as part of an IPM package is based on many factors: available resources, such as money, manpower, technical know how, skills; the agroecosystem; geographical location; and socio-economic situations (Koehler, 1989). In general IPM incorporates three main components: 1) biological control using parasitoids 2) cultural control and host plant resistance using pest resistant varieties of crops 3) risk reduced pesticides are also included as one component for sustainable pest control with human and environmental safety (Tsedeke Abate, 1997; Casida and Quistad, 1998; Ehler, 2006). van Emden (2007b) reviewed many case studies of IPM to control aphids that are economically important on different crops. The case studies showed that implementation of a practical IPM, however, desirable and accepted in terms of service, is still at a very early stage in respect to aphids on most crops.

2.4.8. Use of *Millettia ferruginea* (Hochst) for insect pest control

Higher plants contain a wide range of secondary metabolites like flavonoids, essential oils, phenols, tannin, steroids and alkaloids. These secondary metabolites of the higher plants may be exploited as alternative sources of potentially sustainable insecticides including botanical insecticides and antifeedants (Isman, 1994, 2006), one such species is birbira *(Millettia ferruginea).*

Birbira, *Millettia ferruginea* (Hochst) Baker (Fabaeae: Paplionideae), is a large shady tree which grows up to 35m. It is endemic to Ethiopia and widely distributed in the country and performs well in moist lowland and as well as dry, moist and wet semi-highland agro-climatic zones of 1,000 – 2,500 m.a.s.l. (Azene Bekele *et al*., 1993; Legesse Negash, 1995; Azene Bekele, 2007). *M. ferruginea* is a multipurpose tree used as firewood, local construction, tool handles, household utensils and serves as shade tree in coffee growing areas (Azene Bekele *et al*., 1993; Legesse Negash, 1995; Azene Bekele, 2007). It is also used as decorative plants that planted along roads and in agroforestry, and planted to improve soil fertility (Banouzi *et al*., 2008). Furthermore, its pulverized seeds are extensively used as fish poisoning by traditional fishermen (Legesse Negash, 1995; Azene Bekele, 2007; Banouzi *et al*., 2008). Beside these uses, it has a traditional medicinal values in different parts of Ethiopia in different forms to treat some diseases. Its fresh/dry fruits powder prepared with butter is topically applied to treat skin infection (Fisseha Mesfin *et al.,* 2009), fruits powder mixed with honey is taken orally for amoeba and for dressing 'mujele' (chigger) with fruit paste mixed with butter (Tilahun Teklehaymanot and Mirutse Giday, 2007).

Currently, different investigators have extracted and characterized chemicals from birbira root barks and seeds. The analysis of the root bark and seeds of *M. ferruginea* led to the isolation of a chalcone and eight isoflavones from the root bark and seeds (Ermias Dagne *et al*., 1990; Yankep *et al*., 1997) and three rotenoids from immature seeds (Ermias Dagne and Amha Bekele, 1990). Isman (2006) reviewed that as an insecticide, rotenone is considered as a stomach poison to insects and it must be ingested to be effective. The rotenone that was extracted from the rhizomes or roots of tropical legumes in the genera Derris and Lonchocarpus, principally

contain isoflavonoids which was reported as specific inhibitors of cellular respiration (Isman, 1994).

Birbira seeds extract has been observed to be effective in controlling storage insect pests such as adzuki bean beetle, *Callasobruchus chinensis,* (Bayeh Mulatu and Tadesse Gebremedhin, 2000), maize weevil, *Sitophilus zeamais* (Bekele Jembere, 2002) and bean bruchid, *Zabrotes subfaciatus,* (Bekele Jembere *et al.,* 2007). Its aqueous and chloroform extracts from seed kernels has been evaluated against three species of aphids (Bayeh Mulatu, 2007) and diamond back moth (Bayeh Mulatu and Biruk Wubshet, unpublished data). The results revealed that the contact effect of both aqueous and chloroform extracts of birbira seeds powder showed similar efficacy on aphids while the extract with polar solvent bioassay showed less larval mortality than chloroform residue extract against diamondback moth at lower concentration of 0.4 mg/ml. At the highest rate (1.4 mg/ml), chloroform, aqueous and residue extracts caused similar mean mortality. The effectiveness of both aqueous and organic solvent extracts both by contact, as oviposition deterrent and development retardant is very interesting scenario and needs to be investigated further. Birbira was very effective, particularly on pea aphid, by contact (Bayeh Mulatu, 2007). Thus, the insect was considered to study birbira extracts that was obtained using solvents with different polarity.

Plant parts (plant material), targeted insect species, extraction methods, and choice of solvent affect the efficacy of botanical extracts (Shaalan *et al*., 2006; Das *et al.*, 2010). For example, Gemechis Legesse (2008) reported that an aqueous extract of flowers of *Hagenia abssinica* was more effective against *A. pisun* in inhibiting nymph production compared with its leaf and sawdust extracts. Similarly, Bekele Jembere (2002) compared crude extracts from different parts of birbira (seed, bark, leaf and pod bark) for the determination of their relative toxic effect against maize weevil (*Sitophilus zeamais*). It was only the seed extract that showed significant toxic effect against the weevil. In addition to plant materials and target insect species, extraction methods and selection of solvent type have paramount importance in efficacy of phytochemical extractions.

When choosing an extraction method, maintaining the activity of the extracted compound(s) is the priority (Green, 2004). The extraction method that has been widely used is plant tissue homogenization in a solvent. Dried or wet, fresh plant parts are ground in a blender to fine articles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for some time after which the extract is filtered (Das *et al.*, 2010). Another common method is serial exhaustive extraction which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted (Green, 2004). Other researchers employ soxhlet extraction method of dried plant material using organic solvent. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Das *et al.*, 2010).

During the extraction, organic solvents diffuse into the solid material and solubilize compounds with similar polarity. The nature of the solvent used will determine the types of chemicals likely extracted from the plant (Green, 2004). Shaalan *et al*. (2005) reviewed that solvent type for phytochemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds. A converse relationship is said to exist between extract effectiveness and solvent polarity where efficacy increases with decreasing polarity (Mulla and Su, 1999). This is not consistent due to differences between the characteristics of active chemicals among plants (Shaalan *et al*., 2006). The previous works on birbira aqueous and organic solvent crude extracts and their bioassay have showed similar insecticidal effect against barley and cabbage aphids. This result indicated that the presence of potent insecticide chemicals in both solvent extracts of different polarity and the scenario needs to be investigated using more solvents of different polarity. Thus, different solvents of varying polarity might extract the potent chemicals in birbira seed kernel differently and meanwhile affect the effectiveness of the extracts. As a result, solvents with different polarity were used to produce crude extracts from *M. ferruginea* seed kernel and were assayed for efficacy against pea aphid. Therefore, the aim of this study was to evaluate the efficacy trend of the extracts as the polarity of solvents decrease and in which solvent extracts the potent chemicals with better insecticidal efficacy on pea aphid. Hence, this study was proposed and carried out based on the following objectives.

General Objective

- To evaluate the aphicidal and deterrence effects of extracts from *M. ferruginea* seed kernel with different extracting solvents on pea aphid as a possible way of integrated pest management

Specific Objectives

- To evaluate ex-situ toxicity of *M. ferruginea* seed extracts obtained using different solvents against pea aphid
- To evaluate the contact effect of *M. ferruginea* seed extracts obtained using different solvents against pea aphid on potted intact plants
- To assess the effect of *M. ferruginea* seed extracts on pre probe settling response of pea aphid
- To identify the solvents that may extract the potent insecticidal components in *M. ferruginea* seed kernel better

3. MATERIALS AND METHODS

3.1. Test insect collection and rearing

Field pea seeds of variety Mohanderfer were planted in 3kg volume pots filled with soil prior to the collection of aphids from the field. The pea aphid, *A. pisum* was used throughout the present experiments. A small population of *A. pisum* was collected from the field pea and grass pea fields of Holeta Agricultural Research Center (HARC) and from farmers field around the center, respectively and brought to HARC insectary. Laboratory colony of pea aphid was established in a protected insectary using protective cages. They were reared and bred under laboratory conditions on their host plant field peas, *Pisum sativum*, in the insectary with average maximum 21°C and average minimum of 16°C temperature regimes and relative humidity of 60 – 70%. Rearing was done continuously through out the study period on field pea plants as a host.

3.2. Plant material preparation and extraction

The seed kernel of matured seeds of *M. ferruginea* with purple-brown in colour was used in this study. The matured pods of *M. ferruginea* were collected from the premises of HARC and neighboring areas. The pods were then brought to the insectary of HARC and were put aside for few days to dry. When the pods dried well, the seeds were removed from the pods. The seed coats were split to remove the cotyledons; the cotyledons were ground to powder using mortar and pestle under shady condition (Annex 1). After cotyledons were ground, the powder was kept in a sealed polythene bag in a refrigerator at 4°C until used for its crude extractions using solvents of different polarity. The powder was used to prepare different crude extracts and all solvent extracts were prepared in the laboratory as described below.

Different solvents of varying polarities (deionized water, acetic acid, acetone, chloroform, toluene and hexane with the polarity index of 9.0, 6.2, 5.1, 4.1, 2.4 and 0.1, respectively) were used for the extraction of the powder following the procedures used by Bayeh Mulatu (2007). Six samples of 50 grams of the ground cotyledons of *M. ferrugneia* were weighed separately for crude extractions with deionized water, acetic acid, acetone, chloroform, toluene and hexane. Each 50 gram sample was mixed with each 100 ml solvent in separate flasks. The mixtures were

stirred continuously for fifteen minutes using a magnetic stirrer at room temperature until homogenous solutions were formed and left to stand. Then after each mixture was filtered using a fine cotton cloth called 'Shema'. The filtrate solutions of acetone, chloroform, toluene and hexane were transferred to evaporating dishes to evaporate the solvents using a water bath that was adjusted at the respective boiling points of each solvent in the evaporating hood. However, the deionized water and the acetic acid were evaporated using rotary evaporator under a vacuum, in a boiling bath. After evaporating the solvents, solid extracts were left on each container. The solid extracts were transferred separately to flat polythene and kept spread for a day in ventilated room for further removal of the solvents (if any). All the extracts were packed in polythene bags and were kept in a refrigerator at 4^{o}C to maintain their freshness until used for the assays.

3.3. Ex-situ contact bioassay

Prior to running the experiment, young adult aphids were reared on potted pea plants. The potted plants were infested with 20 viviparous females/pot for six different consecutive days before the actual treatment. The viviparous females introduced to potted plants on each day were allowed to give nymphs for 24 hrs. Then parent stocks were removed and the nymphs were allowed to develop into young adults over six days.

Then stock solutions were prepared from the above six extracts by weighing 20, 40, 60, 80, 100,120 and 140mg, and dissolved in 100 ml of tap water in separate glass flasks. The mixtures were stirred continuously using a magnetic stirrer at room temperature until homogenous solutions were formed. All the homogenous solutions of each mixture produced a lemonade colour except for that of acetic acid extract.

