ADDIS ABABA UNIVERSITY SCIENCE FACULTY GRADUATE STUDIES PROGRAM DEPARTMENT OF BIOLOGY

Oviposition, ovicidal and feeding responses of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) to Birbira, *Millettia ferruginea* (Hochst) Baker (Legumionosae: papilionideae) seed powder extracts.

By

Ayalew Tadesse

A thesis submitted to the school of Graduate studies of Addis Ababa University in Partial fulfillment of the requirement for the Degree of Master of Science in Biology.

June, 2010

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Abstract

The use of native plant species, Millettia ferruginea (Hochst) Baker known locally as birbira, its deterrent/toxicity was assayed in relation to oviposition, ovicidal and feeding (larval settlement) responses of PTM. M. ferruginea seed powder extracts obtained using solvents of varying polarities (water, acetic acid, acetone, chloroform, toluene and hexane) at eight serial diluents (w/v: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4mg birbira seed powder/ml of water) were prepared. The PTM eggs for ovicidal and the host plant parts: potato tubers for oviposition, larvae feeding and adult emergence, leaves for oviposition and neonate larvae settlement (settled, deterred and died) responses were treated with seed powder extracts of birbira at each rate of application. From the five replicates, observation were taken on the number of eggs laid, active and terminated galleries, settled, deterred and died neonate larvae, emerged healthy and deformed adults and number of eggs hatched and unhatched. Finally, the mean number of eggs laid on treated and away treated substrates, active and terminated galleries, mean number of successfully settled, rejected and died neonate larvae and % of egg hatchability was analyzed. The LC_{50} values of birbira seed powder extracts for PTM eggs and neonates were also determined, too. Results revealed that the number of eggs laid on tubers and leaves, the number of active and terminated galleries carved by PTM larvae, percentage of unhatched eggs, were inversely proportional to the increase in rates of the extracts, whereas the number of eggs laid on cages and chambers inner surfaces increase with the increase in the rate of application of the extracts. Adult emergence did not show clear pattern as was the case for most of the cases with the larvae and extracts has no impact in deformity of adult except in acetic acid and chloroform. All birbira seed powder extracts applied on host plant leaf inhibit settling of PTM neonate larvae and its lethal effect to neonates increase with increased rates of application and hours of exposure. The results in this study confirmed that M. ferruginea is a potential natural insecticide of many insect pests.

1. INTRODUCTION

Potato *Solanum tuberosum* (L.) has its region of origin in the high plains of the Andes cordillera where Incas cultivated the plant largely for food (Rolot, 2001)**.** Currently potato is one of the world's prime sources of nutrition. Thus, potato is an important target crop for controlling the tuber moth.

Sporleder (2007) described that the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), (Lepidoptera: Gelechiidae), which probably originated in tropical mountainous regions of South America, has become a cosmopolitan pest of potatoes and other Solanaceous crops (Chumakov and Kuznetsova, 2009). Moawad and Ebadah (2007) and many other authors reported that PTM causes serious damage to stored potato through its larval tunneling and feeding which lead to partial or complete rotting by subsequent infestation by fungi and / or bacteria. This highly invasive pest causes significant crop losses in almost all tropical and sub-tropical potato production systems in Africa, Asia and Central and South America (Lal, 1987; Rolot, 2001; Horgan *et al.*, 2007). Even though, potato crop in most potato growing areas of Ethiopia is attacked by a number of insect pests, the major one is Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Bayeh and Tadesse 1992; Emana and Amanuel, 1992; Bayeh *et al.*, 2008). The only insect that can do significant damage to stored potato tuber is the PTM in South Africa (Visser, 2004) and the major pest problems in Andean potato cultivation are caused by the potato tuber moth (PTM), resulting in serious crop losses (Ortiz, 1999).

An enormous cost is necessary to prevent additional losses of potato crops and others. The use of conventional synthetic insecticides is the most prevalent method of pest control. However, several considerable insecticide-related problems such as the lack of novel insecticides, the high cost of synthetic pyrethroids, and concern for environmental sustainability, the unacceptability of many organophosphates and organochlorines, and increasing insecticide resistance on a global scale are prompting bio-insecticide researchers to reconsider botanical alternatives (Shaalan *et al.*, 2006b). Finally, the rising organic agriculture movement demands insecticide residue free food (Van Naters and Carlson, 2006). An alternative strategy is required for such noxious pest of potato in stores, where the use of repellent plants is an environmentally sound alternative to the application of natural insecticides for the control of PTM (Pucci *et al.,* 2003; Guerra *et al.,* 2007).

Botanical extracts induced a wide range of sub-lethal effects on insect pests' larval mortality, larval duration, pupicidal activity, pupal duration, adult emergence, sex ratio, adult mortality, and malformation (Shaalan *et al.,* 2005b). According to a surveyed literature (published from 1915 to 1993) by Das (1995), botanical preparations from 35 plant species are effective against the pest PTM either in the storage or in the laboratory. In some studies chopped and dried leaves were used, while in others leaf/seed extracts, fruit peel, bulb, root and rhizome were used; plant preparations are effective in reducing the pest damage, or killing at different stages of the PTM (Das, 1995). Aqueous extracts of neem leaves, Colocasia and their mixtures reduced percentage infestation of PTM, in field and also the numbers of survived larvae, pupation and adult emergence percentages (El-Sinry and Rizk, 2002).

The potential egg production and egg hatchability of potato tuber moth, was significantly lowered when PTM of either sex were exposed into vapors of orange peel oil emanating from 160µl oil and oviposition and egg hatching was totally inhibited when female moths of both sexes were exposed to 220µl of the oil (Sharaby, 1998). Crude plant extracts from the exotic invasive tree *Melia azadarach*,(Syringa), was tested and the bioassays showed that pupal weight was negatively influenced and that growth of PTM larvae was retarded after feeding on potato leaves dipped in 10g/ liter syringe leaf extracts (Visser, 2004).

Moreover, native plants like birbira, *Millettia ferruginea* (Hochst) Baker are potential insecticides of many insect pests. Various workers confirmed toxicity of birbira extracts against stored pests such as, grain Legumes, the bean bruchids, *Chalosobruchus chinensis (*L.) (Bayeh and Tadesse, 2000), *Sitophilus zeamais* (Motsch) in maize seeds, (Bekele, 2002), bean bruchid, *Zabrotes subfaciatus* (Bekele *et al.,* 2007). Aqueous seed powder extract of *M. ferruginea* caused 100% *Macrotermes* termite mortality at all levels of applications (1 ml, 2 ml and 3 ml) and doses 10%, 15% and 20% levels of extraction (Gedeon Yohannes, 2006). A seed-water suspension of 10% *M. ferruginea* was toxic to Enset root mealybugs, *Cataenococcus ensete* (Ferdu Azerfegne *et al.,* 2009a).

Both aqueous and organic extracts of birbira seed powder were found to be effective by contact and as stomach poison (Bayeh, 2007). Based on these results Bayeh (2007) suggested that the bioactive compounds extracted by water might be different from those extracted by chloroform or the potent compounds that are extracted by the two solvents might be of the same group of bioactive chemicals, but because of their nature, might dissolve in solvents of different characteristics. Insect pests with diverse feeding habits and feeding place requirements were tested for *M. ferruginea* seed extracts, its contact effect on the three aphid species which feed externally , barley aphid, *Diuraphis noxia* (Mordwlk); brassica aphid, *Brevicoryne brassicae* (L.) (Bayeh, 2007); and pea aphid, *Acyrthosiphon pisum* (Harris), again for stomach poisoning effect directly on chewing insect pest, Diamond back moth, *Plutella xylostelle* (L.), larvae, (Bayeh and Biruk, 2009, unpublished) and the PTM larvae which feeds concealed in leaves, stems and tubers of the host plant (Bayeh, 2009, unpublished).

Therefore, there are enough evidences to justify the extracts from birbira seed powder obtained using aqueous and organic solvents contain potent natural insecticide. However, among the different solvents used water was found to be most effective as compared to all other solvents (Bekele *et al.*, 2002). The investigation in assaying extracts that may be obtained with the use of a range of solvents with different levels of polarity is important. This is because plant parts and target insect species affect the effectiveness of botanical extracts (Shaalan, *et al.*, 2006a). Besides this, selection of solvent type has vital importance in effectiveness of botanical extractions. Shaalan *et al.*, (2005a) reported that solvent types for botanical extractions should be carefully selected because different solvent types can significantly affect the success of extracted plant compounds. A converse relationship is said to exist between extract potency and solvent polarity where efficacy increases with decreasing polarity (Mulla and Su, 1999). Maher and Thiery (2004) evaluated the activity of chemical stimuli from grape berries on the oviposition of *Lobesia botrana* (Lepidoptera: Tortricidae) and reported that only polar extracts that have been obtained by soaking berries in polar solvents such as methanol or water stimulated oviposition, whereas more apolar extracts obtained with chloroform or hexane had no significant effect.

The effectiveness of both the aqueous and organic solvent extracts, both by contact and stomach poison affect oviposition, feeding and development delaying is very interesting scenario and needs to be investigated further. Birbira was very effective as stomach poison and/or by contact particularly on potato pest, the PTM larva which feeds concealed in leaves, stems and tubers of the host plant. Thus the insect pest, *P. operculella*, potato tuber moth (PTM) with concealed feeding habits is preferable to other major insect pest that are also concealed feeders to bioassay oviposition and feeding deterrence effects as well as ovicidal effects of *Millettia ferruginea* seed powder extracted with both polar and non-polar solvents.

The female PTM preferably lay their eggs on the under surface of foliage and tuber eyes of potato. Consequently oviposition might get reduced or prevented on *M. ferruginea* seed powder extract treated potato tubers and potato leaves. Similarly if oviposition is not 100% prevented then the eggs might fail to hatch or reduced egg hatchability might occur. Moreover, if egg hatchability is not 100% reduced the hatched neonate larvae settlement might get reduced or prevented. These all may result in the suppression of PTM population by reducing biological performance of PTM on *M. ferruginea* seed powder extract treated potato tubers and potato leaves. This could be confirmed by comparing the number of oviposited eggs on treated tubers and untreated tubers, oviposited eggs on treated potato leaves and untreated potato leaves, hatchability and un-hatchability of treated eggs laid on potato leaves and neonate settlement on treated leaves and untreated leaves as well as based on effects of concentration variations on treated tubers, treated leaves and treated eggs.

Thus, this study was planned to determine the potency of polar and non-polar solvent extracts of birbira seed powder in affecting both oviposition, ovicidal and feeding (larval settlement) responses of potato tuber moth.

1.1. Objectives of the study

1.1. 1. General objectives

The over all objective of this work is to help generate an alternative and sustainable control method that will reduce damage to potato crops in the field and in store by potato tuber moth using botanical extracts that are locally available.

1.1.2. Specific objectives

1. To determine oviposition deterrent effect of *M. ferruginca* seed powder obtained with solvents of different polarity on PTM adults.
2. To determine feeding deterrent effect of *M. ferruginea* seed kernel obtained with solvents of different polarity on PTM larvae.
3. To determine ovicidal effect of *M. ferruginea* seed kernel extracts obtained with solvents of different polarity against PTM eggs.
4. To identify the solvent(s) that may exhibit the most potent natural insecticide in *M. ferruginea* seed kernel.

2. LITERATURE REVIEW

2.1. Potato crop production importance and constraints

FAO (2008) pointed out that Potato (*Solanum tuberosum*) originated in the highlands of Peru, South America, where it has been grown and consumed for more than 8,000 years. According to Pliska (2008) potato involved on its successful journey around the world in the 16th century, when the Spanish brought it to Europe from the South American Andes. From here, the potato found its way to Asia in the 17th century and to Africa in the 19th century. The crop's comparably short vegetation period allows farmers throughout a wide range of different climatic conditions to find an appropriate season for its cultivation (Sporleder, 2007; Pliska, 2008). The fourth most important food crop after rice, wheat and maize, potatoes are of invaluable importance for the diets and livelihoods of millions of people worldwide (Van Naters and Carlson, 2006; Pliska, 2008). Today, the annual production of potato is approaching 300 million tons (FAO, 2008). Potato has many functions as it is a high value vegetable, an important food and cash crop. Potato has played an important role for food security, poverty elimination, and creation of employments. Potato is major source of carbohydrates and a single medium-sized potato contains about half the daily adult requirement of vitamin C while other staples such as rice and wheat have none (FAO, 2008). Potato is very low in fat, with just 5% of the fat content of wheat, and one-fourth the calories of bread. Boiled potato has more protein than maize, and nearly twice the calcium (FAO, 2008).

FAO (2008) reported that global potato production has grown markedly in the Asia Pacific Region in the past 40 years, particularly due to increased production in developing countries. The main producer is China, with a crop yield of 71 million tones, which amounts to over 20% of global production (Pliska, 2008).

Potato is also both an important tuber vegetable for local food security as well as a good cash crop for smallholder farmers in Ethiopia. Potato in our country is also very essential food and cash crop, favorite choice especially by the people in the mid-to-high landers. It is mainly consumed as boiled, salad, stews and recently potato chips and crisps are getting attention from roadside vendors who frie and sell it on the spot. Potato production in Ethiopia covers an area of about 1600, 000 ha (Ferdu *et al.*, 2009b). Potato average yield/ha (9 tones) in Ethiopia is much lower than the world average yield/ha (15 tones) (Ferdu *et al.*, 2009b), not comparable with the world average yield.

Reduced yield of potato per/ha have been indicating that there are still a lot of problems related to the production of potatoes that need to be addressed to realize the potential of the crop particularly in developing countries like in Ethiopia. The most important constraints responsible for the low productivity of potato are the low yielder potato varieties right now under use and susceptibility to the major disease and insect pests (Ferdu *et al.*, 2009b). In Ethiopia over 42% of potato tubers were expected to have been exposed to PTM infestation (Sileshi and Teriessa, 2001).

With the aim of setting up more rational approaches to control insect pests, Pucci *et al.,* (2003) noted that basic research is needed on the key factors regulating population dynamics of major deleterious insect pests; a numbers of phytophagous insects are known to feed on potato crops in the field and store. Therefore, potato crop production and storage is constrained by insect pests, most notably: cotton aphids (*Aphis gossypii*), pepper and potato aphid, (*Macrosiphum euphorbiae*), green peach aphid (*Myzus persicae*), death’s head halok moth (*Acherontia atropos*), cutworms (*Agrotis segetum* and *Euxoa spp*.), red ants (*Dorylus spp. m.breyinodosus,* egg plant epilachna (*Epilachna fulvosignata*), potato epilachna (*Epilachna hirta*), Teff epilachna (*E. similes*), Metallic leaf beetle (*Lagria vilosa*), pollen beetle (*Mylabris flavoguttata*) and potato tuber moth (*Phthorimaea operculella*) (Bayeh and Tadesse 1992; Emana and Amanuel, 1992; Bayeh *et al.,* 2008), but in Ethiopia only one pest merit close attention, this is PTM, *P. operculella* (Zeller).