To evaluate the direct contact toxicity potential of birbira extracts, ten young adult apterous females (about six days old) were collected from the pre-infested plants and transferred into each glass Petri dish (9 cm in diameter) and the Petri dishes were arranged in completely randomized block design in the insectary spread on a bench. Stock solutions of seven rates of each solvent extract was topically applied using a micropipette at 5µl per aphid. Tap water was

included as blank control. Each rate was replicated six times in a completely randomized block design. The treatments were performed for six consecutive days considering each day as a block. Since the application and data collection cannot be completed for the whole experiment in a day, the experiment was blocked by days. Then the days were used as replication (Gomez and Gomez, 1984). The treatments were initiated in the mornings at 10:00 am. Evaluation was made 5 hrs after treatment to record aphid mortality that takes place due to treatments effect. According to Bayeh Mulatu (1997), it took more than 7 hrs for a Russian Wheat Aphid, *Diuraphis noxia*, before salivation phase and even more time to sustained feeding. The aphids were not provided with food after treatment in order to avoid mortality that may be caused while transferring to food sources. Data were collected on number of aphid died after 5 hrs of the treatment of each day. Aphids were examined 5 hrs after treatment and those that did not move or respond to gentle touch with a camel-hair brush were considered dead. The percent mortality of aphids that occurred in tape water treated ones (contrls) was used to correct for the natural death of aphids in all the treatments and to get percent mortality due to the treatment effects. Thus, mortality data were corrected using Abbott's formula (1925) cited in Hoekstar (1987) for the natural mortality.

$$\text{Corrected \% mortality} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \times 100$$

Where: n = Insect population alive, T = treated, Co = control

The LC_{50} of solvent extracts were calculated using probit analysis (Finney 1971, cited in Rahman and Talukder, 2006) with a log10 transformation of concentrations of the extracts.

3.4. Aphid settling bioassay

The effect of crude extracts on the response of pea aphid was bioassayed using excised leaves of field pea. The test was conducted at HARC insectary. Pea plants of Mohanderfer variety were planted in pots and were allowed to grow for two weeks in cages in the green house. Apterous female aphids were also reared on the same variety that was raised in parasitoid free caged potted plant in the insectary. LC_{50} dose of each solvent extract of *M. ferruginea* seed powder was calculated from the topically applied ex-situ contact toxicity bioassay experiment. The LC_{50} doses obtained from the probit analysis of deionized water, acetic acid, acetone, chloroform,

toluene and hexane extracts of birbira were weighed and their test solutions were prepared by dissolving in 100 ml tap water each. Aphid settling was assessed using a choice and no-choice test in Petri dishes under laboratory conditions (Hunter and Ullman, 1992). Plants that were free of aphids were removed from the green house pots. Two leaves were selected from the third node from the terminus and leaves were cut from the stem using new razor blades. Some of the leaves were dipped into the appropriate test solution and some other leaves in a tap water as a control. The leaves were allowed to dry for 30 minutes at ambient temperature in the isectary. A choice and no-choice aphids settling tests were conducted in Petri dishes under laboratory conditions. The leaves were placed in individual Petri dishes (diameter of 9 cm) with the petioles inserted in wet cotton to keep the leaves longer without shrinking (DelCampo *et al*., 2003).

3.4.1. Choice and no-choice test

One control and one treated leaves dipped in each test solution were placed approximately at equidistance from the center in each Petri dish. In fact, the two extremes of the Petri dishes along their diameters were labeled as treated and control to separate treated leaf from control one. Ten apterous young adults (about six days old), starved for one hour prior to the choice test, were placed midway between the two leaves and the dishes were covered so that the insects had an equal choice between treated and control leaves (Hunter and Ullman, 1992) (Annex 2). Keeping aphids off their host plants for at least half an hour markedly increase their ability to select a host (Klingauf, 1987). Each treatment was replicated ten times at each test solution for 100 aphids per test and the Petri dishes were arranged in completely randomized design (CRD). An aphid location in the dish (on control leaf or on treated leaf) was recorded at 30 min, 1 hr, 2 hrs, and 24 hrs intervals after access to the leaves .

In the no-choice assay, the insects were tested in the same conditions and procedures of the choice test, including the treatments. However, only one treated leaf per Petri dish for each test solution was placed at the center. A leaf dipped in tap water was included as a control in a separate Petri dish. Ten apterous young adults (about six days old), starved for one hour prior to the no-choice test, were placed at a corner of the Petri dishes and the dishes were covered

(Annex 3). This was replicated ten times at each test solution and for the control for 100 aphids per test and the Petri dishes were arranged in completely randomized design (CRD). Data on the number of aphids that settled on the treated leaf in the Petri dish were recorded at 30 min, 1 hr, 2 hrs, and 24 hrs intervals after access to the leaf.

3.5. Contact toxicity of extracts on intact plants

Five seeds of field pea Mohanderfer variety were planted into each circular pot of 20 cm in diameter on sterilized soil. The pots were kept in greenhouse at average maximum temperature of 29^{o}C and average minimum temperature of 3^{o}C. When the plants were two weeks old, the potted plants were taken to the insectary and 10 apterous young females of about six days old and that were reared on separate potted plants in the same insectary were placed on the top of potted plants. The aphids and the plants were caged in a clear cylindrical plastic cage of 60 cm tall and 15 cm in diameter with the open end and the six side holes covered with muslin for ventilation and left aside for 24 hrs before sprayed (Annex 4). The treatment was done using small sprayers that were obtained from local market following the procedures used by Gemechis Legesse (2008).

Prior to treating the plants, calibration of the amount of the extract of the plant material was done using distilled water to know the necessary volume to completely wet the plants using these sprayers before the actual treatment was done. The calibration was done by repeatedly spraying to find close estimate of the application rate, and 7 ml was enough to completely wet the upper and lower surfaces of all leaves and the stems of the five plants in a pot. The potted plants and young aphids within the cages were sprayed with one of the six different solvent extracts at the LC_{50} rate obtained from the result of the ex-situ contact toxicity test (experiment 2.3) and the control plants were treated with tap water following the procedures used by Kemal Ali and Louw (2005) with some modifications. Each treatment was replicated five times and the pots were arranged in a completely randomized design (CRD) and placed in the same insectary used for rearing the aphids. Mortality was recorded by inspection and probing the insects using the camel-hair brush to check death of individuals every 24 hrs for four consecutive days after treatment following the procedures used by Gemechis Legesse (2008).

3.6. Statistical Analysis

Insect mortality data were corrected using Abbott's formula, transformed into arcsin $\sqrt{p}$ (p = percentage mortality) before variance analysis (ANOVA), when deemed necessary (Gomez and Gomez, 1984; Rangaswamy, 1995). Then the transformed data of direct contact toxicity effect of birbira extracts were analyzed using a two factor completely randomized block design using different solvent extracts and rates of application as the two factors (SAS system for windows version 9.0, 2002). Tukey honest significance difference (HSD) at 5% level of significance was used for mean separations whenever significant results were encountered. The concentration-mortality lines were calculated using probit analysis (SPSS Ins., Version 13, 2004) with a log10 transformation of concentrations of birbira extracts. Furthermore, the lethal concentrations of the respective extracts that killed 50% of the aphid population and their 95% confidence intervals were also determined using probit model of SPSS 13 for windows. The paired-choice settling deterrence tests of the extracts were analyzed using paired sample *t* test (Horton, 1995). However, the no-choice settling deterrence and the LC_{50} contact toxicity on intact plants of the extracts were analyzed using one-way ANOVA.

4. RESULTS

4.1. Ex-situ direct contact toxicity effect of birbira seed powder extracts

The Two-Way ANOVAs computed on the percent mortality data from the six treatments showed that there was no interaction between treatments and doses, but the main effects, the treatments and doses resulted in highly significant aphid mortality by contact (Annex 6). Mean percent mortality record of young adult pea aphids at 5 hrs after treatment, due to contact toxicity of the different birbira solvent extracts, topically applied, is shown in Table 2. All the extracts of birbira dissolved in tap water caused significantly ($p<0.05$) higher mortality to young adult *A. pisum* in 5 hrs time than the untreated control. However, the young adult aphids responded differently to the birbira extracts and the deionized water extract was by far the most toxic that caused about 98.15% mortality followed by the acetic acid extract which was 88.61%. Mortality caused by birbira extracts in acetic acid and acetone was not significantly ($p<0.05$) different, whereas, acetic acid brought significantly more death than the chloroform, toluene and hexane. Birbira extract in hexane caused the least mortality records than other extracts.

The probit statistics, estimate of LC_{50}, their 95% confidence limits, and the slope of regression lines for the different solvent extracts of birbira seed cotyledon 5 hrs after topical application to young adult pea aphids, *A. pisum*, are presented in Table 1. The LC_{50}, which is the median (50%) lethal concentration, or concentration killing 50% of the exposed organisms at a specific time of observation, is most often used to express the toxicity. From probit analysis of ex-situ contact bioassay at 5 hrs after treatment, it was found that the deionized water extract of birbira was the most toxic followed by acetic acid extract (Table 1). The hexane extract had the lowest toxic effect on *A. pisum*. The extract of deionized water had the highest toxic effect against pea aphids and the lowest LC_{50} value (0.04 mg/ml). Higher concentrations contributed more significantly to the efficacy of extract on the mortality of *A. pisum* and they appeared to be the most important factors in the degree of toxicity obtained with different solvent extracts. When probit regression lines of the six different extracts were calculated, they showed a linear relationship between mortality percentage and extract concentration at 5 hrs after treatment (Fig.1). From the analysis, the regression line equations of different extracts of birbira extract at different doses were $Y = 1.37X \pm 1.80$ for deionized water extract, $Y = 1.28X \pm 1.26$ for acetic

acid extract and Y = 1.09X ±0.72 for acetone extract, Y = 1.24X ±0.73 for chloroform, Y = 1.21X ±0.29 for toluene and Y = 1.04X -0.04 for hexane. Comparing all regression lines at 5 hrs after treatment, the regression line for deionized water extract showed higher probit mortality. The hexane extract treatment was the least toxic. The order of toxicity of different solvent extracts of birbira against pea aphid was deionized water > acetic acid > acetone > chloroform > toluene >hexane from the LC_{50} probit analysis.

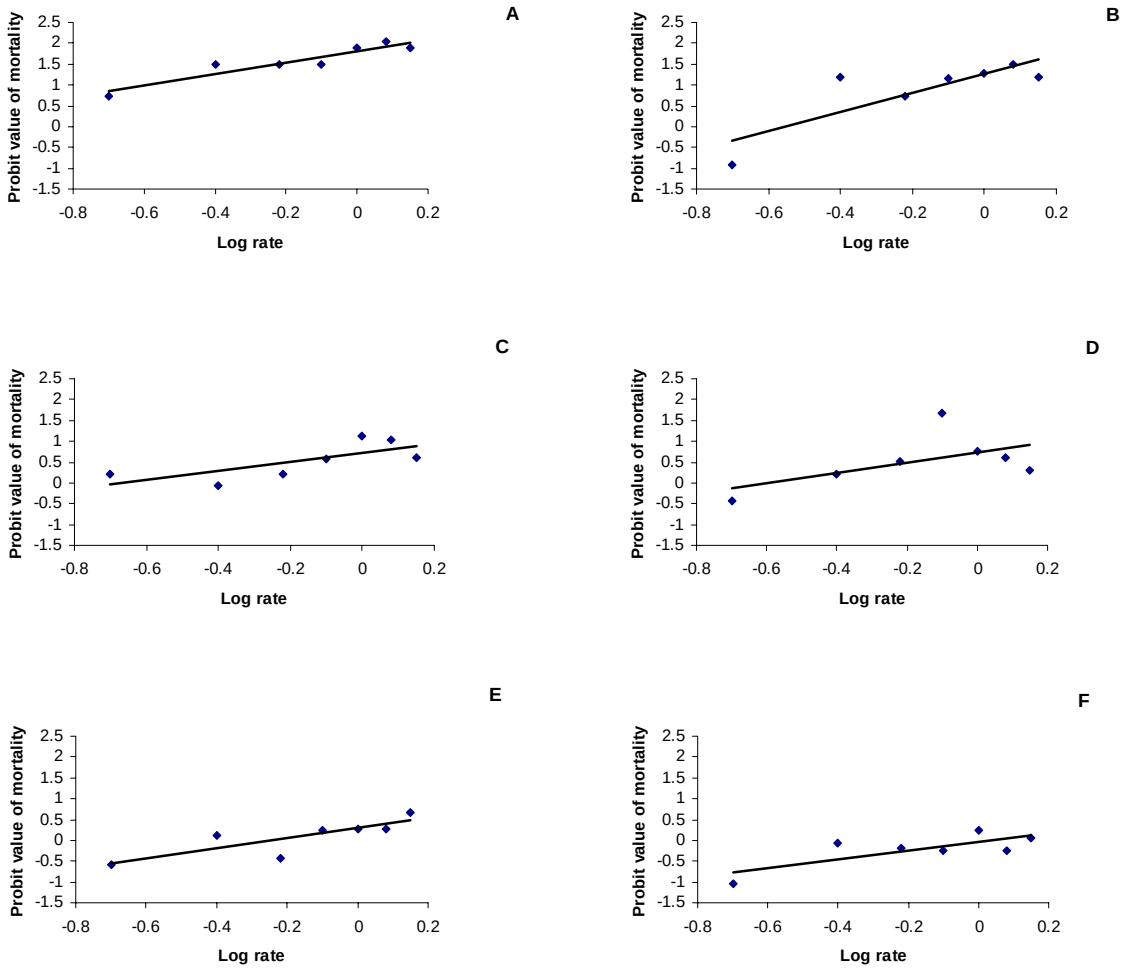


Fig. 1 Probit transformed mortality responses of pea aphids to the toxicity effect of deionized water (A), acetic acid (B), acetone (C), chloroform (D), toluene (E) and hexane (F) extracts from birbira seed powder dissolved in water and topically applied

Table 1. Probit analysis for direct toxicity at 5 hrs after topical application of different solvent birbira extracts to pea aphid, *Acyrthosiphn pisum*

Extract type	LC_{50} (mg/ml)	95% fiducial limit (mg/100 ml water)	Regression coefficient	Intercept	R^2
Deionzed water	0.04	0.45 - 10.61	1.37	1.80	0.85
Acetic acid	0.12	3.15 - 25.37	1.28	1.26	0.70
Acetone	0.25	9.79 - 46.68	1.09	0.72	0.55
Chloroform	0.32	13.59 - 57.62	1.23	0.73	0.61
Toluene	0.57	29.35 - 103.12	1.21	0.29	0.69
Hexane	1.00	56.13 - 203.91	1.04	-0.04	0.58

Table 2. Percent mortality of young adult *A. pisum* due to contact toxicity effect of different birbira extracts dissolved in water and topically applied

Extract type	Mean ± SE percent mortality
Deionzed water	98.15 ± 1.15a
Acetic acid	88.61 ± 3.21 b
Acetone	79.60 ± 3.26bc
Chloroform	69.92 ± 2.57cd
Toluene	54.98 ± 3.14de
Hexane	41.23 ± 3.19e
Control	0 ± 0f
F ratio	40.28
P value	0.0001

The original aphid mortality data were corrected by Abbott's formula and then transformed into arcsin √percentage mortality values before ANOVA and Tukey Student Test (HSD).

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

The extract obtained using deionized water caused significantly higher mortality than the untreated control. Among the different doses of the extract, significantly higher mortality of aphids by contact was observed at 1.2 mg/ml than the 0.2 mg/ml, which was not significantly different from the other rates (Table 3 and Annex 7).

Table 3. Percent mortality of young adult *A. pisum* topically treated with different rates of deionized water extract of birbira

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	86.57±8.39b
0.4	98.59±4.06ab
0.6	98.58±4.06ab
0.8	98.45±5.27ab
1	99.16±4.26ab
1.2	99.90±0.79a
1.4	99.16±4.26ab
control	0±0c
F ratio	57.3
P value	0.0001

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

From the acetic acid extracted birbira, aphid mortality in the untreated control was significantly lower than all the rates used. The minimum rate applied killed significantly less proportion of aphids than all the higher rates used (Table 4 and Annex 8). For the acetone extracted birbira there was aphid mortality difference between the untreated control and the higher rates above 0.8 mg/ml applied. Rates below 1.0 mg/ml did not bring significant mortality of aphids than the untreated control (Table 5 and Annex 9).

Table 4. Percent mortality of young adult *A. pisum* topically treated with different rates of acetic acid extract of birbira

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	36.64±6.70b
0.4	93.88±5.64a
0.6	83.75±7.42a
0.8	94.39±8.18a
1.0	96.09±5.95a
1.2	97.27±5.39a
1.4	95.37±6.72a
control	0±0c
F ratio	24.77
P value	0.0001

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

Table 5. Percent mortality of young adult *A. pisum* due to direct topical application of acetone extract of birbira at different doses

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	62.60±9.38bc
0.4	47.71±6.43c
0.6	58.75±3.62c
0.8	82.92±9.65abc
1.0	92.70±5.98ab
1.2	94.01±7.29a
1.4	97.14±5.97a
control	0±0c
F ratio	8.82
P value	0.0001

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

Chloroform extract of birbira has also caused significant aphid mortality at all rates used than in the control. The lowest mortality was obtained at the minimum rate used which was not different from the 0.4 and 1.4 mg/ml rates. Significantly higher mortality was caused by the extract at the rates between 0.8 and 1.2 mg/ml. Their killings at these rates were similar (Table 6 and Annex 10).

Table 6. Percent mortality of young adult *A. pisum* due to direct topical application of chloroform extract of birbira at different doses

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	36.35±2.60b
0.4	58.49±2.79ab
0.6	70.58±3.20ab
0.8	82.26±6.53a
1	87.52±7.48a
1.2	81.10±7.17a
1.4	66.16±7.72ab
control	0±0c
F ratio	18.13
P value	0.0001

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

Toluene extract of birbira caused the highest aphid mortality at the highest rates applied, which was significantly different from the 0.2 and 0.6 mg/ml rates. The mortality at the lowest rate of the extract was not significantly different from the control (Table 7 and Annex 11). Hexane extract of birbira caused the highest aphid mortality at the 1.0 mg/ml rate than the lowest rate applied and the control. The differences between all the rests of the rates applied were not statistically significant (Table 8 and Annex 12).

Table 7. Percent mortality of young adult *A. pisum* due to direct topical application of toluene extract of birbira at different doses

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	21.09±9.81bc
0.4	59.42±7.98ab
0.6	32.78±3.41b
0.8	59.54±6.62ab
1.0	66.48±8.31ab
1.2	62.35±5.24ab
1.4	82.18±7.79a
control	0±0c
F ratio	7.23
P value	0.0001

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

Table 8. Percent mortality of young adult *A. pisum* due to direct topical application of hexane extract of birbira at different doses

Dose (mg/ml of water)	Mean ± SE percent mortality
0.2	8.00±8.20bc
0.4	50.03±9.56ab
0.6	38.92±8.34ab
0.8	38.94±6.70ab
1.0	64.64±6.61a
1.2	39.79±5.44ab
1.4	56.01±8.44ab
control	01±0c
F ratio	4.47
P value	0.0009

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

4.2. Aphid settling bioassay

4.2.1 Paired choice test

Paired-choice aphid settling deterrence bioassay conducted using excised leaves of field pea was based on the LC_{50} of deionized water, acetic acid, acetone, chloroform, toluene and hexane extract of birbira in the insectary. Aphids settle on a plant and go into deep penetration only when they accept it as a food source. Therefore, the number of aphids that settle and feed on a given substrate is a good indicator of its suitability.

The 24 hrs paired choice assay on excised leaves showed that the six different extracts of birbira had various effects on aphid settlement on field pea leaves. The LC_{50} of acetone, chloroform and toluene extracts did not deter the aphids from the onset of the experiment until its termination. Their activity was non-significant as indicated by the P-values of the statistical analysis (Table

9). The LC_{50} of the hexane extract deterred aphid settling at 30 min, 1 hr and 24 hrs but not at 2 hrs after exposure. Its activity was significant ($p<0.05$) compared to the control leaf as indicated by the P-values. The deterrence activity of deionized water extract was different from hexane extract. The deionized water extract was found to deter aphid settlement significantly only at 30 min and 2 hrs after exposure to the treatment. However, for the acetic acid extract significant effect was observed at 2 hrs and 24 hrs after treatment. In general, the result showed that the mean number of aphids settled on both the control and treated leaves decreased over the observation period. This might be due to the observed mortality of aphids at 24 hrs after exposure (Annex 5).

Table 9. Settling deterrent effect of six different birbira extracts against *A. pisum* over time after treatment (paired-choice test)

Extract type		Time after exposed to the treatments			
		30 min	1 hour	2 hours	24 hours
Deionized water	Control	5.8±0.55	5.5±0.67	5.3±0.47	3.9±0.43
	Treated	3.2±0.89	3.6±0.56	3.6±0.27	3±0.60
	P	0.02	0.14	0.03	0.30
Acetic acid	Control	5.1±0.64	5.2±0.59	5.3±0.54	4±0.33
	Treated	3.4±0.56	3±0.61	3±0.47	2.2±0.36
	P	0.16	0.1	0.03	0.02
Acetone	Control	4.1±0.75	3.9±0.67	4.3±0.75	3.3±0.65
	Treated	4.7±0.62	4±0.67	4±0.56	3.1±0.57
	P	0.64	0.94	0.81	0.87
Chloroform	Control	4.3±0.79	4.6±0.62	5.1±0.41	3.5±0.50
	Treated	3.9±0.60	4.2±0.39	3.8±0.47	2.6±0.45
	P	0.77	0.68	0.11	0.11
Toluene	Control	4.9±0.79	4.9±0.79	4±0.88	3.9±0.58
	Treated	4.5±0.69	4.1±0.82	4.7±0.80	2.8±0.46
	P	0.79	0.63	0.68	0.31
Hexane	Control	6.3±0.65	6.4±0.65	5.6±0.75	3.9±0.46
	Treated	3±0.36	2.7±0.47	3.3±0.70	1.7±0.26
	P	0.01	0.01	0.13	0.00

Numbers for "test" and "control" represent mean of aphids settled on test or control leaf (choice test) using Paired t test.

P-values show the significance differences between the numbers of aphid settled on either of the leaves at 5% level of significance.