The damage caused by PTM is very severe as larvae’s feeding on the tuber can completely damage, making it unmarketable and inconsumable by humans. Besides this the damage on seed tubers is directly on the developing sprouts which die as a result hence depriving farmers tuber seeds to plant or sell for other growers. PTM can cause significant economic damage. Alvarez *et al.* (2005) states that economic damage caused by *P. operculella* can be significant, because in some areas losses of up to 50% of the stored crop have been reported in Yemen and Peru; 86% in Tunisia, Algeria, and Turkey; 90% in Kenya; and 100% in India and the Philippines. Over 42% of the potato tubers were exposed to PTM infestation in Ethiopia (Sileshi and Teriessa, 2001). On averages, 8.7% of the tubers were lost due to field infestation (Sileshi and Teriessa, 2001).

In Ethiopia, PTM was recognized as important first in the warmer areas where potato is grown and now days PTM has established as an important pest in major potato growing areas because of the long distance transportation of seed tubers from limited source sites mainly in the cool highlands of North and West Shewa to many areas across the country (Bayeh *et al.*, 2008). Since PTM activity slows at temperature below 18^0C, cool weather is an effective control in some areas (Guenthner *et al.*, 2003). A survey was made also in some of potato growing areas of southern Ethiopia and PTM was found to be the only pest of potato (Emana Getu and Amanuel Girma, 1992). The activity of male adults of PTM was monitored using sex pheromone baited traps at Holetta, Melkassa Agricultural Research Center and in the major potato growing areas of West Amhara Region, as a result the existence of PTM was reported year round in the seed tuber stores and in green fields at different peaks in different areas (Bayeh *et al.,* 2008).

2.2. PTM damage symptoms on potato

The PTM, *P. operculella* is one of the most important constraints to potato productivity worldwide (Rondon *et al.*, 2007a) and the most important economic damage occurs toward the end of the growing season when tubers become exposed and are infested by PTM prior to or during harvest (Lacey *et al.,* 2008), however unless infestations of potato plants are high, losses in yield due to plant incapacitate are not the main concern with PTM (Guenthner *et al.*, 2003; Rondon *et al.*, 2007a; Lacey *et al.,* 2008).

Potato tuber moth larvae damage both tubers and foliage. Larvae of PTM mine leaves, stems, and petioles and excavate tunnels through potato tuber (Rondon *et al.*, 2007b) and infested tubers may result in removal of entire loads if the level of infestation is high (literature reviewed by Lacey *et al.,* 2008). Foliage damage includes transparent blisters, eaten out patches on leaves and cavities in leaf stalks (Alvarez *et al.*, 2005; Rondon *et al.*, 2007a; Rondon *et al.*, 2007b). The tops of heavily infested potato plants die prematurely, which causes yield reduction (Rondon *et al.,* 2007a) and tubers developing under these plants become infested by larvae from the top of the plant, or by new larvae developing from eggs laid on the tubers. Although PTM larvae prefer the upper foliage it feed on leaves throughout the canopy (Rondon *et al.*, 2007a), they mine the leaves, leaving the epidermal areas on the upper and lower leaf surface intact. That means the larvae feed on the mesophyll, forming blotch mines in the leaves (Varela and Bernays, 1988). Therefore, infested plants can be recognized by the mines the larvae make in the leaves and stems and by the webbing together of adjacent leaves (Chumakov and Kuznetsova, 2009).

PTM infestation drops to near zero when potato plants inter senescence (Pucci *et al.,* 2003). The foliage feeding despite its appearance does not often seriously injure the potato plant (Guenthner *et al.*, 2003) but the serious damage is inflicted when the larvae burrow in to the soil and feed on the developing tubers. Finally, the larvae tunnel deep in to the flesh creating dirty looking or black tubers filled with larval excrement (Rondon *et al.,* 2007a) and fungi, allowing secondary infections on tubers to occur (Palacios *et al.*, 1998; Guenthner *et al.,* 2003) hence rendering them unacceptable in the market place as it becomes unsuitable for human consumption or for seed (Palacios *et al.*, 1998; Guenthner *et al.,* 2003;). The most economically important damage occurs predominantly through the larvae's feeding on the tuber. In the field and in storage facilities, the larvae excavate tunnels throughout the potato tuber, often leaving mounds of frass near the tunnel entrances (reviewed by Oatman and Planter, 1989; Alvarez *et al.*, 2005; Rondon *et al*., 2007a; Chumakov and Kuznetsova, 2009), this damage makes fresh potatoes unmarketable and inconsumable by humans.

In addition to their physical damage to the tubers, the tunnels allow the introduction of bacteria and fungi into the tuber (Palacios *et al.*, 1998; Guenthner *et al.*, 2003; Alvarez *et al.*, 2005). Palacios *et al.* (1998) reported that damage caused by PTM in potato tuber increased from 0.8% after 1 month of storage to 90% 3 month later (end of the storage period), this results suggest that the pest could have entered the stores on or with in tubers as eggs or larval instars so that development of the second generation in stores is believed to be responsible for the damage in tubers (Rondon *et al.*, 2007a).

In the field tuber infestation normally occurs during summer, whilst stored potatoes can be affected all year round. Egg laying on the tubers and development of the larvae continues as potatoes are harvested and put into storage (Rondon *et al.,* 2007a). Chumakov and Kuznetsova (2009) outlines that at higher altitudes the PTM is introduced to potato fields every year with the seed and potato tuber moths are transported to the stores in harvested tubers and returned to the fields in seed tubers. Chumakov and Kuznetsova (2009) states that in all developmental stages, PTM spreads mainly by transportation of potato tubers, fresh fruits of tomato and on tobacco packages and boxes taken from infected farms and areas. PTM activity is likely to increase in all regions where the pest prevails to day (Sporleder, 2007) due to temperature rising. Sporleder, (2007) reported that hot summers allow a rapid PTM population increase which surpasses control thresholds in response to climate changes.

Managing agricultural insect pests with any tactic should be viewed in the context that 30% of all crops are lost to pests in developed countries (Osborne and Cuda, 2003). The losses in other countries are probably significantly higher but we cannot afford to sustain this level of losses to pests in order to feed rapidly increasing human population. Local materials easily available are weapons needed in this battle.

Rondon (2007) describes that satisfactory control of PTM is significant because larval infestation of tubers renders potatoes unmarketable i.e. there is zero tolerance for the presence of PTM larvae in raw processing product because they are classified as foreign material.

Fig.1 Black tunnels and feeding damage from PTM.

Source: (Alvarez *et al.*, 2005)

Fig.2 Exposed tubers are damage predisposed o tuber moth

Source: (Rondon *et al*., 2009a)

2.3. Host plant range of PTM

Larvae of the PTM are oligophagous, specializing in feeding on plants of the family Solanaceae only (Cooper *et al.*, 2009; Chumakov and Kuznetsova, 2009). Although potato tuber moth larvae are primarily associated with potatoes (Chumakov and Kuznetsova, 2009), they have been observed feeding on other plants such as domesticated tomato, *Solanum lycopersicum* (L.), eggplant, *Solanum melogena* (L.) and tobacco, *Nicotiana tabacum* (L.) (Alvarez *et al.*, 2005; Das and Raman 1994 cited in Cooper *et al.*, 2009) peppers and wild solanaceous plants have served as host plants for *P. operculella* (Alvarez *et al.*, 2005).

By Varela and Bernays (1988) a study was made on neonate larvae of *P. operculella* that hatched from soil-laid eggs then host finding and dispersal from hosts and non-hosts were first examined and results indicated that from the first-instar larvae hatching from soil-laid eggs, 80% found the potato plant while roughly 50% found each of the other three plants (datura, tobacco, and tomato) again dispersal from potato, datura, and tobacco was very low, while on tomato it was higher and a high mortality was observed in the 24 h period (Varela and Bernays, 1988). Host plant range expansion by PTM apparently did not result in the formation of a tomato-adapted race; in potato- and tomato-dominated areas of Ethiopia the survival and development time of PTM larvae on tomato did not differ for population (Bayeh, 2000 unpublished data cited in Bayeh *et al.,* 2004). The attributing factor for obvious absence of a tomato-adapted PTM race may be due to the regular movement of moths between host plants (the literature reviewed by Bayeh, 2004).

2.4. **Description and life cycle of Potato tuber moth (PTM)**

2.4.1. Description of PTM

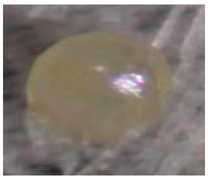
Adult potato tuber moths are silvery-gray in color, have a narrow body, and are approximately 10 mm in length (Madison, 2006; The state of Victoria, 2008). The wingspan is 12-15 mm. and the wings are grayish brown in color, wings of settle down PTM are roof-like (Madison, 2006; The state of Victoria, 2008; Chumakov and Kuznetsova, 2009). Hind wings are almost as wide as forewings, with folded external edge and a fringe longer than it is wide, forewings have dark spots (two to three dots on males and a characteristic “X” pattern on females) and both pairs of wings have fringed edges (Rondon *et al.,* 2007a). Antennae are gray with well-designated joints. The adult potato tuber moths are found to be most active during the night. Alvarez *et al.* (2005) and Chumakov and Kuznetsova (2009) describes that PTM are seldom seen because they are active after sunset and at dawn but sometimes they can be collected near potato fields during the day using a net for capture since adults are fast fliers (Alvarez *et al.,* 2005).

Fig. 3 Female PTM and Male PTM Fig. 4 PTM wing design during flight

Source: (Rondon *et al.*, 2007a)

The last abdominal segment is almost equal to one third the length of the abdomen in the male (Chumakov and Kuznetsova, 2009), while in female, the anal segment is of normal length. The apex of male abdomen is strongly pubescent, covered in dense, hairy bunches. The male possesses a pair of brush organs which are located at the frontal edges of the upper surface of hind wings, and which are often expanded to fan-shaped during copulation attempts with female (Ono, 1979).

Eggs are oval, spherical, translucent, 0.4-0.6 mm in length and 0.4 mm in width. Freshly laid eggs white and turn to yellowish to brown and finally turn black just prior to hatching (Rondon *et al.*, 2007a; Chumakov and Kuznetsova, 2009).



Fig, 5 PTM egg

Source: (Rondon *et al.*, 2007a)

Mature or older larva are pink or flesh-colored or yellowish green, with a pale longitudinal stripe along the median of its back and with a brown head, 10-13 mm in length, 1.5 mm in width (Rondon *et al.*, 2007a; Chumakov and Kuznetsova, 2009).

Fig. 6 Larvae actively feeding on potato leaves

Source: (Alvarez *et al.*, 2005; Rondon *et al.*, 2007a)

Larvae feed on leaves throughout the canopy but prefer the upper foliage (Rondon *et al.*, 2007a). PTM larvae form tunnels under epidermis of leaves and stalks, eating away parenchyma. One larva makes 3-4 tunnels, gradually filling them with excrement (Chumakov and Kuznetsova, 2009). The larvae more often penetrate tubers through tuber eyes. On sprouting tubers, all neonates entered through the eyes (Horgan *et al.*, 2007). They create twisting tunnels in tubers. Web-like connecting of leaves and damage to stalks and sprouts occur too.

PTM pupae are smooth and brown and often are enclosed in a covering of fine sediment, 5.5-6.5 mm in length and it develops in a silky cocoon of grayish silvery color that reaches 10 mm in length (Chumakov and Kuznetsova, 2009).

Fig. 7 PTM pupa

Source: (Rondon *et al.*, 2007a)

2.4.2. Life cycle of PTM

Potato tuber moth undergoes complete metamorphosis. PTM reproduces continuously and has overlapping generations. In most Lepidoptera, females are receptive for mating at dusk or at night (Talekar, 1998) and insects display complex mating behaviors. Ono (1985) reported that the processes of female searching by male potato tuber moths, *P. operculella,* include the involvements of behavioral components to copulation like antennal cleaning, quiescence, walking, wing fanning, contact with female, hair brush display, copulation attempt, and copulation. The adult females lay eggs 2 to 4 days following emergence (Chumakov and Kuznetsova, 2009). On the other hand Horgan *et al.* (2007) describes further that PTM females begin laying eggs 2 days after emerging from their cocoons and can live 30 days (Chumakov and Kuznetsova, 2009). Laying eggs takes place on the lower sides of plant leaves and sometimes on leaf stalks, stems, exposed potato tubers, lumps of soil in field, on potato tubers at the buds and on bags in storehouses (Rondon *et al.*, 2007a; Chumakov and Kuznetsova, 2009). Female PTM can crawl through soil cracks or burrow short distances through loose soil to find tubers on which to deposit eggs (Rondon *et al.*, 2007a). Female fecundity is 150-200 eggs, with 165 on average (Chumakov and Kuznetsova, 2009).

Once the eggs hatch, the larvae feed on the foliage or tubers depending on where the eggs were deposited. All neonates entered undamaged tubers through the eyes, where as in damaged tubers, neonates entered through the cracks (Horgan, *et al.*, 2007). Pupation occurs amongst dead potato leaves on the soil, or on stored potato tubers and the pupa is a silk cocoon covered with soil particles and debris for camouflage (Rondon *et al.*, 2007a). Moreover, pupation occurs inside hidden cocoons located in various shelters such as under dust, on bags/sacks, in floor cracks (Chumakov and Kuznetsova, 2009). Chumakov and Kuznetsova (2009) described that the PTM does not normally enter diapause. This allows it to continuously reproduce provided that there is suitable temperature and food is available such as potato tubers in seed/ ware potato storehouses (Sporleder, 2007).

The PTM life cycle requires three weeks at 27^0C (the literature reviewed by Keasar and Steinberg, 2007). In Ethiopia at room tempreture the full developmental time takes about 35 days between February and May which it takes about 115 days between June and December (personal communicaion). According to Chumakov and Kuznetsova (2009) the optimum conditions for PTM development are of 22-26^0C and air humidity levels of 70-80%. However, considering temperature factors Pucci *et al.*, (2003) indicated that if ambient temperature is at 25°C or higher, they will stop ovipositing and their development ceases when the temperatures drop below 10°C. The larvae mature in 16 to 24 days (Alvarez *et al.*, 2005) or 11-14 days (Chumakov and Kuznetsova, 2009), while larvae pass through 4 instars, the pupal stage lasts 6 to 9 days.

2.4.3. Temperature adaptation of PTM

PTM has adapted to a large climatic range although its biological activity increases with temperature. Since PTM activity slows at temperatures below 18%, cool weather is an effective control in some areas (Guenthner, 2003). Average temperature rise of 1 or 2^0C would increase the number of PTM generations per year from 3.3 to 3.7 or 4.2 while PTM activity would increase 6.9 or 61 fold (Sporleder, 2007) and prefers hot dry summers and generation may be increase 6-8 in a field at this season. Although, the number of generations each year and the length of the life cycle are influenced by temperature and the life cycle can be as short as 2 weeks in summer or as long as 7 months in winter (Alvarez *et al.*, 2005). Warmer temperatures allow potato tuber moth (PTM) to complete their development in 4 to 5 weeks in NeawZeland but probably not more than two in the more temperate climates of North America (Alvarez *et al.,* 2005).

2.5. Potato tuber moth control methods

Rondon *et al.* (2007a) stated that the most effective control method combines cultural, biological, and chemical approaches. Guenthner *et al.*, (2003) noted that PTM management consists of practices that reduce adult populations on plant foliage and larvae ability to reach the tubers.

2.5.1. Cultural control methods

Cultural pest control involves changing or modifying cultivation practices which directly or indirectly reduce the insect pest population. Cultural practices, such as elimination of cull piles and volunteers (sanitation), soil moisture at and after vine clearing, length of time between skin hardening and harvest, rolling or covering hills, crop rotation, etc., were the earliest control measures advocated for reducing PTM (Rondon *et al.*, 2007a). Hanafi (1999) indicated that many cultural practices that are used by farmers to improve the yield and quality of potato can also limit the development of PTM and minimize damage to tubers.

Many cultural practices capable of reducing potato tuber moth populations in the field have been reviewed by (Alvarez *et al.,* 2005; Rondon *et al.,* 2007a; The state of Victoria, 2008; and Chumakov and Kuznetsova, 2009) and include the following:

- Prepare land thoroughly before planting
- Do not plant infested seed potatoes.

- Ø Plant tubers deeply (at least 10cm) and regularly mound the soil so that tubers are always covered by at least 5cm of soil.
- Ø Provide adequate irrigation to avoid soil cracking.
- Ø Harvest potatoes as soon as possible once the crop has matured.
- Ø Do not leave harvested potatoes in the field overnight.
- Ø Do not cover harvested potatoes with potato tops or other debris.
- Ø Remove infested tubers from the field
- Ø Plough in debris as soon as possible after harvest to prevent larvae pupating in dry stems left in the field.
- Ø Destroy volunteer potatoes and host plants.

Alvarez *et al.* (2005) and Chumakov and Kuznetsova (2009) outlined further that it is important to keep tubers under at least 5cm (2inches) of the soil that is free of cracks. Irrigation that continually keeps soil moist and the surface sealed is also a control method (Alvarez *et al.*, 2005). Varieties with deep stolons make it more difficult for infestations. Harvesting before PTM populations build up is another control method. Also, volunteer plants and cull piles need to be managed to remove potential food sources and habitat (Alvarez *et al.,* 2005) and Chumakov and Kuznetsova, 2009). When tubers become exposed and are infested by PTM prior to or during harvest, up to 100% may be infested if preventive measures, such as deep planting and ridging, are not used (reviewed by Oatman and Planter, 1989; Guenthner *et al.,* 2003). Potato stems and tubers showed no damage at harvest as the result of the adequate use of cultural practices such as irrigation, hilling up and timely harvest (Hanafi *et al.,* 1999; Alvarez *et al.,* 2005; Rondon *et al.,* 2007a; and The state of Victoria, 2008). In addition, stores close to the potato fields may serve as source of food and refuge for the pest through out the year (Palacios *et al.,* 1998) that may have had an influence on the low level of damaged tubers in the field.

Palacios *et al.* (1998) stated that prevalence of the pest in the absence of a crop suggests that the pest survives in crop residue between field plantings and observation from the remaining population in the field showed the presence of larvae and pupae in dried tubers and stems 3 months after harvest. Besides this PTM is known to survive as pupae in the soil (Rondon *et al.*, 2007a), this indicates that moth population can survive in crop residues in the absence of a host plant, thus timely sanitation to collect and destroy plant residue and volunteer plants is an important management option.

2.5.2. Chemical control methods

2.5.2.1. Synthetic chemical insecticides

Insecticides remain the chief means of control for potato tuber moth (Alvarez *et al.,* 2005). Currently used synthetic insecticides to control PTM in Israel have been reported including the organo-phosphate methamiophos and the carbamate cartap (the literature reviewed by Keasar and Steinberg, 2007), where as in Ethiopia the synthetic insecticide diazionon 60% EC used effectively to control PTM in the field (Ferdu *et al.,* 2009b). Valnuron is IGR chemical applied to the potato leaves which has been tested as control methods of PTM (Coll *et al.,* cited in Keasar and Steinberg, 2007). In Australia farmers are advised to use insecticides only occasionally or under high PTM pressure (The state of Victoria, 2008). In the state of California potato growers are recommended to control PTM using insecticides by respecting the threshold of 15-20 PTM male adults per trap per night (Rondon *et al.,* 2007a). PTM can be controlled by insecticides but the treated potato tubers in contact with synthetic insecticides can be used only as seed because of the health hazard from residues if used for ware (reviewed by Lal, 1987).

In New Zealand PTM are an important pest of potatoes because of its high reproductive potential and the inability of insecticides to prevent tuber infestation (Foot 1979 cited in Herman *et al.,* 2005). However, as the farmers gain more experience with both agronomic practices and chemical insecticides, they are likely to rely more heavily on cultural practices and less on insecticides to control PTM damage (Hanafi, 1999). Unfortunately, there are reports from many countries of PTM resistance to several insecticides (Haines, 1977 cited in Lal, 1987; USDA, 2006). Chemical pest control has resulted in more than 500 insect species becoming resistant to one or more pesticides and almost without exception; attempts to eradicate insect pests have failed (Van Lenteren, 2005). Increased numbers of insecticide applications in attempts to control PTM damage on potato tubers was shown to increase the infestation, possibly because of harmful impact on its natural enemies (Coll *et al.,* 2000 cited in Keasar and Steinberg, 2007). Pucci *et al.* (2003) describes that problems in connection with pests and diseases management are heightened, especially in the places where monocultures of potatoes create a heavy dependence on synthetic insecticides which causes serious ecological, toxicological and economic concerns.

Table 1 Resistance of PTM to insecticides.

Insecticides	country	References
Azinphos-methyl (organophosphorus) And carbaryl (Carbamates)	Mexico	Llanderal-cazares *et al.*, 1996. Journal of Entomological science
Fenvalerate, cypermethrin, permethrin And deltamethrin (pyrethroid)	Peru	Collantes *et al.*, 1986. Crop protection

Source: (USDA, 2006).

Management recommendations should be based on field-specific information. *P. operculella* populations vary greatly from field to field and from area to area. Therefore crops should be monitored regularly and before making management decisions such as spraying. In areas under threat by PTM, growers are encouraged to monitor the insect numbers using pheromone traps (Rondon *et al.*, 2007a). Pheromone traps are used to monitor PTM populations in the field to help time insecticide applications (Herman *et al*, 2005).

2.5.2.2. Botanical insecticides

Botanicals are natural insecticides derived from plants. They are characterized by rapid breakdown and therefore are considered safer to the environment than the synthetic insecticides (Herman *et al.*, 2005; Guleria and Tiku, 2009; Sharma and Gupta, 2009).

Alabi *et al.* (2005) point out that the use of botanicals in crop protection has now gained a popular ground in the world of agriculture as an alternative to the use of toxic, persistent and synthetic compounds. According to Alabi *et al.* (2005) several factors are now responsible for making the use of alternative methods more attractive particularly in developing countries. 1. Limited external reserves and poor exchange rates of their respective currencies. 2. Complete removal of subsides on synthetic herbicides, insecticides and fungicides for majority of farmers in many African countries.
3. Rejection of chemicals contaminate stored food’s commodity.

Amiri-BeSheli (2008) described that the deleterious effects of plant extracts on insects can be manifested in several ways, including toxicity, mortality, and reduction of reproduction, fecundity and fertility (Shaalan *et al.*, 2005b).

Botanical insecticides are uniquely characterized in that the content of the bioactive substances vary with season, growing conditions, age at harvest, differences in extraction methods and storage conditions thus posing difficulties in dosage standardizing (Clegg and Mackean, 1994). Solar radiation for instance accelerates their decomposition, it is therefore essential that the potency of a botanical extracts like *M. ferruginea* be determined locally through testing different concentrations on infested plants and comparing the effects. Botanicals extraction time was another main parameter in the extraction procedure. The extraction time can either be as short as few minutes or very long up to 24 hours (Lee *et al.*, 2005 cited in Chan *et al*., 2009).

Shaalan *et al.* (2005b) reported that azadirachtin acts as an insect feeding deterrent and growth regulator, based on many research reports the treated insect usually cannot molt to its next life stage and dies (Buss and Park-Brown, 2006).

There are also numerous botanicals identified to be effective against crop pests. Some of them are described below: Botanical insecticides obtained from extracts of *Pongamia glabra*, *Azadirachta indica* and *Chrysanthemum cinerariifolium* and their biological efficiency was compared against insect pests of *Spodoptera littoralis*, *Myzus persicae* and *Tetranychus urticae* on greenhouse plants, results showed that in all the tested extracts at the highest concentration caused 100% mortality (Pavela, 2009) and in the other tested concentrations, on day 12 after application, the highest efficiency was determined for *M. persicae* pongam oil, for *T. urticae* and *S. littoralis* neem oil (Pavela, 2009). The insecticidal activity of *Rhamnus dispermus* (Rhamnaceae) extracts was enhanced against peach trunk aphid, *Pterochloroides persicae* (Homoptera: Lachnidae) by increasing the concentration of the extracts and the exposure time (Ateyyat and Abu-Darwish, 2009). Crushed barks of *Rhamnus dispermus* Ehrenb (Rhamnaceae**)** were extracted exhaustively in a Soxhlet extractor with hexane, chloroform, acetone and ethanol respectively, among these both the acetone and ethanol extracts showed higher mortality (69 and 71%, respectively) than the hexane and chloroform extracts (40 and 56%, respectively) after 72 h of exposure at the highest concentration (Ateyyat and Abu-Darwish, 2009). Moreira *et al.* (2007) reported that the insecticide property of the plant species basil, *Ocimum selloi* (Benth.), rue, *Ruta graveolens* (L.), lion's ear, *Leonotis nepetifolia* (L.) R.Br., jimson weed, *Datura stramonium* (L.), baleeira herb, *Cordia verbenacea* (L.), mint, *Mentha piperita* (L.), wild balsam apple, *Mormodica charantia* (L.), and billy goat weed or mentrasto, *Ageratum conyzoides* (L.) were evaluated with hexane and ethanol extracts. Among the eight plants screened, only the hexane crude extract of *A. conyzoides* showed insecticide activity, with 76 and 88.67% mortality of *R. dominica*, at 4 and 24 hours after the exposure, respectively. In agreement with this work, (Saxena *et al.*, 1992 cited in Moreira *et al.*, 2007) observed some acute toxic effect of polar extracts, obtained with petroleum ether and acetone of *A. conyzoides* against *Culex quinquefasciatus* (Say) (Diptera: Culicidae) (Moreira *et al.*, 2007).

Rotenone is a broad-spectrum contact and stomach poison that is effective against leaf-feeding insects, such as aphids, certain beetles (asparagus beetle, bean leaf beetle, Colorado potato beetle, cucumber beetle, flea beetle, strawberry leaf beetle, and caterpillars, as well as fleas and lice on animals (Buss and Park-Brown, 2006), also most insects are highly susceptible to low concentrations of pyrethrins. However, the toxins cause immediate knockdown or paralysis on contact, but insects often metabolize them and recover (Buss and Park-Brown, 2006).

Moawad and Ebadan (2007) states that the 0.02 and 0.05%conc. of cardamon oils exhibited the best reduction in percentage of eggs hatchability (67.47 and 86.74%) and cardamom and rosemary oils of 1.5%conc.dust (0.5, 1 and 1.5% oils mixed with talcum powder and then dusting potato tuber) on potato tuber obtained the lowest percentage of PTM larval penetration, % pupation and adult emergence. It is suggested that dusting a thin layer of tested natural oils may act as a defensive barrier, by causing confusion or disturbance to the foraging neonate larvae as reviewed by Moawad and Ebadan (2007) indicated that dusting Neemerich oil (*Azadirachta indica*) on potato tuber caused ovicidal and larvicidal action against PTM. Dried, chopped leaves and flowers of *Minthostachys spicata*, and *M. glabrescens* (Lamiaceae) in Peru that have been covered potato tubers in store compared with a control treatment of maize straw (5%) reduced the percentage of tuber damage (5% vs. 12%) (Guerra *et al.*, 2007) and essential oils of *Minthostachys spicata, M. glabrescens* and *M. mollis* at natural concentrations deterred PTM oviposition, reduced the number of eggs laid by about

80% compared with the control treatments in both assays regardless of the Minthostachys species difference in protection (Guerra *et al.,* 2007).

Indigenously developed bio-insecticides are environmentally sound; nature friendly and economically feasible, therefore inclusion of traditional knowledge in the insect pest control programme may be assured, as it reduces dependence on synthetic chemical insecticides and deterioration of ecology. Ramasoota (2001) searched the perception of 50 farmers and their family members involved in farming activities in Thailand and results indicated that both group of farmers, who either adopted or refrained from using botanical insecticides had experienced health disorders from low doses of chemical insecticides poisoning and 80% of farmers decided to adopt to use botanical insecticides to reduce health impact and operation cost. However, Deng *et al.* (2009) clarified that botanical insecticides and other indigenous products used in traditional control of insect pests was studied and it has shown that majority of farmers still never uses botanicals to control insect pests in the field (76%) and storage (79%), respectively. This indicates that though botanicals are advantageous to use, their replacing synthetic insecticides may require longer time.

2.5.3. Biological control

Biological control means the use of parasitoids and predators and the use of micro organisms pathogenic to insect pests. It is the most successful, most cost effective and environmentally safest way of pest management (Van Lenteren, 2005).

2.5.3. 1. Microbial-insecticides

Rondon *et al.* (2007a) described that insect diseases caused by bacteria, viruses, and nematodes have been developed to control insect pests, including PTM. For instance Bt are naturally occurring soil borne organisms that can control some insects in larval stage (Guenthner *et al.,* 2003; Alvarez *et al.,* 2005).