4.2.2. No-choice

No-choice aphid settling deterrence bioassay using excised leaves of field pea was also conducted with the LC_{50} of deionized water, acetic acid, acetone, chloroform, toluene and hexane extract of birbira. The results of the no-choice deterrence bioassay effects of the LC_{50} of these extracts of birbira on aphid settling is presented in Table 10. The extracts had no deterrence effect on the aphids as none of the extracts significantly deterred the aphids in all the treatments than the control within 30 min, 1 hr and 2 hrs after exposed to the treatment (Annex 13, 14 and 15, respetively). However, within 24 hrs after treatment, acetone, chloroform and toluene extracted birbira showed aphid settling deterrence effect than the control leaves (Annex 16). As a result, significantly fewer aphids settled on treated than control leaves. Deionized water, chloroform, toluene and hexane extracted birbira did not cause any gradual significant change in number of aphids settled among 30 min, 1 hr, 2 hrs and 24 hrs after treatment. Significantly, fewer numbers of aphids settled on acetic acid and acetone extracted birbira at the 30 min than at the 2 hrs and 1 hr after the treatment, respectively, but not significantly different number of aphids settled 24 hrs after the treatment. However, in the control significantly higher number of aphids settled on the leaves at 2 hrs and 24 hrs than 30 min and the highest aphid number was recorded at 24 hrs.

Table 10. Number of pea aphids, *A. pisum*, settled on control and treated leaves with different extracts of birbira (no-choices test)

Type of extracts	N	Number of aphids settled (Mean ± SE)				F ratio	P-value
		30 min AT	1 HAT	2 HAT	24 HAT		
Deionized water	10	7.60± 0.52Aa	8.30± 0.42Aa	8.50± 0.31Aa	8.10± 0.38Aab	0.87	0.47
Acetic acid	10	5.70±0 .78Ba	6.70± 0.76ABa	8.50± 0.43Aa	7.30± 0.72ABab	2.92	0.047
Acetone	10	4.80± 0.53Ba	7.70± 0.63Aa	8.00± 0.71Aa	6.80± 0.66ABb	5.09	0.01
Chloroform	10	5.60± 0.99Aa	7.20± 0.87Aa	8.00± 0.60Aa	6.70± 0.72Ab	1.55	0.22
Toluene	10	6.40± 0.79Aa	7.40± 0.62Aa	8.00± 0.52Aa	6.20± 0.49Ab	1.9	0.15
Hexane	10	5.80± 0.70Aa	7.00± 0.77Aa	7.60± 0.70Aa	7.90± 0.50Aab	1.88	0.15
Control	10	7.30± 0.54Ba	8.40± 0.52ABa	9.10± 0.23Aa	9.50± 0.22Aa	5.58	0.00
F ratio		1.96	0.91	0.87	4.00		
P value		0.09	0.49	0.52	0.00		

AT = after treatment, HAT = Hour after treatment

The upper and lower cases are for along rows and within columns comparison, respectively. Means followed by the same upper case along rows and lower case letters within a column are not significantly different at the 5% level of probability using Tukey Student Test (HSD).

4.3 Contact toxicity of the extracts on intact plants

Cumulative percent mortality records at 24, 48, 72 and 96 hrs of young adult pea aphids sprayed with LC_{50} doses of birbira extracts from the probit analysis (Table 1) while on intact pea plants is presented in Table 11. The One-Way ANOVAs at these observation periods are also annexed in Annex 17, 18, 19 and 20, respectively. All the extracts of birbira except hexane caused significantly ($p<0.05$) higher mortality to the aphids within three and four days time after treatments than the untreated control. Within 24 hrs after spray, none of the extracts showed significant ($p<0.05$) mortality of aphids than the untreated control. Within 48 hrs of exposure, significantly higher mortality was recorded for deionized water and acetic acid extracted birbira than hexane and the control which was not different from chloroform extracted birbira. The acetone extract caused significantly ($p<0.05$) higher mortality than untreated control, and similar effect with other extracts. But, extracts of chloroform, toluene and hexane showed non-

significant ($p<0.05$) mortality compared to untreated control. Within 72 and 96 hrs after spray, hexane extract did not show any significant effect than the untreated control while the rest of the extracts caused significantly higher mortality than both did.

This result showed that the toxic effects of all the extracts of birbira except hexane extract increased with time. Deionized water, acetone and chloroform extracted birbira killed significantly ($p<0.05$) higher aphids at 96 hrs than at 24 and 48hr after spray, but not different from that of at 72 hrs. Acetic acid extracted birbira caused significantly ($p<0.05$) higher aphid mortality on the forth day than on the first day after spray which was not different from the second and third day. In general, the results showed that toxicity related mortality in pea aphid became clear and significant in the latter observation times.

Table 11. Cumulative percent mortality of young adult *A. pisum* sprayed with LC50 extracts of birbira while the aphids were on intact potted plants

Type of extracts	Cumulative percent mortality of aphids (Mean ± SE)				F ratio	P-value
	24 HAT	48 HAT	72 HAT	92HAT		
Deionized water	12.00±4.90Ca	24.00±2.45BCa	31.11±2.22ABa	40±2.72Aa	13.21	0.00
Acetic acid	8.00±4.90Ba	20.00±5.48ABa	28.89±5.67ABa	40±5.67Aa	6.22	0.01
Acetone	8.00±4.90Ca	16.00±5.10BCab	26.66±2.72ABa	37.77±2.72Aa	10.34	0.00
Chloroform	4.00±2.45Ca	12.00±2.00BCabc	24.44±1.60ABa	31.11±4.16Aa	13.32	0.00
Toluene	8.00±2.00Ba	12.00±3.74Babc	22.22±4.97ABa	33.33±4.97Aa	7.63	0.00
Hexane	2.00±2.00Aa	2.00±2.00Abc	0±0.00Ab	4.44±4.44Ab	0.48	0.70
Control	0±0.00Aa	0±0.00Ac	0±0.00Ab	0±0.00Ab		
F ratio	1.41	6.49	14.38	18.77		
P value	0.25	0.00	0.00	0.00		

AT = after treatment, HAT = hour after treatment

The original aphid mortality data were corrected by Abbott's formula and then transformed into arcsin √percentage mortality values before ANOVA and Tukey Student Test (HSD).

The upper and lower cases are for along rows and within columns comparison, respectively. Means followed by the same upper case along rows and lower case letters within a column are not significantly different at the 5% level of probability using Tukey Student Test (HSD).

5. DISCUSSION

The present study revealed the ex-situ contact toxicity effect of six different solvent extracts of birbira (*M. ferruginea*) on young adult pea aphids. The consistently recorded higher survival of the aphids in the untreated controls indicated the strength of the test setting used in this study. All the extracts of birbira dissolved in tap water caused significant ($p<0.05$) insecticidal activity against *A. pisum* young adults in 5 hrs time than the untreated control. Nevertheless, the young adult aphids responded differently to the birbira extracts. From different crude extracts of the seed powder, the deionized water extract was the most active causing 98.15% mortality of the tested aphids followed by acetic acid extracted birbira, which caused 88.61% aphid mortality. This is consistent with the findings of Bayeh Mulatu (2007) in that the aqueous extract of birbira was the most toxic against *A. pisum.* Mortality caused due to acetic acid and acetone extracted birbira dissolved in water was not significantly ($p<0.05$) different, whereas, acetic acid brought significantly more death than the chloroform, toluene and hexane. Birbira extract in hexane caused the least mortality records than other extracts though it was not significantly different from the toluene extract.

From the young adult pea aphids, *A. pisum*, treated with different doses of six birbira extracts, mortality was confirmed to be significantly dependent on the rates of application of each extract. Therefore, mortality caused after the correction of the natural death of the aphids in the six extracts is credited to the concentration gradient dependent insecticidal effect of the extracted birbira by the solvents on the young aphids. However, the lethal concentration (LC_{50}) of the extracts that kill 50% of the treated aphids were different for the six birbira extracts. The LC_{50} values were much lower for the birbira extract by deionized water than the rest of the extracts. Among all the extracts, hexane extracted birbira had the highest LC_{50}, but with the least insecticidal efficacy record.

In this study, toxic activity of birbira extracts decreased with decreasing polarity (from deionized water to hexane) of the extracting solvents used. Hence, this result revealed a direct relationship between the effectiveness of the extracts and polarity of the solvents used in the extraction. Previously, Mulla and Su (1999) reported a converse relationship between the effectiveness of

plant extracts and polarity of the solvents used in extraction. Accordingly, the toxic effect of botanical extracts increased with decreasing polarity of the solvents used for extraction. Contrarily, Shaalan *et al*. (2006) reviewed that the inconsistency of this fact due to differences between the characteristics of active compounds among plants and target insect species. Other workers also showed that activity of plant extracts increased with increasing polarity of the extracting solvent. Green (2004) found that serial exhaustive extraction, which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a polar solvent (methanol), ensured that a wide polarity range of phytochemical compounds could be extracted. Bekele Jembere (2002) reported that polar solvents (acetone, ethanol, water) extracts of birbira (*M. ferruginea)* seeds were the most toxic to *Sitophilus zeamais* within 48 hrs after treatment. According to the author, acetone extracted birbira was the most effective among these extracts by causing 100% mortality to the weevil within 24 hrs after treatment. However, Bekele Jembere *et al.* (2006) found that the polar solvent, distilled water extracted birbira was the most effective to sorghum chaffer grub, *Pachnoda interrupta* (Olivier) among the different polar and non-polar organic solvents used for the extractions. Similarly, Bayeh Mulatu (2007) reported that aqueous extract of birbira was the most toxic than its chloroform extract against *A. pisum.* In line with this, the findings of present study indicate that the most polar solvent, water, extracted birbira is found to contain the most toxic components of birbira than non-polar solvent extracts.

It was observed that almost all the doses of deionized water, acetic acid, and chloroform extracted birbira caused statistically similar toxic effects, except at 0.2 mg/ml in which 86.57%, 36.64% and 36.35% mortality were recorded, respectively. The same extracts caused the highest percentage mortality of 99.90% and 97.27% at 1.2 mg/ml and 87.52% at 1.0 mg/ml of water for deionized water, acetic acid and chloroform extracts, respectively. The highest mean percent mortality due to acetone extracted birbira in water was observed at the rate of 1.0 mg/ml which was significantly higher ($p<0.05$) than the rates below 0.8 mg/ml. Toluene extract showed the higher mean percent mortality of aphids at the maximum rate use though rates 1.2, 1.0, 0.8 and 0.4 mg/ml were not significantly ($p<0.05$) different from the highest rate used. Hexane extracted birbira caused 64.64% mortality of aphids at 1.0 mg/ml of water in which it was significantly higher than 0.2 mg/ml of the extract and untreated control. Generally, these results indicated that the extracts caused higher mortality at their higher rates used revealing their rate dependences.

Particularly, the deionized water extract of birbira caused the highest percentage mortality (99.90%) at the rate of 1.2 mg/ml. This is a pointer to the fact that increasing the dose to some level is likely to show some increment in the potency of the extract. From this result, it can be deduced that deionized water extracted birbira is effective at doses greater than 0.4 mg/ml to cause more than 98% mortality on pea aphids when topically applied. The results also indicated that effectiveness of the extracts increased with the rates. Earlier work by Bekele Jembere (2002) revealed that mortality of *Sitophilus zeamais* due to water and acetone extract increased with increasing rates. Bayeh Mulatu and Biruk Wubshet (unpublished data) also showed that water, chloroform and extracts in chloroform extracted birbira at the highest rates of 1.2 and 1.4 mg/ml in water caused the highest larval mortality of diamond back moth, *Plutella xylostella* L., that fed on cabbage leaves treated with these extracts.