Sabbour (2006) reported that potato cultivation in both the field and in the store treated with the bio-insecticides *Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisopilae, margosan-O* added to the fertilizers (compost banana, livestock's manure, poultry wastes, urea and ammonium nitrate) during the fertilization periods; resulted in significant reduction of the densities of the PTM and spoiled potatoes were significantly decreased in the treated areas. Salama *et al.*, (1994) further describes that the mode of action of *B. thuringiensis* involves biochemical changes in the haemolymph of P. operculella where the level of proteins in the haemolymph markedly decreased during larval treatment with *B. thuringiensis* (the literature reviwed by Salama *et al.*, 1994). In Columbia, a pathogen, *Baculovirus phtorimaca* is being developed against PTM larvae as a means of biological control (Rolot, 2001). However, microbial control of PTM is not yet developed for commercial use, but has potential in the future (Rondon *et al.*, 2007a). Biological insecticides in particular were more effective in preventing losses by insects in stores in cases where the initial level of infestation was relatively low.

The expansion and deployment of transgenic *Bacillus thuringiensis* (Berliner) (Bt) varieties in cotton, corn and potatoes has lead to a new dimension to insect Pest Management (reviewed by Manyangarirwa *et al.*, 2006). According to Alvarez *et al.* (2005) high mortality has been found in PTM larvae that fed on tubers and leaves of transgenic potato plants expressing toxin genes from *Bacillus thuringiensis* (Bt). Alvarez *et al.* (2005) in a choice experiment showed that PTM larvae a preferred the control plant over the transgenic Bt potato plant and the insecticide Bt was consistently the most effective in killing PTM larvae. Besides this Christou (2006) noted that the implementation of insect-resistant transgenic crops has been increasing annually at double-digit rates since the commercial release of first-generation maize and cotton expressing a single modified *Bacillus thuringiensis* toxin (Bt) nine years ago. According to Christou (2006) transgenic crops expressing Bt are more environmentally friendly because of their specificity and absence of direct effects on biological control agents and non-targets (Guenthner *et al.*, 2003).

2. 5.3.2. Parasitoids and predators

Rondon *et al.* (2007a) pointed out that one of the first assessments for a successful integrated pest management program, following establishment of a monitoring program, should be to determinate the role of natural enemies. Minimal insecticide usage allows numbers of parasitic wasps and predators to build up. This requires choosing insecticides that preserve natural enemies.

The PTM has a significant amount of parasites and predators (Chumakov and Kuznetsova, 2009). A few parasitoid wasps have been collected from PTM in the Pacific Northwest, but the importance of parasitoids in potato fields is unknown. Also unknown is the role of common predators such as lady beetles, big-eyed bugs, and ground beetles in controlling PTM. However, Chumakov and Kuznetsova (2009) listed out that many predator insect species such as ladybirds, lacewings, red and blue beetles, ants, and parasitic wasps are natural enemies of the PTM. In Ethiopian potato system, the specialist parasitoids that imposed to control PTM was found efficient three times lower than those of generalist predators, particularly coccinellids (*Coccinella septempunctata* and *Cheilomenes* spp.), *Chrysoperla carnea* Lacewings, spiders and ants (Bayeh, *et al.*, 2004), that caused up to15% vs. 40% mortality of larvae on exposed potato plants, respectively. Among the parasitoid species recorded *Diadegma mollipla* (Holmgren) (Ichneumonidae), a braconid species and other morphotypes constituted 66.2%, 18.3% and 15.5% respectively of all recovered parasitoids from foliage of exposed potato plant (Bayeh *et al.*, 2004).

Chumakov and Kuznetsova (2009) stated that parasitoid wasps such as *Copidosoma* spp. and *Apanteles* spp. are important in PTM control in other parts of the world. The poly-embryonic parasitoid *Copidosoma koehleri* (Blanchard) has been used with some success to reduce populations of *P. operculella* in South America (Alvarez et al., 2005). Besides this Pucci *et al.* (2003) described that *C. koehleri* is a native parasitoid of South America and is now used in mass releases throughout the world. *P. operculella* was parasitized by *C. koehleri* in low level, (20-60%) in South Africa; in Zimbabwe it was successful in controlling the PTM that no synthetic insecticides were needed; moderate results were registered in different areas of Australia; but unfortunately with disappointing result in Italy; but later the specimens were reared until obtaining enough individuals to perform inundative releases in different districts of southern Italy. However, results were very modest (literature reviwed by Pucci *et al.*, 2003). The report from Israel, too, was not encouraging (Keasar and Steinberg, 2007).

Kuhar *et al.*, (2004) reported that *Trichogramma ostriniae* (Peng and Chen) released in the fields of potatoes in 2002 and 2003 significantly reduced the number of tunnel holes.

2.5.4. Resistant cultivar selection.

Natural host plant resistance factors from the Solanum species can be induced into the cultivated potato through traditional breeding. *Solanum berthaultii* (Hawkes), S. commersonii (Dunal), *S.*

sparsipilum (Bitter), *S. sucrense* (Hawkes) and *S. tarijense* (Hawkes) have reported resistance to potato tuber moth (Malakar and Tingey, 1999; the literature reviewed by Cooper *et al.*, 2009;). Potato crop exposure to PTM has been reported to reduce through selection of PTM resistant potato varieties (Hanafi, 1999). Varietals selection offers some opportunity to reduce PTM damage (Rondon *et al.*, 2007a). In agreement with this the relative rates of potato tuber damage due to *P. opercullela* were assessed in 30 varieties of potato grown at Alemaya, eastern part of Ethiopia by Sileshi and Teriessa (2001), result indicated that there were significant differences observed amongst genotypes with range of 6%-62% field infestation in tubers (Sileshi and Teriessa, 2001). More than 120 potato cultivars have been tested in the Columbia Basin, and levels of PTM tuber damage have differed among cultivars (Rondon *et al.*, 2007a). Varietal differences in susceptibility to PTM damage may be due to differential feeding by larvae or to adult egg-laying preferences (Rondon *et al.*, 2007a).

The plant related factors, however, have not been fully identified in cultivated potato. But many wild potato species bear sticky trichomes and pubescence on their leaves and stems that play a role in anti-herbivory (Malakar and Tingey, 2000). Glandular trichomes on foliage of the wild potato species, *S. berthaultii*, deter oviposition by *P. operculella* and negatively affect other important performance parameters. Tricomes of this wild potato mechanically transferred to foliage of a susceptible potato cultivar reduced egg laying by 97% (Malakar and Tingey, 2000). Removal of trichomes also led to increased mobility of larvae on the leaf surface, more leaf feeding, shorter larval development and larger pupae (Malakar and Tingey, 2000).

Horgan *et al.* (2007) pointed out that two potato species (*S. berthaultii* and *S. tarijense*) represent a strong potential source of antiherbivore resistance without major production penalties. *S. berthaultii* bears two types of glandular trichome (type A and type B) that together reduced oviposition by the PTM where females were often completely deterred from ovipositing on foliage with >300 trichomes per cm^2. In contrast, neonate establishment on *S. berthaultii* was generally positively related to trichome densities, indicating that trichomes may be a poor defense against *P. operculella* when the moth oviposits in soil and neonate larvae select the host plant. *S. tarijense* has only one type of glandular trichome (type A) and eglandular hairs (Horgan *et al.*, 2007). The foliage and tubers of *S. berthaultii* and *S. t. tuberosum S. berthaultii* hybrids are more resistant to tuber moth than non-hybrid cultivars (review by Horgan *et al.,* 2007). However this factors associated with tomato leaves such as glandular tricomes, have no effect on the larval establishment of PTM on the plant (Bayeh *et al.,* 2006).

This resistance provides an important genetic trait potentially useful for management of PTM in cultivated potatoes (Malakar and Tingey, 2000). Therefore cultivar selection may be a way to reduce PTM damage as Guenthner *et al.,* (2003) reported that potential yield increase due to PTM-resistance is the largest benefit category in both Egypt and South Africa. Trichome-based tomato resistance offers the potential to reduce synthetic insecticide use, but its compatibility with biological control remains poorly understood (Verheggen *et al.,* 2009).

2.5.5. Integrated pest management (IPM)

IPM is a pest population management system that utilizes all suitable pest control techniques in a compatible manner to reduce pest populations and maintains them at levels below those causing economic injury (Smith and Reynolds, 1966). IPM is the smart approach to managing insect pests because it uses observation, knowledge and thinking instead of the brute force of toxic synthetic insecticide sprays alone. IPM in potatoes is similar to that of other crops: understanding the components and maintaining a balance within an ecosystem and the environment to produce high yields. This implies biological control is a major player while chemical insecticides are utilized as a last resort so that the balance of the agro-ecological system is maintained for as long as possible (FAO, 2008). Economic and damage thresholds are used to determine when management actions should be taken. Although biological control is the first and preferred line of defense in insect pest control, often not all pests, diseases or weeds in a certain crop can be kept below damaging levels by biological control alone (FAO, 2008).

Pheromone traps are used to monitor potato tuber moth populations in the field to help time insecticide applications (Herman *et al.,* 2005). The trap consists of a dishpan of water with a little soap to break the surface tension of the water. Place a tent over the trap to help catch adult moths and place the pheromone inside to attract them. If you catch 10 - 20 per day per trap you should treat the field (Herman *et al.,* 2005). Insecticide treatment must occur before vine kill. Planting varieties that set tubers deep in the soil and hilling the plants up well to protect the tubers from PTM can help reduce damage.

Insecticides with growth regulating properties (IGR) may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development. Tunaz and Uygum (2004) indicated that insect growth regulators may come from a blend of synthetic chemicals or from other natural sources, such as plants. For instance, Azadirachtin (AZ), a tetranortriterpenoid limonoid from the Indian neem tree (*Azadirachta indica* A. Juss.), is the active ingredient of IGRs commercially available. Neem-or AZ based IGRs are very selective ecdysone antagonists and have a broad spectrum of activity as reviewed by (Tunaz and Uygum, 2004). They were found to have a very broad spectrum of activity against agricultural, stored product and house-hold pests (Awad *et al*., 1998 cited in Tunaz and Uygum (2004)), and several other insect species.

Therefore, other insect pest reducing methods are needed. The methods to prevent or reduce development of insect pests as summarized by Van Lenteren, (1993) are the followings:

1. Prevention

Prevent introduction of new pests (inspection and quarantine), start with clean seed and plant material, start with pest free soil (steam sterilization and solarization), and prevent introduction from neighboring crops.

2. Reduction

Apply cultural control (crop rotation and others), use plants which are (partly) resistant to pests, and apply one of the following control methods:

a. Mechanical control (mechanical destruction of insect pest organisms)

b. Physical control (heating)

c. Control with attractants, repellants and antifeedants

d. Control with pheromones

e. Control with hormones

f. Genetic control

g. Biological control (natural enemies and competitor)

h. (Selective) chemical control

3. Guided or supervised control: control based on sampling and spray thresholds.

4. Integrated control: control based on the integration of methods which cause the least disruption of ecosystems.

2.6. Description and use of *Millettia ferruginea* (Hochst) Baker

Higher plants are a rich source of novel insecticides (Jacobson, 1990; the literature reviewed by Khoshnoud *et al.*, 2008). In Ethiopia there is an endemic tree locally called birbira, *M. ferruginea* (Hochst) Baker; which belongs to the family *Legumionosae*, sub family *papilionideae.* MacLachlan (2002) described that birbira is a large shady tree which grows up to length of 25 m in height or so and the trunk of birbira is mostly straight, with smooth grey bark; short brown or golden hairs cover some surfaces of the flowers, fruits and leaves; the pods of the birbira are large and sometimes open with a loud popping sound. Pod may contain 5 to 10 seeds that are dark red to orange colored, flattened and somewhat round like a flat disk, but sometimes almost square with rounded corners (MacLachlan, 2002). Seed of birbira should not be dried in direct sunlight, but under shade at room temperature either for propagation or botanical preparation.

There are two subspecies of *M. ferruginea* (MacLachlan, 2002). The range of subspecies *ferruginea* is found more to the north, in Tigre, Gondar, Gojam, Shewa, Welega, Harerge, and Ilubabor and it is widely distributed in the country and performs well in moist lowland as well as dry, moist and wet semi-highland agro climatic zones of 1000 - 2500 m a. s. l. (MacLachlan, 2002; Bekele *et al.*, 2007) and has many hairs on the lower surface of the leaflets; hairs on the flowers are lighter in color than the other subspecies, a golden brown. Subspecies darassana is found more in southern Ethiopia, but is found north to Shewa. This subspecies has only a few hairs on the leaflets. Hairs on the flowers in this subspecies are darker, blackish brown (MacLachlan, 2002; Azene *et al.*, 1993 as reviwed in Bekele *et al.*, 2007).

Legesse Negash (2002) also reported that in central-south Ethiopia *M. ferruginea is* frequently found in association with some useful annual and perennial crops like maize, sorghum, barley and coffee which indicates the usefulness of birbira tree in the conservation and improvement of soil fertility and in the productivity of traditional farming systems. Besides this insecticidal and other benefits derived from *Millettia* trees when integrated into farming systems of local farmers are broadly reviewed by Legesse Negash (2002) as fish poisoning, use in apiculture, and as fodder supply especially during the flowering period of the tree when fresh twigs, leaves and flowers are harvested and fed to sheep and goats. Banzouzi *et al.* (2008) explained in general that the genus *Millettia* including *M. ferruginea* has an important place in the Pharmacopoeias of sub-Saharan Africa, with numerous therapeutic indications, such as anti-tumoral, anti-inflammatory, anti-viral, bacterial, insecticidal and pest-destroying with a wide range of biological activities.

Much of the understanding about plants that have useful properties comes from native populations of people living in each given locality (Cseke *et al*., 2006). Natives through out the tropics have long used rotenone-containing plants as fish poisons (Perry *et al.*, 1998).This is true in Ethiopia also where the local people around lake Tana and other areas are still using birbira for fish poisoning traditionally in tributary rivers (personal observation) and concerned workers from ministry of agriculture are controlling fish poisoning activities, this is not due to poisoning effect of fishes to human but, their essential seasonal reproduction time. Scattering of powdered botanical material containing rotenone on the water allowing lazy fishing and the dead fish were collected and subsequently eaten produced no ill effects on the consumers (Dewick, 2001).

Dewick (2001) reported that rotenone and rotenoids are relatively harmless to mammals unless they enter the blood stream, being metabolized rapidly up on digestion. Traditionally in Ethiopia *M. ferruginea* fruit powder mixed with honey is given orally for amibiase (Banzouzi *et al.*, 2008). Rotenone is one of the dominant compounds found in the seeds and stem barks of *M. ferruginea* as reviewed in Bekele *et al.* (2002). Rotenone thus provides an excellent biodegradable insecticide and is used as such either in pure or powdered plant form (Dewick, 2001).

Bekele *et al.* (2007) reported that acetone extracts of *M. ferruginnea* and *Tephrosia vogellii* seeds were the most active resulting 100% mortality of the bean bruchid, *Zabrotes subfaciatus* and the active acetone extract of *M. ferruginea* seeds was further fractionated and the most toxic fraction was subjected to chromatographic and spectroscopic methods, resulting in the identification of rotenone as the most active compound against bean bruchid pest.

Birbira is available in most part of the country and in general, its plant derived ingredients was confirmed to control insect pests of agriculturally and medically importance, such as, potato tuber moth (Bayeh, 2009, unpublished), bean bruchids, *Chalosobruchus chinensis* (L.) (Bayeh and Tadesse, 2000), stem borers, *Sitophilus zeamais* (Motsch) (Bekele, 2002), three aphid species (Bayeh, 2007), as remedy for treating and healing Chigger flea, *Tunga penetrans* (L.) infections (reviewed in Legesse Negash, 2002), Enset mealy bug (Ferdu *et al*., 2009a) and diamond back moth (Bayeh and Biruk, 2009, unpublished).

Insecticidal bioactive agents derived from plants are processed into various forms which include: preparations of crude plant material, plant extracted oils and pure chemicals isolated from plants (Johanson, 1998).

The traditional uses of *M. ferruginea* in Ethiopia was reported by Banzouzi *et al.* (2008) that physiologically mature fruits were used for the treatment of pain, application of fruit paste mixed with some better and for amibiase, fruit mixed with honey is given orally. Leaves sap expressed for the treatment or earache and in case of bacterial infection of nails, they are bandaged with a paste of leaves and seed extracts were used as insecticidal properties (Banzouzi *et al*., 2008). The stems decoction against tooth aches (Lock, 1959, Neuwinder, 2000, Raponda-Walker and Sillans, 1961 cited in Banzouzi *et al*., 2008).

The extract of active compounds contained in birbira seed powder was used without further refinement in this work and might be extended to be used by small scale farmers. The most effective ingredient could be isolated and might be in the future formulated as commercial products.

There are several ways of administering insecticides to an insect and/or host plants. Feeding method has been used as one way of employing insecticides to evaluate the toxicity of ingested insecticides (Perry *et al.*, 1998). Therefore feeding assays using crude extract have been resulted in fed insect pest larvae with reduced growth, eating and utilization of ingested and digested treated substrate in a dose-dependent manner which implied that the crude extract act both as deterrent and toxic activities (Wheeler and Isman, 2001).

The test insects with different feeding habits and niche requirements such as aphids, sucking plant saps; diamond back moth, feeding externally on foliage and PTM with concealed feeding habits on tuber and leaves were treated to their different developmental stages and their host plants with birbira extracts to suppress their population below economic damage level. On this three major insect pests and host plants contact toxicity on aphids (Bayeh, 2007) and dipping methods to control DBM and PTM (Bayeh, 2009 unpublished) were employed. Perry *et al*. (1998) states that a commonly employed method is topical application, where the insecticide is dissolved in a relatively non-toxic solvent, such as acetone and when small plant feeding insects, stored product insects, house fly larvae, insect eggs etc…are considered, dipped in aqueous solutions, emulsions or suspensions of the insecticides for short periods of time may be employed. Topical application of sinigrin enhanced feeding of the larvae on the leaf disks of a non-host plant lettuce, *Lactuca sativa* (L.), but not on those of *Barbarea vulgaris* (Serizawa *et al.*, 2001), the reason that a feeding deterrent(s) in *B. vulgaris* leaves, which is extractable with chloroform, is responsible for this unacceptability (Serizawa *et al.*, 2001).

Poland (2006) reported that larval tunnels disrupt translocation of applied insecticides such as toxic botanical extracts. Penetration of active compounds into the leaves plays an important role in their systemic activity. That means transcuticular penetration into the leaf tissues plays a key role in foliar applied systemic and locally systemic insecticides (Zelena and Veverka, 2007). However to maximize the effectiveness of botanical treatments, sprays should thoroughly cover all plant surfaces, including the under sides of leaves

Toxicity interaction of insecticides with a given biological system is dose related. Sharaby (1988) account that the percentage of lesser cotton leaf worm, *Spodoptera exigua* (Hbn) egg hatchability decreased as the essential oil of lemon grass, *Cymbopogen citratus* concentration increased. According to Sharaby (1988) there was no significant difference in percentage of egg hatchability between untreated and treated egg masses with 0.25% of oil concentration. The highest dosage (0.3 %) of the essential oil of the *Vernonia amygdalina* induced the highest mortality in the maize weevil, *Sitophilus zeamais* (Asawalam and Hassanali, 2006). Extracts of *Pongamia glabra*, *Azadirachta indica* and *Chrysanthemum cinerariifolium* against insect pests of *Spodoptera littoralis*, *Myzus persicae* and *Tetranychus urticae* showed that the highest concentration caused 100%

mortality (Pavela, 2009). Application of the crude methanolic extract from *B. vulgaris* leaves deterred *Plutella xylostella* feeding on the leaf disks of cabbage (*Brassica oleracea* L.) in a dose-dependent manner (Serizawa *et al.*, 2001). Besides this, dose related interaction of insecticides was reported by Bayeh (2009 unpublished) that eggs oviposited on treated tubers in cages; the number of active galleries by PTM larvae and the number of pupae formed was lower at higher rates application of birbra extracts than the control and lower rates, on the other hand eggs oviposited away from treated potato tubers in cages, the number of terminated galleries and pupal formation increases at lower rates of birbira extracts application. Therefore the use of crude extracts of assay for the effect of different plant species with botanical property is an appropriate method.

3. MATERIALS AND METHODES

3.1. Study location

The study was conducted at Holetta Agricultural Research Center (HARC) in Insect Science Laboratory and green house and Addis Ababa University in Insect Science Research Laboratory.

3.2. Insect rearing

Potato tuber moth infested tubers of varieties of Gudenie and Jalenie were collected from HARC potato farm land during the harvesting season of 2009 for rearing. The tubers were 35kg and kept in sacks made of kaki cloth and in transparent plastic cages in the Insect Science Laboratory at HARC, at ambient temperature and humidity. Then the 4^{th} instar larvae moving out of the tubers for pupation and the pupae found on tubers surface and sacks were watched and collected regularly. During collection of 4^{th} instar larvae and pupae in stores, care was taken to reduce physical damage of eggs, larvae, pupae and even adults if present. Between 4^{th} week of December and end of April every developmental stage was seen in abundance in the rearing facilities. Besides this rearing was also done on an intact potato plant and excised leaves.

The emerging adults were enclosed in mica chambers to allow them lay eggs on tubera and leaves. As source of food they were provided with 10% sugar solution soaked in cotton balls. Once adults emerged mating occurred and within a few hours, depending on temperature, females seek a host on which to lay eggs (Rondon *et al.*, 2007a). The fresh, green potato leaves were replaced with care in vials arranged in mica chambers every other day and growing larvae from old leaves were transferred on to the fresh ones. This was carried out until most larvae pupated. The pupae were then transferred to Petri dishes and maintained in the Insectary at ambient temperature and humidity until adults emerged. The emerging adults both from the Petri dishes and potato storing sacks were used to establish the continuous cultures in the Holetta Insectary both for running, oviposition, ovicidal effects, and feeding deterrent assays and for culture maintenance.

To test oviposition deterrence of birbira seed powder extracts of the six solvents on treated tubers and excised leaves, 192 adults were used for each setting. A total of 32 (16 :16) per solvent extract of birbira and 2 pair of adults per treatment serial dilution were introduced into a transparent plastic cages and chambers for oviposition on tubers and leaves, respectively.

Potato seed sprouts of Gudenie variety were also collected from diffused light store at HARC and planted in staggered date in pots in the green house for rearing PTM on foliage and obtain healthy, insecticide free potato leaves for ovicidal, oviposition and larval settlement bioassays. All rearing and experimenting equipments were disinfected with 75% alcohol and washed with distilled water in order to reduce mortality factors and rear healthy PTM. During rearing PTM the necessary precaution was given for over crowding problems which causes physical damage, physiological stress and resource depletion. This might hinder continuous supply of eggs, larvae, pupae and adults, possibly due to waste accumulation, space sharing, complete damage of foliage or tubers.

Sex identification of adults for oviposition experiments and of pupae during rearing was done. Identification was made using a hand held magnifying lens and dissecting microscope. Therefore, adult size, abdominal segment and size, color markings on fore wings, structures on frontal hind wings, a characteristic movement interaction among PTM adults and pupae structures were applied for female and male PTM separation. Based on the above criteria, females are slightly larger in size than males, the tip of male abdomen is covered with thick hairy bunches but no hairy covers in females and the apex of male abdominal segment is longer than the rest of abdominal segments but in female the last abdominal segment is of same length to other segments, the fore wings posses 2 or 3 dark spots on males and a characteristic 'X' pattern on females, there are brush structures on frontal hind wings of males, not found in females, males fanned their wings while walking around females. The pupae that were emerging as males posses slightly curved apex where as pupae that were emerging as females do not posses curved apex.

Adult female and male PTM were collected using aspirator to be introduced in to oviposition chambers and cages, the most reliable sex identification method used in this research was the appearance of anal segment of the abdomen and their body size.

3.3. Plant material collection and extraction

Physiologically matured birbira, *M. ferruginea* pods containing seeds were collected in the location of HARC and placed in polythene bags and preserved at 4^0C.

The seed coat was removed and ground into fine powder manually using a mortar and Pestle and the powder was kept in polythene bags at 4^0C. Birbira seed powder was soaked and allowed to dissolve separately in hexane, toluene, chloroform, acetone, acetic acid and de-ionized water with their polarities of 0.1, 2.4, 4.1, 5.1, 6.2, and 9.0, respectively. Birbira seed powder weighing 50gm was put into each Pyrex glass beakers, containing 100ml of the considered solvents. Then, first stirred with glass road and further dissolved in the respective solvents using magnetic stirrer (Stuart Scientific UK) for 30 minutes and then allowed to settle. The supernatants were then separated and poured into separate Pyrex glass beakers, filtered through fine porous muslin cloth. Acetone, chloroform, toluene and hexane were placed on water bath evaporation set at their respective boiling points and the extracts obtained using the remaining two (de-ionized water and acetic acid) were dried in a rotary evaporator to remove the solvents.

The concentrated solids were used to prepare serial dilutions of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, 1.0mg/ml, 1.2mg/ml and 1.4mg/ml of water and readied for application on potato tubers and leaves and eggs to assay their effects on the biological performance of PTM eggs, larvae and adults. The extracts prepared from *M. ferruginea* seed powder using aqueous and organic solvents produced homogeneous and lemonade looking solutions (Bayeh, 2007). In this study, too when birbira seed powder extracts of both aqueous and organic solvents were prepared confirmed to be homogeneous and lemonade looking solutions. The acetic acid extract was found to have some amount of non homogenized particles or residues, while the most extract produced the same homogeneous and lemonade looking solution same to others. Therefore, the homogenous mixtures containing Pyrex flasks were put into a refrigerator at 4^0C until use and attained the ambient room condition on the spot of treatment application on potato tubers, leaves and PTM eggs.

3.4. Oviposition and feeding bioassay on tubers

Potato, *Solanium tuberosum* tubers of the variety Jalenie weighing 28 kg of healthy and insect pest free 300 tubers were stored under room temperature and relative humidity for three weeks. After three weeks, 240 tubers were washed with tap water, air dried and readied for oviposition and feeding assay. The extracts were prepared at the serial rates of dilutions (w/v): 0.0mg, 0.2mg, 0.4mg, 0.6mg, 0.8mg, 1.0mg, 1.2mg, and 1.4mg birbira seed powder/ml water at a time. Then five comparable sized tubers were dipped into each of the bowls containing the different concentrations of birbira seed powder extracts for 1hr. Then, the dipped tubers were taken out and air dried on paper towels. The five tubers that received same treatment were put with enough space between tubers into a transparent plastic cage. This was done to allow enough space for the tubers and to minimize movement of larvae between tubers, which is a rare case to occur because larvae once settled with in a tuber often remain concealed feeding in the tuber that first successfully tunnels until they matured and enter pupation.

Ovipositional response to potato tuber in the Insectary Laboratory was measured in no choice settings, where PTM adults and hatching larvae were allowed to contact tubers treated similarly with one treatment rate. Therefore, this assay setting was a no choice arena and 4 PTM, newly emerged adults (2 :2) were introduced into each cage. Females were allowed to oviposit on tubers treated with different six treatments and eight serial concentration levels for identical period (72 hrs) and then their ovipositional responses for different extracts of birbira seed powder were compared on the basis of the number of eggs laid during an identical period. Untreated tubers were serving as control and placed in similar transparent plastic cages. Each tuber was considered as a replicate. The number of eggs laid per tuber and away from tuber on the inner surfaces of each cages was counted and recorded. On a total of 240 potato tubers were experimented. The tubers were then kept for 35 days in dark room away from direct sun light in order to avoid greening on their surfaces of tubers. Regular observation was carried on the expected eggs hatching, pupation date and after 35 days and any change was recorded.

On potato tubers, the PTM larvae feed just below the surface and sometimes deep into the flesh. The tubers can appear black since the PTM larvae tunnels are filled with larval feces. Therefore, it is necessary to cut tubers to carefully check for signs of PTM larvae damage and the presence of larvae or pupae (Rondon *et al.*, 2007a), in diffused light stores or in the field during harvest as well as in the laboratory works. Accordingly, data was collected on the number of galleries carved by PTM larvae (terminated and dug deep and wide/active galleries). The larvae were maintained on tubers until pupated. The pupae were also maintained to record adult emergence. The adults emerged were recorded classified in to healthy and deformed.

3.5. Oviposition and Larval settling bioassay on leaves

The test of both oviposition and settling deterrence or allowance was conducted using green and fresh potato leaves which were similarly treated with the different extracts at the different serial dilutions that were prepared. When significant green foliage was available, larvae prefer to feed in the foliage, rather than in tubers (Rondon *et al.,* 2007a). Therefore, to assay responses of PTM oviposition, 5 leaves per serial dilution were taken from different potato plants of same age node below the apex of the main stem. The fresh green leaves were dipped for 1hr. with the different extracts at the different serial dilutions described above and prepared in bowls. Each treated leaf was dried and put in glass vials with drops of water and stopped using a cotton plug placed randomly in flat tray. Five glass vials that receive the same treated leaves were put within a clear mica chamber and 2 pairs of young adults of PTM were introduced inside each chamber with complete access to same treated leaves. 10 % sugar solution was soaked in cotton ball and put at the center of vials. Mating is expected to occur soon or longer for about 10hrs. Females begin laying eggs 2 days after emerging from their cocoons (Horgan *et al.,* 2007). After 3 days, the adults were removed from each chamber and the total number of eggs laid during the oviposition period on the leaves and elsewhere in the containers were counted and recorded.