The paired-choice aphid settling deterrence activity of birbira based on the LC_{50} of deionized water, acetic acid, acetone, chloroform, toluene and hexane extracts against young adult *A. pisum* was evaluated using excised leaves of field pea bioassay. In the assay, the LC_{50} birbira extracts of deionized water, acetic acid and hexane produced a significant reduction in the number of aphids settled on the treated leaves, though these three extracts did not show significant settling deterrence of aphids at all time of the observations. Similarly, limited settling deterrence activities of the extracts were observed in no-choice settling deterrence test. The test results showed that none of the extracts significantly affected aphids' settlement in all the extracts than the control at 30 min, 1 hr and 2 hrs after aphids' exposure to the treatments. However, at 24 hrs after treatment, significantly fewer aphids settled on acetone, chloroform and toluene extracted birbira treated than the control leaves although the differences among all the extracts were not significant. There were also no significant stepwise reductions in the number of aphids settled on treated leaves over time for each extract whereas some significant stepwise increment in the number of aphids settled on the control leaves occurred over time.

Previous studies on oviposition and feeding deterrence effects of birbira extracts on different species of insect pests have shown its deterrence effect. Bayeh Mulatu and Tadesse Gebremedhin (2000) reported that the oils of birbira extract was among the most effective in partially or completely deterring egg laying, and bean beetle, *Callosobruchus chinensis* L., emergence from

the laid eggs. Similarly, Tebkew Damte and Mekasha Chichaybelu (2002) showed that birbira seed powder gave complete egg laying deterrence of the same beetle for six months in stored chickpea. Bayeh Mulatu and Biruk Wubshet (unpublished data) showed that oviposition deterrence effect of water extracted birbira on diamond back moth, *Plutella xylostella,* was dependent on the concentration of extract. Accordingly, at lower rates of 0.2 and 0.4 mg/ml low egg laying deterrence effect was observed. However, the effect increased with the rates that reached complete deterrence at rates of 1.2 and 1.4 mg/ml. Similarly, Bayeh Mulatu (unpublished data) tested water and chloroform extracts of birbira in water as feeding deterrence against potato tuber moth, *Phthorimaea operculella* (Zeller) and found that the active galleries were higher for the control and lower rate (0.2 mg/ml) than the higher rates. These results showed that phytochemical compounds in the crude extracts of birbira were responsible for the observed deterrence effects. Similarly, the present study also indicates that the presence of deterrent compounds in the extracts that were negatively affected the settlement of aphids on the treated leaves.

The efficiency of the six different solvent extracts of birbira based on their LC_{50} were evaluated against young adults of *A. pisum* in the insectary on intact potted field pea plants. The contact toxicity of the tested birbira extracts to the aphids revealed that all the extracts except hexane extracted one caused significantly ($p<0.05$) higher mortality of aphids within three and four days time after treatments than the untreated control. Nevertheless, the differences among the extracts were not significant. Still the more polar solvents had the leading cumulative mortality records and the trend was maintained in the decreasing order of solvents polarity.

The mortality observed in this study, on intact plants, followed some trend of increase over time after exposure starting from the 24 hrs to 96 hrs in all the extracts except that of hexane extract. Similarly, Gemechis Legesse (2008) reported that mortality of aphids exposed to extract of different parts of *Hagenia abyssinica* followed some trend of increase with time of exposure starting from the 24 hrs to 72 hrs in the experiments. However, compared with birbira, all the leaf and flower aqueous extract and ethanol extract of the sawdust of *H. abyssinica* caused low mortality (less than 33%) to the adult *A. pisum* on field pea plants treated with the extracts. Bayeh Mulatu (2007) showed that water and chloroform extracted birbira effectively suppressed pea

aphid populations on field pea plants in production fields. The assessment of mortality on intact plants has shown clearly that the death of aphids from the extracts increased on the fourth days. This is despite the availability of food source indicating that there are present potent allellochemicals in birbira that may act in a prolonged period after the one time application. This coupled with the observed deterrence effect confirm the importance of birbira extracts as insecticide with multiple actions.

As the results from probit analysis of LC_{50} doses of the extracts shown, the efficiency of the extracts decreased from the most polar solvent, deionized water, to the non-polar solvent, hexane. From this results, it can be deduced that deionized water extracted birbira become more effective than the rest extracts when applied at the higher rates.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study revealed that all the deionized water, acetic acid, acetone, chloroform, toluene and hexane extracted birbira has different degree of pesticidal and some settling deterrence properties against *A. pisum*. All of the six extracts of birbira powder tested were effective to varying degree in their aphid killing potency. The contact toxicity of the extracts of the birbira seed powder observed in ex-situ and on intact plants in this study was generally dependent on the polarity of extracting solvents and concentrations.

Some of the results observed were comparable with what had been reported earlier against aphids and/ other pests. In general, the polar solvent extracts of birbira had better contact toxicity against *A. pisum* than birbira extracts of the non-polar solvents. Therefore, based on the results obtained, it can be concluded that birbira seed powder contains toxic compounds, which are natural insecticides that act by contact coupled with deterrence effect against *A. pisum*. Thus, the use of aqueous crude extract of birbira seed powder needs to be pushed further for practical use by small-scale farmers because of its inexpensive and effective, and easy adaptability.

6. 2 Recommendations

- The use of aqueous crude extract of birbira seed powder needs to be pushed further for practical use by small-scale farmers because of its inexpensive and effective, and easy adaptability. Moreover, as an application solvent water was effective in dissolving potent allelochemicals in the extract.
- Furthermore, studies should be conducted to identify effective formulation mechanisms of the active ingredient of the plant material.
- Studies on formulation stability under tropical and subtropical conditions need to be undertaken.
- Further studies on possibility to combine formulated birbira extracts with other pest management tactics should also be considered.

7. REFERENCES

Almohamad, R., Verheggen, F. J., Francis, F. and Haubruge, E. (2007). Predatory hoverflies select their oviposition site according to aphid host plant and aphid species. *Entomol. Exp. Appl*. **125**: 13–21.

Anyim, A. (2003). Effects of insecticidal treatment on the yield and control of the major pest of soybeans *Glycine max* (L. Mevill) in south Eastern Nigeria. *Int. J. Agric. Rural Dev*. **4**:100-109.

Asfaw Telaye, Geletu Bejiga and Alem Berhe (1994). Role of cool-season food legumes and their production constraints in Ethiopia. **In**: *Cool–season food legumes of Ethiopia*, pp.3–18, (Asfaw Tilaye, Geletu Bejiga, Saxena, M. C. and Solh, M. B., eds). `Proceedings of the first National cool-season food legumes review conference, 16-20 December 1993; Addis Ababa, Ethiopia. ICARDA: Aleppo, Syria.

Azene Bekele (2007). *Usesful trees and shrubs for Ethiopia: identification, propagation and management for 17 agroclimatic zones.* Nairobi: RELMA in ICRAF Project, Nairobi. Pp. 552.

Azene Bekele, Bavnie, A. and Tengnas, B. (1993). *Usesful Trees and Shrubs for Ethiopia: Identification, propagation, management for agricultural and pastoral communities.* Regional Soil Conservation Unit, Swedish International Development Authority (SIDA), English Press, Nairobi. Pp. 356.

Ba-Angood, S.A. and Stewart, R.K. (1980). Effect of cereal aphid infestation on grain yield and percentage protein of barley, wheat, and oats in southwestern Quebec. *Can. Entomol.* **112**: 681–686.

Bale, J.S., Ponder, K.L. and Pritchard, J. (2007). Coping with Stress. **In**: *Aphids as Crop Pests*, pp. 287 - 310, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Banouzi, J.T., Prost, A., Rajemiarimiraho, M. and Ongoka, P. (2008). Traditional uses of the African *Millettia* species (Fabaceae). *Int. J. Bot*. **4**: 406-420.

Bayeh Mulatu (1997). Localization of resistance factors in leaf tissue profile of selected barley lines against the Russian Wheat Aphid (*Diuraphis noxia* Mordwilko; Homoptera: Aphididae). M.Sc. Thesis. Wageningen Agricultural University. Pp. 57.

Bayeh Mulatu (2007). Contact bioassay of an endemic plant to Ethiopia on three aphid species. *Ethiop. J. Biol. Sci.* **6**: 51-62.

Bayeh Mulatu (unpublished data). Birbira, *Millettia ferruginea* (Hochst.) Baker rude seed extracts affect oviposition and feeding by the potato tuber moth, *Phthorimaea operculella* (Zeller).

Bayeh Mulatu and Biruk Wubshet (unpublished data). Effects of birbira seed kernel powder aqueous and organic crude extracts on feeding and oviposition responses of Diamondback moth.

Bayeh Mulatu and Tadesse Gebremedhin (2000). Oviposition-deterrent and toxic effects of various botanicals on the Adzuki bean beetle, *Callosobruchus chinensis* L. *Insect Sci. Applic.* **20**:33-38.

Beck, S.D. (1965). Resistance of plants to insects. *Annu. Rev. Entomol.* **10**:207-232.

Bekele Jembere (2002). Evaluation of the toxicity potential of *Millettia ferrginea* (Hochst) Baker against *Sitophillus zeamais* (Motsch.). *Int. J. Pest Manage*. **48**: 29-32.

Bekele Jembere, Daniel Getahun, Merid Negash and Emiru Seyoum (2006).Toxicity of Birbira (*Millettia ferruginea*) seed crude extracts to some insect pests as compared to other botanical and synthetic insecticides. 11th NAPRECA Symposium Book of Proceedings, Antananarivo. Pp. 88-96.

Bekele Jembere, Namukobe, J., Kiremire, B.T. and Ermias Dange (2007). Extracts of *Millettia ferruginea*, *Tephrosia vogelli* and *Tephrosia pentaphylla* against the bean bruchid, *Zabrotes subfaciatus. Sinet: Ethiop. J. Sci.* **30**: 49-54.

Bernays, E.A. and Funk, D. (1999). Specialists make faster decisions than generalists: experiments with aphids. *Proc. Royal Soc. Lond. Series B* **266**: 151–156.

Bernays, E.A. and Funk, D. (2000). Electrical penetration graph analysis reveals population differentiation of host-plant probing behaviors within the aphid species *Uroleucon ambrosiae*. *Entomol. Exp. Appl.* **97**: 183–191.

Blackman, R.L. and Eastop, V.F. (2000). *Aphids on the World's Crops*: *An Identification and Information Guide*, 2nd edn. John Wiley and Sons, Chichester. pp.466.

Blackman, R.L. and Eastop, V.F. (2006). *Aphids on the World's Herbaceous Plants and Shrubs*. John Wiley and Sons, Chichester, pp.1439.