Larvae feed on potato leaves and to assay responses of larval settlement, leaves were excised from potted potato plants grown in the green house, free of any insecticides and insect pests. As reviewed by Smith *et al.* (1994) various researchers have found no differences between insect feeding on excised and intact foliage, others also have reported differences in among insect feeding on intact Vs excised plants (the literature reviewed by Smith *et al.,* 1994).

Larval settlement response also done on green and fresh potato leaves similarly treated with water, acetic acid, acetone, chloroform, toluene and hexane extracts of birbira at the eight different serial dilutions (w/v: 0.0, 0.2, 0.4, 0.6. 0.8, 1.0, 1.2, 1.4 mg of bibira seed powder/ml of water as diluents) and put five Petri dishes labeled on with each rates and treatment types, were placed randomly on a flat table (Annex 13). Prior to laboratory setting was arranged for the neonate larvae settlement experiment, more number of cut fresh green leaves in glass vials with drops of water and stopped using a cotton plug were arranged for oviposition. Eggs laid on leaves were collected every day. The same aged eggs were kept until hatching in Petri dishes and in clear plastic cages. At ambient temperature and relative humidity in HARC Insectory embryonic development was completed after six days, just on the seventh day. Newly emerging larvae (neonate), five per leaf were carefully transferred to the birbira extract treated leaflet using a soft paint brush and the time of initial contact with the leaf was recorded and each larva was observed and the time it takes for larvae to settle and feed under the leaf sheath or abandon were recorded after 1, 2, 3, 4, and 5 hrs intervals using a hand-held magnifying lens, until the neonate established on or abandoned the leaf. The hatching neonate larvae were burrows into the leaves and settle to feed. However, this was occurring in less than five hours. Therefore, data was taken on the number of feeding larvae, repelled larvae (found on Petri dishes away leaves) per leaf. Larvae may die while attempting to settle and this was also recorded.

3.6. Ovicidal bioassay

For ovicidal effect assays, a potted potato plants were readies a priory. Leaves for oviposition were cut and arranged in glass vials with water and stopped using a cotton plug. Glass vials bearing untreated leaves were placed in 8 transparent mica cages to get enough number of egg masses.

Newly emerged PTM adults of males and females were introduced into each chamber. Females were allowed to lay eggs for three days in the provided substrates and removed, when they are found alive. The egg masses laid on provided green and fresh untreated potato leaves were recorded and then a leaf or leaves bearing 25 eggs were dipped for 3 seconds into each birbira extract of the serial dilutions prepared in the bowls. Similarly, for the control a leaf or leaves carrying on 25 eggs were treated with water. After 3 seconds, leaves with 25 eggs that received same treatments were again held in vials with water and stopped using a cotton plug placed in each chamber and then were incubated at room conditions.

Hatching periods may vary depending on temperature variation in different areas, season’s. In this study most of the eggs started hatching after six days of embryonic development at ambient temperature and relative humidity. Hatched and unhatched eggs were counted to determine the percentage of hatchability. The experiment was carried out in a completely randomized design. Twenty five eggs per rates and a total of 1200 eggs (200 eggs per treatment) were tested.

4. Data analysis

Data were analyzed using JMP IN (2000) version 5.0 and SPSS (2003) version 13.0. The collected data was checked for normality and those parameters that may require transformation was log normalized to run ANOVA (Sokal and Rolf, 1994 cited in Bayeh in unpublished, 2009). For data on tubers, one-way analysis of variance was done on the number of eggs oviposited per tuber. The number of terminated and active galleries was also computed and analyzed using one way ANOVA. The differences among treatments were determined with a Tukey test at 5% probability level. The number of adults emerged (healthy and deformed) tabulated and compared for each extracts and rates applied.

Eggs laid away from treated tubers on the inner surfaces of cages and away from treated leaves on inner surfaces of chambers compared on line graphs. For data on leaves which include oviposited number of eggs and settled neonate larvae per leaf was under go similar validation and analysis of variance carried out. The susceptibility of a PTM eggs to a treatments of each serial concentrations was analyzed by hatched and unhatched eggs count to determine the percentage of hatchability.

Natural mortality in the control was adjusted % mortality of eggs/neonates were computed and used according Abbott's formula (Abbott 1925, cited in Perry *et al.*, 1998). Mortality for eggs and neonates in the control treatment was used to correct mortality in the other treatments. This adjustment was obtained using Abott's formula (% Corrected mortality = $(P-P_0 /100- P_0)*100$, where P represents % mortality in the control and P_0 = % mortality of insects in the untreated control). Using probit regression (SPSS 13.0) the LC50 values foe the six extracts were determined. Dose dependent eggs/neonate larval mortality curves were produced using the probit out puts.

5. RESULTS

5.1. Oviposition and feeding responses of PTM to Birbira extracts treated tubers

5.1.1. Oviposition response

The effect of birbira seed powder extracts on the oviposition by *P. operculella* on tubers is presented in Tables 2. The number of eggs laid per tuber was more significantly affected by treatment ($F_{47, 192}$ = 6.49, P< 0.0001) and by rate ($F_{47, 192}$ = 12.14, P< 0.0001). However, the number of eggs oviposited was not found to be affected significantly by the interaction of extracts and the rate of application ($F_{47, 192}$ = 1.31, P< 0.1282).

There was no significant difference in the number of eggs laid on tubers treated with birbira extracts at the rate of 0.2mg/ml applied ($F_{5, 24}$ = 2.61, P> 0.0506) and the control ($F_{5, 24}$ = 0.20, P> 0.955). There was no significant difference in the number of eggs laid on tubers dipped at the rate of 0.4mg/ml of all extracts applied ($F_{5, 24}$ = 1.00, P> 0.4372), whereas on tubers treated at the rate of 0.6mg/ml of chloroform and water extracts significantly lower the number of eggs were found oviposited ($F_{5, 24}$ = 5.76, P< 0.0012). There was no significant difference in the oviposited eggs on tubers treated at the rate of 0.8mg/ml of all the extracts ($F_{5, 24}$ = 1.43, P> 0.2477) and significantly lower the number of eggs were deposited on tubers treated with chloroform extract at the rate of 1.0mg/ml applied compared to the other extracts ($F_{5, 24}$ = 3.29, P> 0.02). On tubers treated at the rate of 1.4mg/ml significantly lower the numbers of eggs were laid with water, chloroform and hexane ($F_{5, 24}$ = 7.06, P< 0.0003) extracts treated tubers. The eggs deposited on tubers treated with hexane, chloroform, and water extracts were found less in number than the other extracts treated tubers (Table 2), whereas on tubers treated with toluene and acetic acid extracts there was no significant difference in the number of eggs laid among all the rates including the control.

Table 2 Effects of birbira seed powder extracts obtained using polar and non-polar solvents on oviposition response of PTM on Potato tuber.

Rate (mg/ml)	Number of eggs laid per tuber (mean ± SE)*					
	AABSP	ABSP	CHBSP	HBSP	TBSP	WBSP
0.0	10.00±0.70aA	9.60±0.81aA	9.80±0.66aA	9.20±0.58bA	9.20±0.86aA	9.60±0.50aA
0.2	12.00±3.33aA	7.80±2.05aA	4.40±1.36bA	11.00±0.63aA	7.80±1.68aA	4.40±1.72abA
0.4	7.80±0.40aA	4.20±1.24abA	3.80±1.11bA	7.20±1.59bA	6.80±3.00aA	7.00±1.58aA
0.6	7.80±0.96aB	4.20±0.86bB	2.00±0.31bA	7.60±1.66abB	7.00±1.70aB	2.00±0.54bA
0.8	6.00±2.66abA	4.00±0.77bA	2.00±0.70bA	5.60±2.11bA	6.60±1.53aA	2.40±0.81bA
1.0	6.00±1.76acB	3.80±1.74bB	1.00±0.00cA	3.20±1.06bB	6.80±1.24aB	2.00±0.63bB
1.2	4.00±1.30aA	3.80±0.86bA	2.40±0.60bA	0.80±0.20cA	5.20±1.77aBA	2.40±0.60bA
1.4	5.20±1.42aB	3.80±0.48bB	0.80±0.20cA	0.40±0.24cAB	4.60±1.02aB	1.60±0.40cA

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD); ABSP-acetic acid extracts of birbira seed powder; ABSP- acetone extracts of birbira seed powder; CHBSP- chloroform extract of birbira seed powder; TBSP- toluene extract of birbira seed powder; WBSP- water extract of birbira seed powder.

5.1.2. Active and terminated galleries carved on tubers by PTM larvae

The effect of birbira extracts of polar and non-polar solvents on the terminated galleries and active galleries carved by PTM larvae on treated tubers is presented (Tables 3 and 4). The mean number of active galleries was found to be not significantly affected by the treatment types ($F_{47, 192}$ = 1.25, P> 0.28). Instead it was more significantly affected by rates ($F_{47, 192}$ = 30.49, P<.0001) and by the interaction of extracts and the rate of application ($F_{47, 192}$ = 2.24, P< 0.0003). The numbers of terminated galleries that occurred on potato tuber treated with birbira seed powder extracts were found significantly affected by the rates of application ($F_{47, 192}$ = 8.59, P< 0.0001). There was no significant difference in the number of terminated galleries affected by extracts ($F_{47, 192}$ = 0.63, P> 0.6708) and it was marginally significantly affected by the interaction of extract by rate of application ($F_{47, 192}$ = 8.59, P< 0.04) and by rates alone ($F_{47, 192}$ = 8.59, P< 0.0001).

Active galleries carved by the PTM larvae at each rate applied for comparison between treatments was found that no significant difference in the mean number of active galleries carved by PTM on the control potato tubers ($F_{5, 24} = 1.09$, P> 0.389) (Table 3). At the rate of 0.2mg/ml significantly more active galleries were recorded on hexane extract than all the other treatments. The lower active galleries were found in water and toluene extract treated tubers ($F_{5, 24} = 3.58$, P< 0.0146). At the rates between 0.4mg/ml and 0.8mg/ml there was no significant difference among the different extracts. At the higher rates (1.0mg/ml - 1.4mg/ml) significantly more active galleries were found in toluene extract tubers except at the rate of 1.4mg/ml in which the water extract allowed more active galleries to be carved on tubers. This is unlike the lower rates of water extract between 0.8mg/ml and 1.2mg/ml that reduced significantly the number of active galleries carved.

There was no statistically significant difference in the mean number of terminated galleries carved by the larvae on tubers treated with acetic acid birbira extract at both lower and higher rates applied including the controls ($F_{7, 32} = 7.68$, P> 0.6514). Similar results were obtained for the acetone extract ($F_{7, 32} = 1.274$, P> 0.2942) and chloroform extract except at the rate of 1.4mg/ml at which significantly lower number of terminated galleries were found ($F_{7, 32} = 2.38$, P< 0.0441). There was no also significant difference in the number of terminated galleries tunneled by the larvae in tubers that were dipped in hexane extract at the rates of 0.2mg/ml, 0.6mg/ml and the control ($F_{7, 32} = 8.62$, P< 0.0001). Significantly lower numbers of terminated galleries were however found in tubers treated at rates 0.4mg/ml, 0.8mg/ml, 1.0mg/ml and 1.2mg/ml. Similarly, significantly lowest numbers of terminated galleries were found in tubers treated at rate 1.4mg/ml applied ($F_{7, 32} = 8.62$, P< 0.0001). The number of terminated galleries found in tubers that were dipped in toluene birbira seed powder extracts at the rate of 0.2mg/ml, 1.0mg/ml and 1.4mg/ml was significantly lower ($F_{7, 32} = 3.83$, P < 0.0039), while in other rates no significant difference was found. There were significantly lower mean numbers of terminated galleries found on tubers treated with water extract at all the rates and the control ($F_{7, 32} = 11.08$, P < 0.0001).

There was no statistically significant difference among the controls set with each of the treatments ($F_{5, 24}$ = 1.48, P> 0.2309). Significantly lower numbers of terminated galleries were found in tubers dipped in toluene birbira seed powder extracts at the rate of 0.2mg/ml than all the other rates ($F_{5, 24}$ = 4.07, P< 0.0081). At the rate of 0.4mg/ml significantly lower numbers of terminated galleries were recorded for water extract than the other rates ($F_{5, 24}$ = 3.26, P< 0.0218). Similar responses were also found at all the higher rates except at the rate of 0.8mg/ml (Table 4).

Table 3 Active galleries carved by PTM larvae on potato tubers treated with birbira seed powder extracts obtained using polar and non-polar solvents.

Rate (mg/ml)	Number of deep galleries per tuber (Mean ± SE)					
	AABSP	ACBSP	CHBSP	HBSP	TBSP	WBSP
0.0	2.40±0.50aA	3.40±0.24aA	4.00±0.44aA	3.20±0.48aA	3.00±1.00aA	2.60±0.24aA
0.2	2.80±0.48aB	3.60±0.50aAB	3.40±0.81aAB	4.60±0.67aB	1.80±0.20abA	2.00±0.44abA
0.4	2.40±0.50aA	1.20±0.58abA	3.40±0.92aA	0.60±0.40bA	3.20±1.06aA	2.40±0.67aA
0.6	1.00±0.63abA	1.20±0.58abA	1.20±0.58bA	2.40±0.87abA	0.00±0.00bA	1.60±0.24abA
0.8	0.80±0.80abA	0.60±0.60bA	0.80±0.58bA	0.80±0.37bA	1.00±0.63abA	0.60±0.40bA
1.0	0.00±0.00bA	0.80±0.58bA	1.40±0.50abA	0.00±0.00cA	0.00±0.00bA	0.80±0.37abA
1.2	0.00±0.00bB	0.60±0.40bAB	0.00±0.00bB	0.00±0.00cB	1.60±0.50abA	0.20±0.20bB
1.4	0.00±0.00bA	0.00±0.00bA	0.00±0.00bA	0.00±0.00cA	1.00±0.63abA	1.40±0.50abA

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Table 4 Terminated galleries carved by PTM larvae on potato tubers treated with birbira seed powder extracts obtained using polar and non-polar solvents.

Rate (mg/ml)	Number of terminated galleries per tuber (mean ± SE)					
	AABSP	ACBSP	CHBSP	HBSP	TBSP	WBSP
0.0	10.00±1.14aA	8.20±0.86aA	9.20±1.06aA	7.40±0.50aA	8.00±0.54aAB	9.80±0.86aA
0.2	10.40±2.37aB	8.80±1.88aB	11.40±1.63aB	8.00±0.89aB	3.00±0.00bA	5.60±1.28bBA
0.4	10.80±2.22aB	8.00±1.22aBA	7.20±0.73aBA	5.00±0.89bA	6.20±1.01abBA	4.40±1.02bA
0.6	8.20±1.77aA	8.60±1.56aA	8.80±2.51aA	9.60±1.16aA	4.40±1.40abA	3.00±0.44bA
0.8	7.80±0.86aA	4.80±1.24aA	6.20±2.31aA	3.40±0.24bA	4.00±1.30abA	2.80±0.48bA
1.0	7.40±1.91aAB	10.00±1.73aB	6.00±1.22aAB	3.60±1.28bA	3.20±0.73bA	3.00±0.70bA
1.2	8.60±2.03baB	10.20±1.59aB	5.80±1.06aAB	3.60±0.67bA	4.00±0.70abA	2.20±0.37bA
1.4	6.20±2.17aA	7.20±1.71aA	3.80±0.73bA	2.00±1.14bA	3.40±0.40bA	2.00±0.54bA

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at $P< 0.05$ using Tukey Kramer (HSD).

The emergence of PTM adults (Healthy, Deformed) from treated potato tubers with birbira seed powder extracts is presented (Annex 9). In all the treatments the emerged adults are not deformed except in acetic acid and chloroform extracts treated tubers. However, the adult emergence did not show clear pattern as was the case for most of the cases with the larvae.

5.2. Oviposition response of PTM to Birbira extracts treated potato leaves

The effect of birbira seed powder extracts on the oviposition by *P. operculella* on potato leaves is presented in Tables 5. The oviposition response on leaves by *P. operculella* was significantly influenced by rate ($F_{47, 192} = 14.66$, $P< 0.0001$) and interaction of extract and rate of application ($F_{47, 192} = 4.12$, $P< 0.0001$), whereas oviposition was not significantly affected by treatment alone ($F_{47, 192} = 0.05$, $P> 0.99$).

The rate applied on potato leaves per treatment was compared along rows for the six extracts of birbira seed powder and there was no significant difference among the control which was treated with water ($F_{5, 24}$ = 0.63, P> 0.68). There was also no significant difference in the mean number of eggs oviposited on potato leaves treated with extracts at the rates of 0.2mg/ml ($F_{5, 24}$ = 0.89, P> 0.50). The lowest numbers of eggs were oviposited on tubers treated with acetic acid and water extracts ($F_{5, 24}$ = 16.28, P< 0.0001). Particularly, acetic acid, acetone, chloroform and water extract prevented oviposition. More eggs were oviposited on leaves treated with lower rates of the extract than the three higher rates ($F_{7, 32}$ = 14, P < 0.0001). The response for acetone extract treated leaves was similar to acetic acid extract effects. All extracts except toluene and hexane showed that more significantly lower number of eggs was oviposited on leaves treated with the rates 1.0-1.4mg/ml than all the lower rates (Table 5).

Table 5 Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the oviposition response of PTM on Potato leaves

Rate (mg/ml)	Mean(±SE) number of eggs laid on potato leaf					
	AABSP	ABSP	CHBSP	HBSP	TBSP	WBSP
0.0	9.40±0.87aA	9.20±0.58aA	9.20±0.86aA	9.20±0.86aA	8.80±0.73bA	9.20±0.37aA
0.2	1.20±1.20bA	1.60±1.02bA	1.60±1.60bA	2.60±0.81bA	3.80±0.66dA	1.40±0.67bA
0.4	0.60±0.60bB	4.60±1.88bB	4.60±0.97abB	5.00±0.83dB	13.20±0.58aA	0.60±0.60cB
0.6	4.60±1.36abB	3.60±1.07bB	7.00±2.02aC	3.00±0.70bB	9.40±0.40bC	0.20±0.20cA
0.8	2.80±1.31bA	1.60±0.67bA	6.00±2.25aA	3.20±0.86bA	5.00±0.31dA	1.20±0.58bA
1.0	0.00±0.00cA	0.00±0.00cA	0.00±0.00bA	1.20±0.80cA	2.00±0.83cA	0.40±0.24cA
1.2	0.00±0.00cA	0.40±0.24cB	0.00±0.00bA	0.40±0.40cA	2.40±0.67cC	0.00±0.00cA
1.4	0.00±0.00cA	0.20±0.20cA	0.80±0.80bA	0.20±0.20cA	2.00±0.44cB	0.20±0.20cA

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

5.3. Oviposition deterrence effect of birbira seed powder

This was measured in terms of the number of eggs oviposited away from treated potato tubers or leaves. This was found to increase with increasing rates of application of the different extracts both on tubers and leaves of potato. These shows clearly the levels of oviposition deterrence each of the extracts have effected. The present study results have shown that the degree of deterrence varied between the extracts and rates of application on tubers as well as on potato leaves (Annex 7).

5.4. Ovicidal effects of extracts of Birbira seed powder on PTM eggs

The mean number of hatched eggs was significantly lower at the tested rates ($F_{7, 40}$ = 4.55, P< 0.001) and it was also significantly lowered by both extracts and rates of application ($F_{7, 40}$ = 4.306, P< 0.003). The highest percentage of unhatched eggs was recorded with range of 60% to 100% by all solvent extracts of *M. ferruginea* seed powder considered in this study except hexane extract which is below 40% in all the rates of applications. The highest percentage of eggs were killed or failed to hatch after treated with birbira seed powder extracts of acetone, water and acetic acid (Annex 8). The preliminary test of eggs dipped with water birbira seed powder extracts were found unhatched or failed to hatch. When it was checked under a light microscope the eggs surface was found shrank and a depression on the surface was observed.

5.4.1. Toxicity of birbira seed powder extract on PTM eggs

LC_{50} value was calculated using SPSS version 13.0 after the raw data was transformed and corrected for natural mortality. Results indicated (Table 6) that water extracts of birbira seed powder was more toxic to the *P. opercullela* eggs, where the LC_{50} was 0.23, closely followed by acetone (0.24). The least toxic was obtained with hexane extract. The rate dependent mortality values of PTM eggs are presented as dosage mortality curves obtained through probit analysis. The log dose dependent mortality of eggs supposed to assume linearity (Fig. 8).

Acetone and water extracts dose mortality curve showed high number of egg mortality at the lowest rate of application than the other extracts. Similarly, chloroform extract dose mortality curve indicated that an increased slope as rate increased. The plain like curve at all rates of application was indicated in hexane extract (Fig.8).

Table 6 Probit models for the six solvent extracts obtained after parameter estimates converged 32 repeated iterations (dose mortality of eggs).

PTM development stage	Extract type	LC_{50} of birbira extract	95% confidence limits
Eggs	Water	0.23	0.12 - 0.37
	Acetic acid	0.41	0.22 - 0.58
	Acetone	0.24	0.13 - 0.40
	Chloroform	1.25	0.76 - 2.00
	Toluene	0.93	0.60 - 1.40
	Hexane	2.95	1.30 - 6.42

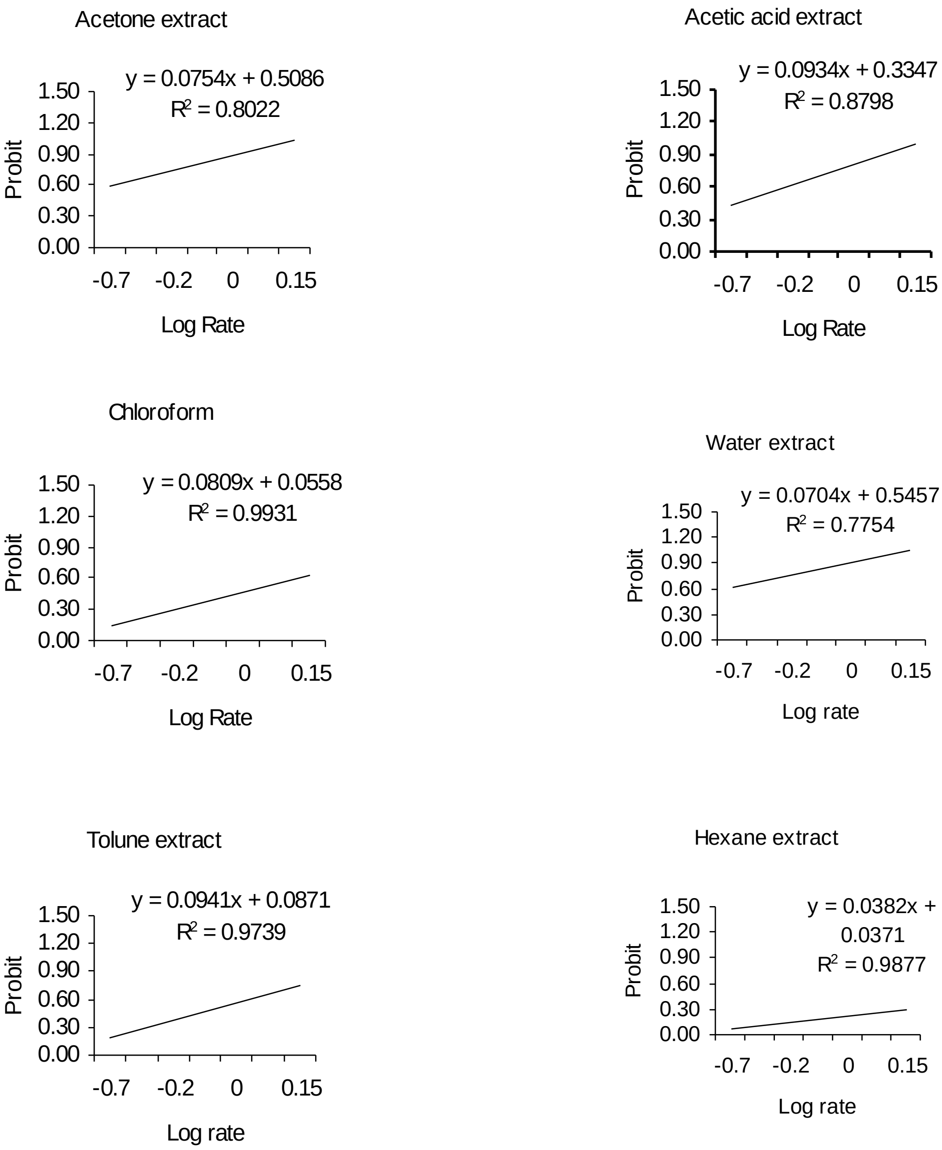


Fig. 8 Linear regression means percent on egg mortality caused by birbira seed powder extracts.

5.5. Effects of Birbira seed powder extracts on settling of PTM neonate larvae

There was less number of neonates settling within 1 hour observation at all the rates of application and the control for acetic acid extract of birbira seed powder. However marginally significant difference was observed at the higher rates compared with the control and lowest rates ($F_{7, 32} = 2.33$, $P< 0.04$). Statistically significant settling of neonate larvae were found after 2 hours exposure of acetic acid birbira seed powder treated leaves fed by larvae as compared with that of water treated control ($F_{7, 32} = 3.23$, $P<0.01$). More significant differences were found at the highest rate of application (1.4mg/ml) ($F_{7, 32} = 3.23$, $P<0.01$). During the hours 3 observations no significant feeding response was found at the rate of 0.2mg/ml and the control, whereas in all the higher rates there was significantly poor settling responses ($F_{7, 32} = 8.37$, $P< 0.0001$). More significant differences were found at the highest three rates treated leaves ($F_{7, 32} = 8$, $P< 0.0001$). After 4 hours of observations a significantly lower feeding responses were recorded at rates greater than 0.6mg/ml up to the highest rates, where as at the rates of 0.2mg/ml, 0.4mg/ml and the control no statistically significant feeding responses were found ($F_{7, 32} = 9.41$, $P< 0.0001$).

Table 7 Effects of acetic acid birbira seed powder extract treatment on cumulative settling of PTM neonate larvae on potato leaves.

Rate (mg/ml)	Settled larvae (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.80±0.37 a	2.00±0.54a	4.20±0.48a	4.20±0.48a	4.20±0.48a
0.2	0.20±0.20a	1.60±0.67a	2.20±0.91ab	2.40±0.97a	2.40±0.97a
0.4	0.80±0.37a	1.20±0.58a	1.80±0.73b	2.40±0.60a	2.40±0.60a
0.6	0.80±0.37a	1.00±0.31a	1.40±0.40b	1.40±0.40b	1.40±0.40b
0.8	0.20±0.20b	0.40±0.24a	0.60±0.24b	0.60±0.24c	0.60±0.24c
1.0	0.00±0.00b	0.20±0.20a	0.20±0.20b	0.20±0.20c	0.20±0.20c
1.2	0.00±0.00b	0.20±0.20a	0.20±0.20b	0.20±0.20c	0.20±0.20c
1.4	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00c	0.00±0.00c

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Within the first hour to the second hours observations a similar results were found with acetone birbira seed powder extract and settling inhibition was not statistically significantly higher in the control ($F_{7, 32} = 32.66$, $P<0.0001$) and ($F_{7, 32} = 72.25$, $P< 0.0001$) respectively. Neonate larvae feeding inhibition clearly started even at lower rates with very closer means to the higher rates of applications. However, during the third period of observations, there was significant settling inhibition differences at all the rates of applied (0.2mg/ml to 1.4mg/ml) compared to the water treated potato leaves (control) ($F_{7, 32}$ = 42.47, P< 0.0001). A significant settling responses differences were also maintained during the forth ($F_{7, 32}$ = 126.00, P< 0.0001) and fifth ($F_{7, 32}$ = 322.66, P< 0.0001) hours observations at all the rates against the control.

Table 8 Effects of acetone birbira seed powder extract treatment on cumulative settling of PTM neonate larvae.

Rate (mg/ml)	Settling response after (Mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	1.40±0.24b	3.40±0.40b	3.80±0.58b	4.20±0.37b	4.40±0.24b
0.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.6	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.8	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Mean number of neonate larvae settled in chloroform birbira seed powder extract was not significantly different during hour 1 ($F_{7, 32}$ = 2.50, P< 0.04) and hour 2 ($F_{7, 32}$ = 2.07, P> 0.07) at both the rates treated on excised potato leaves including the water treated control. During 3 hours observations, there were significantly smaller mean number of neonate larvae settled ($F_{7, 32}$ =21.71, P< 0.0001) at both lower and higher rates treated potato leaves. There was also statistically significant mean number of feeding responses differences at the rates greater than 0.4mg/ml treated leaves after 4 hours of observations ($F_{7, 32}$ = 38.58, P< 0.0001). Consistently more larvae settled in the control and at the lowest rate applied.

Table 9 Effects of chloroform birbira seed powder extract treatment on cumulative settling of PTM neonate larvae.