Blackman, R.L. and Eastop, V.F. (2007). Taxonomic issues. **In**: *Aphids as Crop Pests*, pp. 1-29, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Braendle, C., Caillaud, M.C. and Stern, D.L. (2005). Genetic mapping of aphicarus - a sex-linked locus controlling a wing polymorphism in the pea aphid (*Acyrthosiphon pisum*). *Heredity* **94**: 435-42.

Brewer, M.J. and Elliott, N.C. (2004). Biological control of cereal aphids in North America and mediating effects of host plant and habitat manipulations. *Annu. Rev. Entomol.* **49**: 219–242.

Brisson, J. A. and Stern, D. L. (2006). The pea aphid, *Acyrthosiphon pisum*: an emerging genomic model system for ecological, developmental and evolutionary studies. *BioEssays* **28**: 747–755.

Caillaud, C.M. (1999). Behavioral correlates of genetic divergence due to host specialization in the pea aphid, *Acyrthosiphon pisum*. *Entomol. Exp. Appl.* **91**: 227–232.

Caillaud, C.M. and Via, S. (2000). Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphid. *Am. Naturalist* **156**:606–621.

Caillaud, C.M., Boutin, M., Braendle, C. and Simon, J.C. (2002). A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrthosiphon pisum* (Harris). *Heredity* **89**: 346-352.

Casida, J.E. and Quistad, G.B. (1998). Golden age of insecticide research: past, present, or future? *Annu. Rev. Entomol.* **43**: 1–16.

Chapman, R.F., Bernays, E.A. and Simpson, S.J. (1981). Attraction and repulsion of the aphid *Cavariella aegopodii*, by plant odors. *J. Chem. Ecol.* **7**: 881–888.

Coats, J.R. (1994). Risks from naturalversus synthetic insecticides. *Annu. Rev. Entomol.* **39**: 489-515.

CSA (2002). The 2001/2002 Ethiopian agricultural sample enumeration, preliminary results, Part I. Results of country level, statistical report on area and production of pulse crops. Central Statistics Authority (CSA). Addis Ababa, Ethiopia.

CSA (2008). *Agricultural sample survey 2007/08: Report on area and production of crops (private peasant holdings, Meher season)*, vol. I. Central Statistical Agency (CSA), Addis Ababa. Pp. 133.

Das, K., Tiwari, R. K. S. and Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *J. Med. Plant Res.* **4**: 104-111.

DelCampo, M.L., Via, S. and Caillaud, M.C. (2003). Recognition of host-specific chemical stimulants in two sympatric host races of the pea aphid *Acyrthosiphon pisum*. *Ecol. Entomol*. **28**: 405–412.

Dewar, A.M. (2007). Chemical Control. **In**: *Aphids as Crop Pests,* pp. 391-422, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Dixon, A.F.G. (1977). Aphid ecology: life cycles, polymorphism, and population regulation. *Ann. Rev. Ecol. Syst*. **8**: 329-53.

Dixon, A.F.G. (1985a). *Aphid Ecology*. Blackie, Glasgow, 157 pp.

Dixon, A.F.G. (1985b). Structure of aphid populations. *Ann. Rev. Entomol.* **30**: 155-74.

Dixon, F.A.G. (1987). The way of life of Aphids: Host specificity, speciation, and distribution. **In**: *Aphids: Their Biology, Natural Enemies and Control*, Vol. 2A, pp.197-608, (Minks, A.K. and Harrewijn, P., eds.). Elsevier, Amsterdam.

Dubey, N.K., Srivastava, B. and Kumar, A. (2008). Current status of plant products as botanical pesticides in storage pest management. *J. Biopest.* **1**: 182-186.

Ehler, L.E. (2006). Integrated pest management (IPM): Definition, historical development and implementation, and the other IPM. *Pest Manag. Sci.* **62**: 787-789.

Elhag, E.A. (2000). Deterrent effects of some botanical products on oviposition of the cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Int. J. Pest Manage.* **46**: 109-113.

Ermias Dagne and Amha Bekele (1990). *C*-prenylated isoflavones from *Millettia. Phytochemistry* **29**: 2679-2682.

Ermias Dagne, Amha Bekele, Noguchi, H., Shibuya, M. and Sankawa, U. (1990). *O*-Geranylated and *O*-prenylated flavonoids from *Millettia ferruginea*. *Phytochemistry* **29**: 2671-2673.

Fiebig, G.M. and Poehling, H.M. (1998). Host-plant selection and population dynamics of the grain aphid Sitobion avenae (F.) on wheat infected with Barley Yellow Dwarf Virus. *Bull. IOBC/WPRS* **21**: 51–62.

Fisseha Mesfin, Sebsebe Demissew and Tilahun Teklehaymanot (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J. Ethnobiol. Ethnomed.* **5(**28).

Fox, P.M., Pass, B.C. and Thurston, R. (1967). Laboratory studies on the rearing of *Aphidius smithi* (Hymenoptera: Braconidae) and its parasitism of *Acyrthosiphon pisum* (Homoptera: Aphididae). *Ann. Entomol. Soc. Amer.* **60**: 1083-1087.

Funk, D. and Bernays, E.A. (2001). Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae*aphids. *Ecology* **82**: 726–739.

Gabrys, B. and Tjallingii, W.F. (2002). The role of sinigrin in host plant recognition by aphids during initial plant penetration. *Entomol. Exp. Appl.* **104**:89-93.

Gemechis Legesse (2008). Bioactivities of extracts of H*agenia abyssinica* against A*canthoscelides obtectus* (Bruchidae) and A*cyrthosiphon pisum* (Aphididae). M.Sc. Thesis submitted to graduate studies. Addis Ababa University, Addis Ababa University. Pp. 73.

Gomez, K.A. and Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research, 2nd edn.* John Wiley and Sons, Inc, New York. pp. 680.

Green, R.J. (2004). Antioxidant Activity of Peanut Plant Tissues. Masters Thesis. North Carolina State University, Raleigh. Pp. 81.

Hailu Beyene, Workneh Negatu and Shelemew W/mariam (1994). Small-holder production practices and constraints in Ethiopia. **In**: *Cool–season food legumes of Ethiopia*, pp.19–30, (Asfaw Tilaye, Geletu Bejiga, Saxena, M. C. and Solh, M. B., eds). `Proceedings of the first National cool-season food legumes review conference, 16-20 December 1993; Addis Ababa, Ethiopia. ICARDA: Aleppo, Syria.

Hassan, S. (2009). Effect of variety and intercropping on two major cowpea [*Vigna unguiculata* (L.) Walp] field pests in Mubi, Adamawa State, Nigeria. *J. Hortic. For.* **1**: 014-016.

Hazell, S. P., Gwynn, D. M., Ceccarelli, S. and Fellowes, M. D. E. (2005). Competition and dispersal in the pea aphid: clonal variation and correlations across traits. *Ecol. Entomol.* **30**: 293–298.

Hill, D. (1993). *Agricultural insect pests of the tropics and their control*. Second edition. Cambridge University press, Cambridge. pp. 746.

Hill, D.S. and Waller, J.M. (1990). *Pests and Diseases of Tropical Crops*. Field Handbook Longman Scientist and Technical, Vol. 2, UK. Pp. 432.

Hodgson, C. (1991). Dispersal of apterous aphids (Homoptera, Aphididae) from their host plant and its Significance. *Bulletin Entomol. Res.* **81**: 417–427.

Hoekstar, J.A. (1987). Acute bioassays with control mortality. *Water Air Soil Pollution* **35**:311-317.

Horton, D. (1995). Statistical consideration in the design and analysis of paired-choice assays. *Environ. Entomol.* **24**: 179-192.

Hunter, W. B. and Ullman, D. E. (1992). Effects of the neem product, RD-Repelin, on settling behaviour and transmission of zucchini yellow mosaic virus by the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Ann. appl. Biol.* **120**: 9- l 5.

Hutchison, W.D. and Hogg, D.B. (1985). Time-specific life tables for the pea aphid, *Acyrthosiphon pisum* (Harris), on alfalfa. *Res. Popul. Ecol*. **27**: 231-253.

Isman, M. B. (2008). Botanical insecticides: for richer, for poorer. *Pest Manage*. *Sci*. **64**: 8– 11.

Isman, M.B. (1994). Botanical insecticides. *Pesticides Outlook* **5**: 26-31.

Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **51**: 45–66.

Isman, M.B. (2007). Botanical insecticides: for richer, for poorer. *Pest Manag. Sci.* **64**: 8-11.

Katis, N.I., Tsitsipis, J.A., Stevens, M. and Powell. G. (2007). Transmission of Plant Viruses. **In**: *Aphids as Crop Pests,* pp. 353 - 390, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Kemal Ali (1997). Economic threshold for pea aphid. *Pest Mgt. J. Eth*. **1**: 63–68.

Kemal Ali (1999). Population dynamics of *Acyrthosiphon pisum* (Homopter:Aphididae)and its natural enemies on *Pisum sativum* (L.). *Pest Mgt. J. Eth*. **3**: 23-29.

Kemal Ali (2002). An integrated approach to pest management in field pea, *Pisum sativum* (L), with emphasis on pea aphid, *Acyrthosiphon pisum* (Harris). PhD Thesis. University of the Free State, Bloemfontein, South Africa.

Kemal Ali (2006). Insect-pest management research for faba bean and field pea in Ethiopia. **In**: *Food and forage legumes of Ethiopia*: *progress report and prospects*, pp. 247–259, (Kemal Ali, Gemechu Kenneni, Seid Ahmed, Malhotra, R. S., Makkouk, K. and Halila, M. H., eds). Proceedings of the workshop on food and forage legume, 22-26 September 2003, Addis Ababa. ICARDA, Alepo, Syria.

Kemal Ali and Tibebu Habtewold (1994). Research on insect pests of cool-season food legumes. **In**: *Cool–season food legumes of Ethiopia*, pp. 369–395, (Asfaw Tilaye, Geletu Bejiga, Saxena, M. C. and Solh, M. B., eds). Proceedings of the first National cool-season food legumes review conference, 16-20 December 1993; Addis Ababa, Ethiopia. ICARDA: Aleppo, Syria.

Kemal Ali and Louw, S. vdM. (20005). Effects of neem formulation on pea aphid [*Acyrthosiphon pisum* (Harris)] development and reproduction on field pea in Ethiopia. *Ethiop. J. Agric. Sci.* **18**: 73-87.

Kemal Ali Louw, S.M. and Swart, W.J. (2005). Components and mechanisms of resistance in selected field pea *Pisum sativum* lines to the aphid *Acyrthosiphon pisum* (Homoptera: Aphididae). *Int. J. Trop. Insect Sci.* **25**: 114-121.

Kennedy, J. S. and Stroyan, H. L. G. (1959). Biology of aphids. *Annu. Rev. Entomol.* **4**: 139-160.

Kim, S., Roh, J.Y., Kim, D.H.,Lee, H.S. and Ahn, Y.J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryze and Callasobruchus chinensis. J. Stored Pro. Res.* **39**: 293-303.

Kindlmann, P., Jarosik, V. and Dixon, A.F.G. (2007). Population Dynamics. **In**: *Aphids as Crop Pests*, pp. 311 - 330, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Klingauf, F.A. (1987). Feeding, adaptation and excretion. **In**: *Aphids: Their Biology, Natural Enemies and Control*, Vol. 2A, pp. 225-253, (Minks, A.K. and Harrewijn, P., eds). Elsevier, Amsterdam.

Koehler, C.S. (1989). Prospects of implementation of IPM programs from ornamental plants. *Florida Entomol.* **72**: 191-194.

Kyamanywa, S., Balidawa, C.W. and Ampofo, K.J.O (1993). Effect of maize plant on colonization of cowpea plant by bean flower thrips, *Megalurothrips sjostedti*. *Entomol. Exp. Appl.* **69**: 61-68.

Lane, A. and Walters, K.F.A. (1991). Effect of pea aphid (*Acyrthosiphon pisum*) on the yield of combining peas. *Aspects of Appl. Biol.* **27**: 363-368.

Legesse Negash (1995). *Indigenous trees of Ethiopia: biology, uses and propagation techniques.* SLU Reprocentralen, Umea, Sweden. Pp. 285.

Melaku Wale (2004). Relative efficacy of some botanicals, detergent and kerosene in controlling the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) on grass pea Lathyrus sativus. *Int. J. Tropical Insect Sci.* **24**:143-149.

Melaku Wale, Bekele Jembere and Emiru Seyoum (2000). Biology of the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) on cool season food legumes. *Ins. Sci. Appl*. **20**: 171–180.

Melaku Wale, Bekele Jembere and Emiru Seyoum (2003). Effect of density on reproduction and survival of pea aphid on cool season food legumes. *Pest Mgt. Eth.* **7**: 63-69.

Miura, T., Braendle, C., Shingleton, A., Sisk, G., Kamblampati, S. and Stern, D. L. (2003). A comparison of parthenogenetic and sexual embryogenesis of the pea aphid *Ayrthosiphon pisum* (Hemiptera: Aphidoidea). *J. Exp. Zool. (Mol.Dev. Evol.)* **295**: 59–81.

Miyazaki, M. (1987). Morphology of aphids. **In**: *Aphids their biology, natural enemies and control. Volume A*, pp. 1–27, (Minks, A. K. and Harrewijn, P., eds). Elsevier, Amsterdam.

Mondor, E.B. and Roitberg, B.D. (2002). Pea aphid, *Acyrthosiphon pisum*, cornicle ontogeny as an adaptation to differential predation risk. *Can. J. Zool.* **80**: 2131–2136.

Moran, N.A. (1992). The evolution of aphid life cycles. *Annu. Rev. Entomol.* **37**: 321-48.

Mulla, M.S. and Su, T. (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. *J. Am. Mosq. Control Assoc.* **15**:133-152.

Mulugeta Negeri, Adane Kassa, Seneshaw Aysheshim and Fantahun Mengistu (1999). The role of predatory insects in regulating the population of wheat aphid at Ambo. *Pest Mgt. J. Eth.* **3**: 69-76.

Nampala, P., Ogenga-Latigo, M.W., Kyamanywa, S., Adipala. E., Oyobo, N.and Jackai, L.E.N. (2002). Potential Impact of Intercropping on Major Cowpea Field Pests in Uganda. *Afr. Crop Sci. J.* **10**: 335-344.

Niemeyer, H.M. (1990). Secondary plant chemicals in aphid-host interactions. **In**: *Aphid-Plant Genotype Interactions*, pp. 187-205, (Campbell, R.K. and Eikenbary, R.D., eds). Elsevier, Amsterdam.

Nottingham, S.F. and Hardie, J. (1993). Flight behavior of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and nonhost plant odor. *Physiol. Entomol.* **18**: 389–394.

Ogenga-Latigo, M.W., Ampofo, J.K.O. and Balidawa, C.W. (1992). Influence of maize row spacing on infestation and damage of intercropped beans by beans aphids (*Aphis fabae* Scop.) 1. Incidence of Aphids. *Field Crop Res.* **30**: 111-121.

Ogenga-Latigo, M.W., Balidawa, C.W. and Ampofo, J.K.O. (1993). Factors influencing the incidence of black bean aphid, *Aphis fabAae* Scop., on common beans intercropped with maize. *Afr. Crop Sci. J.* **1**: 49-58.

Ogunleye, R.F. (2001). Comparative effectiveness of Gamalin 20, Audrex 40 and Vetox 85 in controlling *Eurema hacabe* (L.) a defoliator of *Parkia biglobossa*. *J. Techno. Sci.* **5**:77-80.

Ogunleye, R.F. (2006). Comparative effectiveness of Cypermethrin and *Zanthoythum zanthoxyloides* (LAM) for the control of the cowpea foliage pest, *Ootheca mutabilis* (Salbergy). *J. Ultra Sci. Phys. Sci.* **18**:9-14.

Pickett J.A., Wadhams L.J., Woodcock C.M. and Hardie J. (1992). The chemical ecology of aphids. *Annu. Rev. Entomol.* **37**: 67-90.

Podjasek, J. O., Bosnjak, L. M., Booker, D. J. and Mondor, E. B. (2005). Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*. *Can. J. Zool*. **83**: 1138–1141.

Powell, G. and Hardie, J. (2000). Host-selection behaviour by genetically identical aphids with different plant preferences. *Physiol. Entomol.* **25**:54–62.

Powell, G., Maniar, S.P., Pickett, J.A. and Hardie, J. (1999). Aphid responses to non-host epicuticular lipids. *Entomol. Exp. Appl.* **91**:115–123.

Powell, G., Tosh, C.R. and Hardie, J. (2006). Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.* **51**: 309–330.

Powell, W. and Pickett, J.A. (2003). Manipulation of parasitoids for aphid management: progress and prospect. *Pest Manag. Sci.* **59**: 149-155.

Price, P.W. (1997). *Insect Ecology.* John Wiley and Sons, New York. pp. 874.

Quarles, W. (2007). Managing aphids with pheromones. *IPM Practitioner* **109**: 1–10.

Quisenberry, S.S. and Ni, X. (2007). Feeding Injury. **In**: *Aphids as Crop Pests,* pp. 331 - 352, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Rahman, A. and Talukder, F.A. (2006). Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculates.* 10pp. *J. Insect Sci.* **6(**03).

Rakhshani, E., Tomanovi, Z., Stary, P., Talebi, A-A., Kavallieratos, N.G., Zamani, A-A. and Stamenkovi, S. (2008). Distribution and diversity of wheat aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) in Iran. *Eur. J. Entomol.* **105**: 863–870.

Rangaswamy, R. (1995). *A Text Book of Agricultural Statistics.* Wiley Eastern, Ltd., New Delhi. pp. 496.

SAS Institute Inc. (2002). SAS system for windows version 9.0. Cary, NC, USA.

Saxena, K.N., Pala-Okeyo, A., Seshu-Reddy, K.V., Omolo, E.O. and Ngode. L. (1990). Insect Pest Management and Socio-economic circumstances of small-scale farmers for food crop production in western Kenya: A Case Study. *Insect Sci. Appl.* **10**: 443-462.

Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M. and Tscharntke, T. (2003). Relative importance of predators and parasitoids for cereal aphid control. *Proc. R. Soc. Lond. (B) Biol. Sci.* **270**: 1905–1909.

Senayit Yetneberk and Asrat Wondimu (1994). Utilization of cool-season food legumes in Ethiopia. **In**: *Cool–season food legumes of Ethiopia*, pp.40-50, (Asfaw Tilaye, Geletu Bejiga, Saxena, M. C. and Solh, M. B., eds). `Proceedings of the first National cool-season food legumes review conference, 16-20 December 1993; Addis Ababa, Ethiopia. ICARDA: Aleppo, Syria.

Shaalan, E.A-S., Canyon, D., Younes, M.W.F., Abdel-Wahab, H. and Mansour, A-H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment International* **31**: 1149-1166.

Shaalan, E.A-S., Canyon, D.V., Younes, M. W. F., Abdel-Wahab, H. and Mansour, A-H. (2006). Efficacy of eight larvicidal botanical extracts from *Khaya Senegalensis* and *Daucus carota* against *Culex annulirostris. J. Am. Mosq. Control Assoc.* **22**: 433-436.

Sloggett, J. J. and Weisser, W. (2002). Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrtosiphon pisum*. *Oikos* **98**: 323–333.

SPSS Institute (2004). SPSS 13 for windows. Chicago, USA.

Sullivan, D.J. (2008). Aphids (Hemiptera: Aphididae). **In**: *Encyclopedia of Entomology*, 2nd edn. pp. 191-215, (Capinera, J.L., ed). Springer, Florida.

Sutherland, O.R.W. (1969). The role of crowding in the production of winged forms by two strains of the pea aphid, *Acrythosiphon pisum*. *J. Insect Physiol*. **15**: 1385–1410.

Sylvester, E. S. (1980). Circulative and propagative virus transmission by aphids. *Ann. Rev. Entomol.* **25**: 257-86.

Talukder, F.A. and Howse, P.E. (2000). Isolation of secondary compounds from aphanamidis polystachygas as feeding deterrents against adult *Tribolium castaneum* (Coleoptera: Tenebroidae). *J. Plant Dis. Prot.*, **107**:498-504.

Tebkew Damte and Mekassha Chichaybelu (2002). The efficacy of some botanicals in controlling Adzuki bean beetle, *Callosobruchus chinensis* in stored chickpea. *Trop. Sci.* **42**: 192-195.

Tesema Tanto and Eshetayehu Tefera (2006). Collection, conservation and sustainable utilization of grain legumes in Ethiopia. **In**: *Food and forage legumes of Ethiopia*: *progress report and prospects*, pp. 15–22, (Kemal Ali, Gemechu Kenneni, Seid Ahmed, Malhotra, R. S., Makkouk, K. and Halila, M. H., eds). Proceedings of the workshop on food and forage legume, 22-26 September 2003, Addis Ababa. ICARDA, Alepo, Syria.

Tilahun Teklehaymanot and Mirutse Giday (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *J. Ethnobiol. Ethnomed.* **3**(12).

Tosh, C.R., Powell. G. and Hardie, J. (2003). Decision making by generalist and specialist aphids with the same genotype. *J. Insect Physiol.* **49**: 659–669.

Tsedeke Abate (1997). Pesticides in Ethiopian agriculture: A researcher's view. *Pest Mgt. J. Eth.* **1**: 49-55.

Tsedeke Abate, Tadesse Gebremedhin and Kemal Ali (1982). *Arthropod pests of grain legumes in Ethiopia: their importance and distribution.* IAR, Addis Ababa.

van Emden, H.F. (2007a). Host-plant Resistance. **In**: *Aphids as Crop Pests*, pp. 447 - 468, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

van Emden, H.F. (2007b). Integrated Pest Management and Introduction to IPM Case Studies. **In**: *Aphids as Crop Pests*, pp. 537 - 548, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Vargas, R.R., Troncoso, A.J., Tapia, D.H., Olivares-Donoso, R. and Niemeyer, H.M. (2005). Behavioural differences during host selection between alate virginoparae of generalist and tobacco-specialist *Myzus persicae. Entomol. Exp. Appl.* **116**: 43–53.

Volkl, W., Mackauer, M., Pell, J.K. and Brodeur, J. (2007). Predators, Parasitoids and Pathogens. **In**: *Aphids as Crop Pests*, pp. 187-234, (van Emden, H.F.; Harrington, R., eds). CAB International, Wallingford.

Weber, K. A., and Hampton, R. 0. (1980). Transmission of two purified carlaviruses by the pea aphid. *Phytopathology* **70**:631-633.

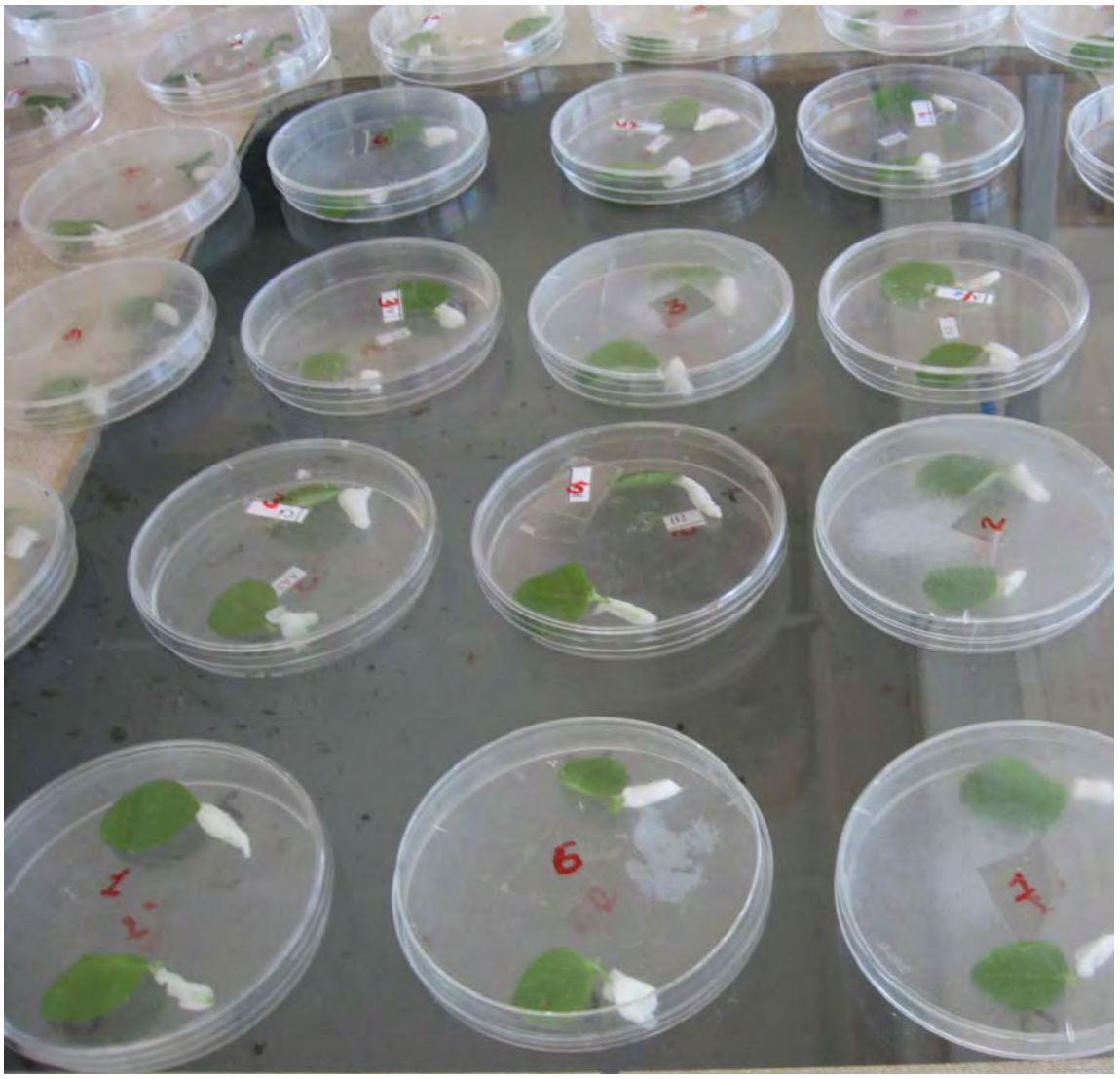
Weisser, W. W., Braendle, C. and Minoretti, N. (1999). Predator-induced morphological shift in the pea aphid. *Proc. Biol. Sci.* **266**: 1175–1181.

Wolfson, J.L., Shade, R.R., Mentze, P.E. and Murdock, L.C. (1991). Efficacy of ash for controlling infestations of *Callosobruchus maculatus*. *J. Stored Prod. Res.* **27**:239-242.

Yankep, E., Fomum, Z. T. and Ermias Dagne Dagne (1997). An *O*-geranylated isoflavone from *Millettia griffoniana*. *Phytochemistry* **46**: 591-593.

ANNEXES

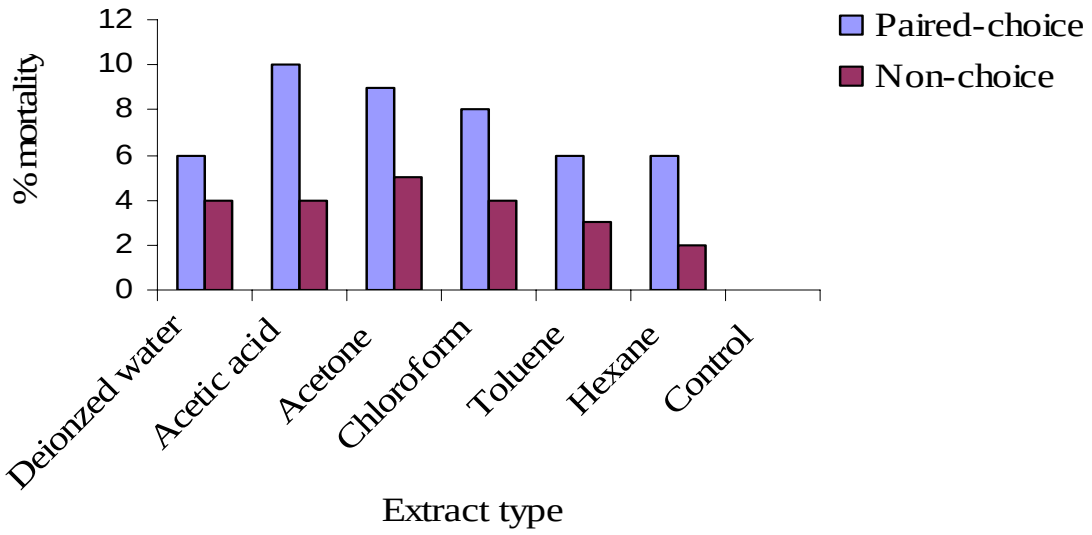
Annex 1: Partial steps in birbira preparation and extractions



Annex 2: Experimental setup in insectary to study the settling deterrence effect of the LC_{50} of birbira extracts on *A. pisum* in paired-choice test

Annex 3: Experimental setup in insectary to study the settling deterrence effect of the LC_{50} of birbira extracts on *A. pisum* in no-choice test

Annex 4: Experimental setup in insectary to study the effect of the LC_{50} birbira extracts treatment on *A. pisum* while on intact plants



Annex 5: Percent mortality of aphids in paired-choice and no-choice tests 24 hrs after treatment

Source of variation	DF	Sum of Squares	Mean Squares	F ratio	P > F
Block	5	6645.08	1329.01	5.67	0.0001
Treatment	5	49075.89	9815.18	41.9	0.0001
Dose	6	23302.14	3883.67	16.58	0.0001
Treatment*Dose	30	9257.65	308.59	1.32	0.1358
Error	210	49191.76	234.25		

Annex 6: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of different solvent extracts of birbira

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	36212.23	5173.18	56.76	0.0001
Block	5	1853.74	370.75	4.07	0.0051
Error	35	3189.70	91.13		
Total	47	41255.67			

Annex 7: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of deionized water extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	33791.25	4827.32	29.48	0.0001
Block	5	3517.55	703.51	4.30	0.0038
Error	35	5731.01	165.74		
Total	47	43039.81			

Annex 8: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of acetic acid extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	27918.46	3988.35	26.25	0.0001
Block	5	5488.12	1097.62	7.22	0.0001
Error	35	5318.27	151.95		
Total	47	38724.85			

Annex 9: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of acetone extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	20760.18	2965.74	15.22	0.0001
Block	5	211.15	42.23	0.22	0.9531
Error	35	6822.12	194.92		
Total	47	27793.46			

Annex 10: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of chloroform extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	17383.91	2483.42	10.93	0.0001
Block	5	3221.61	644.32	2.84	0.0298
Error	35	7954.01	227.25		
Total	47	28559.53			

Annex 11: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of toluene extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	7	13096.65	1870.95	6.74	0.0001
Block	5	2807.95	561.59	2.02	0.0996
Error	35	9721.59	277.76		
Total	47	25626.20			

Annex 12: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults due to topical application of hexane extract of birbira at different doses

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6	59.34	9.89	1.96	0.0855
Error	63	318.60	5.06		
Total	69	377.94			

Annex 13: Summary table for analysis of variance (ANOVA) for settling deterrence activity of different solvent extracts of birbira against pea aphid, *A. pisum*, at 30 min after treatments (no-choice test)

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6	24.74	4.12	0.91	0.4919
Error	63	284.70	4.52		
Total	69	309.44			

Annex 14: Summary table for analysis of variance (ANOVA) for settling deterrence activity of different solvent extracts of birbira against pea aphid, *A. pisum*, at 1 hr after treatments (no-choice test)

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6	14.57	2.43	0.87	0.5236
Error	63	176.30	2.80		
Total	69	190.87			

Annex 15: Summary table for analysis of variance (ANOVA) for settling deterrence activity of different solvent extracts of birbira against pea aphid, *A. pisum*, at 2 hrs after treatments (no-choice test)

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6	73.80	12.30	4.00	0.0019
Error	63	193.70	3.07		
Total	69	267.50			

Annex 16: Summary table for analysis of variance (ANOVA) for settling deterrence activity of different solvent extracts of birbira against pea aphid, *A. pisum*, at 24 hrs after treatments (no-choice test)

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6.00	925.29	154.21	1.33	0.2788
Error	28.00	3257.31	116.33		
Total	34.00	4182.60			

Annex 17: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults 24 hrs after sprayed with LC_{50} of extracts birbira of while the aphids were on intact plants

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6.00	3246.36	541.06	7.07	0.0001
Error	28.00	2142.31	76.51		
Total	34.00	5388.67			

Annex 18: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults 48 hrs after sprayed with LC_{50} of extracts birbira of while the aphids were on intact plants

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6.00	6497.24	1082.87	37.46	0.0000
Error	28.00	809.39	28.91		
Total	34.00	7306.63			

Annex 19: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults 72 hrs after sprayed with LC_{50} of extracts birbira of while the aphids were on intact plants

Source of variation	df	Sum of Squares	Mean Square	F ratio	P > F
Treatment	6.00	8739.259	1456.543	18.77061	0.0000
Error	28.00	2172.716	77.597		
Total	34.00	10911.98			

Annex 20: Summary table for analysis of variance (ANOVA) for mean percent mortality of *A. pisum* young adults 96 hrs after sprayed with LC_{50} of extracts birbira of while the aphids were on intact plants