Rate (mg/ml)	Settled larvae after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	1.00±0.63b	1.60±1.02b	4.00±0.63b	4.40±0.40b	4.40±0.40b
0.2	0.00±0.00a	0.40±0.40a	1.20±0.58ab	1.20±0.58a	1.20±0.59a
0.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c
0.6	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c
0.8	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c
1.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c
1.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c
1.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00c	0.00±0.00c

* Means of five replicate

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

At the rate of 0.8mg/ml there was marginally significantly lower differences in hexane birbira seed powder extract ($F_{7, 32}$ = 2.33, P< 0.04) after 1 h, where as at all other rates, there was no statistically significant difference mean numbers of neonate larvae settled including the control. Settling of PTM neonate larvae in hexane birbra seed powder extract after hour 2 was significantly lower at the higher rates and so was also at the lower rate (0.2mg/ml) ($F_{7,.32}$ = 7.05, P< 0.0001). And there was no significant number of settled neonates at the rate of 0.4mg/ml and the control ($F_{7, 32}$ = 7.05, P< 0.0001). Within hour 3 there were statistically significant differences among lower and higher rates (i.e. 0.2mg/ml, 0.6mg/ml, 0.8mg/ml, 1.0mg/ml, 1.2mg/ml, and 1.4mg/ml) ($F_{7, 32}$ = 9.79, P< 0.0001) and no significant difference was found at the rate of 0.4mg/ml and the control. After 4 hours observations, no significant differences of mean numbers settling neonate larvae was found between 0.2mg/ml, 0.4mg/ml and the control ($F_{7, 32}$ = 13.71, P< 0.0001). The rate of 0.6mg/ml was significantly different with the highest rate (1.4mg/ml) and the control ($F_{7, 32}$ = 13.71, P< 0.0001). There were significantly lower number of larvae settled among rates of 0.8mg/ml, 1.0mg/ml, 1.2mg/ml, and 1.4mg/ml treated leaves ($F_{7, 32}$ = 13.71, P< 0.0001).

Table 10 Effects of hexane birbira seed powder extract treatment on cumulative settling of PTM neonate larvae.

Rate (mg/ml)	Settled larvae after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	1.80±0.20b	4.00±0.44c	4.80±0.20c	4.80±0.20b	4.80±0.20b
0.2	0.80±0.37b	1.80±0.48b	2.60±0.50ac	3.40±0.67b	3.40±0.67b
0.4	1.00±0.44b	2.80±0.86bc	3.40±0.92c	3.80±0.58b	3.80±0.58b
0.6	0.40±0.24b	1.40±0.24b	2.60±0.24c	2.60±0.24c	2.60±0.24c
0.8	0.20±0.20a	0.40±0.24a	0.80±0.37a	0.80±0.37a	0.80±0.37a
1.0	0.60±0.24b	0.60±0.24a	0.80±0.37a	0.80±0.37a	0.80±0.37a
1.2	1.20±0.58b	1.20±0.58b	1.40±0.50b	1.40±0.50a	1.40±0.50a
1.4	0.40±0.24b	0.60±0.24a	0.60±0.24a	0.60±0.24a	0.60±0.24a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

There was no significant difference observed within the first hour in toluene birbira seed powder extract treated potato leaves on settling responses of neonate larvae ($F_{7, 32}$ = 1.64, P> 0.16). Similar results were found during the second hours observation to the first hour visit ($F_{7, 32}$ = 2.34 P< 0.05). More significant differences were found at the rate of 1.0mg/ml within 2 hours observation. Within the third hours of observations, there were statistically significant neonate larvae feeding on a leaves in toluene birbira extract at both lower (0.2mg/ml) and higher (0.6mg/ml, 0.8mg/ml, 1.0mg/ml, 1.2mg/ml, and 1.4mg/ml) rates of treated leaves on settling of the PTM larvae ($F_{7, 32}$ = 8.64, P< 0.0001) and no significant settling differences between the rate of 0.4mg/ml and the control ($F_{7, 32}$ = 8.64, P< 0.0001). After hour 4 a significant differences were found between all the rates of application (lower and higher rates) and the control ($F_{7, 32}$ = 12.98, P< 0.0001).

Table 11 Effects of toluene birbira seed powder extract treatment on cumulative settling of PTM neonate larvae.

Rate (mg/ml)	Settled larvae after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.60±0.40a	1.20±0.20a	2.80±0.37b	4.00±0.63b	4.00±0.63b
0.2	0.40±0.24a	0.60±0.40a	0.80±0.37a	1.20±0.37a	1.20±0.37a
0.4	0.80±0.37a	1.20±0.37a	1.40±0.24ab	1.40±0.24a	1.40±0.24a
0.6	0.20±0.20a	0.20±0.20a	0.20±0.20a	0.20±0.20a	0.20±0.20a
0.8	0.00±0.00a	0.20±0.20a	0.20±0.20a	0.20±0.20a	0.20±0.20a
1.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.2	0.00±0.00a	1.00±0.54a	1.00±0.54a	1.00±0.54a	1.00±0.54a
1.4	0.20±0.20a	0.40±0.24a	0.40±0.24a	0.40±0.24a	0.40±0.24a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Significantly different larval settling responses were found at the first hour observation in water birbira seed powder extract at the rates: 0.4mg/ml, 0.6mg/ml, 1.0mg/ml, 1.2mg/ml and 1.4mg/ml treated leaves ($F_{7, 32}$ = 4.01, P< 0.003), where as at the rates of 0.2mg/ml and 0.8mg/ml and the control there was no significant differences. There were no significant differences at the rates of 0.2mg/ml, 0.6mg/ml, and 0.8mg/ml compared to the control ($F_{7, 32}$ = 4.41, P< 0.0016) during hours 2 observations. Significant feeding responses were found at the lower rates (0.4mg/ml) and higher rates (1.0, 1.2 and 1.4mglml) of water birbira seed powder extract ($F_{7, 32}$ = 4.41, P< 0.0016). There was no significant mean number of neonate larvae settling at the rate of 0.2mg/ml treated potato leaves and water treated control ($F_{7, 32}$ = 12.82, P< 0.0001) (Table 12). Between the forth hours to fifth hours of observations statistically significant settling differences were found at both lower and higher rates of treated potato leaves against the water treated control ($F_{7, 32}$ = 16.40, P< 0.0001) and ($F_{7, 32}$ = 17.51, P< 0.0001).

Table 12 Effects of de-ionized water birbira seed powder extract treatment on cumulative settling of PTM neonate larvae.

Rate (mg/ml)	Settled larvae after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	1.20±0.37b	1.40±0.24b	3.20±0.58b	3.80±0.20b	4.00±0.31b
0.2	1.00±0.44ab	1.20±0.58b	2.00±0.54b	2.00±54b	2.20±0.37c
0.4	0.00±0.00a	0.00±0.00a	0.20±0.20a	0.80±0.58c	0.80±0.58a
0.6	0.00±0.00a	0.20±0.20ab	0.20±0.20a	0.20±0.20c	0.60±0.40a
0.8	0.40±0.40ab	0.40±0.40ab	0.60±0.40ab	0.60±0.40c	0.60±0.40a
1.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
1.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

5.6. Effects of birbira seed powder extracts on cumulative mortality responses of PTM neonate larvae

The mean neonate larval mortality caused by birbira extract obtained using polar and non-polar solvents on potato leaves is presented in Table 13 to 18. No significant mortality differences, at hour 1 and hour 2 periods in acetic acid birbira extract treated leaves on transferred PTM neonate larvae at all rates of application ($F_{7, 32}$ = 1.07, P> 0.39) and ($F_{7, 32}$ = 2.23, P> 0.05) respectively. There were no significant mortality differences also at 3 hours periods, too ($F_{7, 32}$ = 2.71, P< 0.0248). At hour 4 particularly at lower rates (0.2mg/ml, 0.4mg/ml, 0.6mg/ml) and at the highest rate (1.4mg/ml) including the control significant lethal effect was observed ($F_{7, 32}$ = 7.51, P< 0.0001). Particularly more significant difference was revealed at the rate of 0.2mg/ ml ($F_{7, 32}$ = 7.51, P< 0.0001). Among the rates: 0.8md/ml, 1.0mg/ml and 1.2mg/ml there was no significant separation in the mean larval mortality ($F_{7, 32}$ = 7.51, P< 0.0001). Generally there was significant larval mortality among the highest rates of application (0.8mg/ml, 1.0mg/ml, 1.2mg/ml and 1.4mg/ml) after five hours observation ($F_{7, 32}$ = 11.20, P< 0.0001).

Table 13 Mean neonate larvae mortality caused by acetic acid birbira extract on potato leaves.

Rate (mg/ml)	Mortality after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.20±0.20b	0.60±0.40b
0.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00b	1.20±0.73c
0.4	0.00±0.00a	0.00±0.00a	0.20±0.20a	0.20±0.20b	1.60±0.40c
0.6	0.00±0.00a	0.00±0.00a	0.60±0.40a	1.20±0.58ab	1.60±0.50c
0.8	0.80±0.80a	1.80±0.80a	2.00±0.70a	2.20±0.58a	3.20±0.58c
1.0	1.20±0.73a	1.80±0.91a	2.80±1.15a	3.80±0.58a	4.80±0.20a
1.2	1.20±0.96a	1.40±0.97a	1.40±0.97a	2.60±0.87a	3.40±0.60ac
1.4	0.40±0.24a	0.60±0.24a	0.80±0.20a	1.00±0.31ab	4.80±0.20a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

After 1 hr to treatment application, significant larval mortality was observed at the rates between 0.4mg/ml and 1,4mg/ml. In all the latter observations, the acetone extract treated leaves at all the rates applied caused significant neonate larvae mortality when compared with the control (Table 14).

Table 14 Mean mortality effect of acetone birbira extract to neonate larvae of *P. opercullela* on potato leaves.

Rate (mg/ml)	Mortality after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
0.2	0.20±0.20b	1.40±0.87b	3.00±0.77a	3.80±0.20a	3.80±0.20a
0.4	2.80±1.01a	4.20±0.37a	4.60±0.40a	4.60±0.40a	4.60±0.40a
0.6	1.80±0.96a	3.00±0.63a	3.20±0.66a	3.20±0.66a	3.20±0.66a
0.8	4.00±0.63a	4.00±0.63a	4.00±0.63a	4.00±0.63a	4.00±0.63a
1.0	2.00±0.83a	3.20±0.20a	3.40±0.24a	3.40±0.24a	3.40±0.24a
1.2	2.20±0.96a	2.60±0.81a	2.60±0.81a	3.00±0.54a	3.00±0.54a
1.4	2.80±1.15a	3.40±0.92a	3.40±0.92a	3.40±0.92a	3.40±0.92a

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

There was a statistically significant mean neonate larvae mortality difference between the chloroform extract at all the rates and the control ($F_{7, 32} = 3.77$, $P < 0.004$) after the first hour of observation. More significant mean mortality differences were found at hour 2 observation between the control and all the extract rates ($F_{7, 32} = 11.54$, $P < 0.0001$). The mortality differences remain significantly different between the control and the chloroform extract after 3-5 hrs of observation (Table 15).

Table 15 Mean mortality effect of chloroform birbira extract to neonate larvae of *P. opercullela* on potato leaves.

Rate (mg/ml)	Mortality after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00a	0.00±0.00a	0.20±0.20a	0.20±0.20a	0.20±0.20a
0.2	0.40±0.40a	3.40±0.50b	3.40±0.50b	3.40±0.50b	3.40±0.50b
0.4	1.40±0.87b	1.40±0.87c	2.60±0.40b	3.20±0.37b	3.20±0.37b
0.6	2.20±1.01bc	3.80±0.37b	4.00±0.31b	4.40±0.24b	4.40±0.24b
0.8	1.80±0.80b	3.80±0.58b	3.80±0.58b	4.20±0.58b	4.20±0.58b
1.0	3.40±0.92c	4.40±0.40b	4.40±0.40b	4.40±0.40b	4.40±0.40b
1.2	3.80±0.37c	3.80±0.37b	3.80±0.37b	3.80±0.37b	3.80±0.37b
1.4	3.60±0.92c	4.80±0.20b	4.80±0.20b	4.80±0.20b	4.80±0.20b

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

During observation hour 1, there was no significant mean mortality difference in all rates of hexane birbira seed powder extracts applied on potato leaves and the control ($F_{7, 32} = 0.85$, $P > 0.5499$). After 2 hrs of observation mortality of neonate larvae was recorded at the three highest rates of hexane extract applied. This was continued to be the case after 3, 4 and 5 hrs of observation (Table 16).

Table 16 Mean mortality effect of hexane birbira extract to neonate larvae of *P. opercullela* on potato leaves.

Rate	Mortality after (mean ± SE)*				
(mg/ml)	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.20±0.20a	0.20±0.20a
0.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.60±0.60a	0.60±0.60ab
0.4	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.40±0.40a	0.40±0.40a
0.6	0.00±0.00a	0.00±0.00a	0.20±0.20ab	0.60±0.40a	0.60±0.40a
0.8	0.00±0.00a	0.00±0.00a	0.60±0.60ab	1.00±0.63a	1.00±0.63b
1.0	0.00±0.00a	0.40±0.40ab	1.20±0.80ab	3.40±0.40b	3.40±0.40b
1.2	0.20±0.20a	0.60±0.24ab	1.40±0.74ab	1.80±0.66ab	1.80±0.66b
1.4	0.20±0.20a	1.40±0.67b	2.40±0.60b	2.60±0.67b	2.60±0.67b

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at $P< 0.05$ using Tukey Kramer (HSD).

For toluene extracted birbira significant neonate mortality was recorded at 0.8mg/ml, 1.2mg/ml and 1.4mg/ml rates of application ($F_{7, 32} = 5.54$, $P< 0.0003$). The mortality difference turned non significant after 2 hrs but at the observations taken after 3, 4 and 5 hrs, the difference was significant between the control and all the rates of the extract. Particularly after 5 hrs the extract caused highly significant mortality of neonate larvae (Table 17).

Table 17 Mean mortality effect of toluene birbira extract to neonate larvae of *P. opercullela* on potato leaves.

* Means of five replicates

Rate (mg/ml)	Mortality after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.40±0.40a
0.2	0.00±0.00a	0.60±0.40a	1.40±0.74ab	1.60±0.74ab	3.80±0.37b
0.4	0.20±0.20a	0.80±0.20a	1.40±0.40ab	1.80±0.48ab	2.20±0.37ab
0.6	0.00±0.00a	1.80±0.86a	1.80±0.86ab	2.60±0.74b	3.60±0.60b
0.8	1.20±0.37ab	1.20±0.37a	2.40±0.67ab	3.80±0.37b	4.20±0.37b
1.0	0.00±0.00a	2.00±0.70a	2.60±0.60ab	2.60±0.60b	4.00±0.31b
1.2	1.40±0.50ab	1.80±0.58a	2.60±0.40ab	2.80±0.48b	3.00±0.54b
1.4	2.00±0.70b	2.40±0.67a	3.20±0.58b	3.20±0.58b	3.60±0.67b

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Within the first hours of observation, water birbira seed powder extract did not cause significant mortality differences on neonate larvae among rates applied on excised treated leaves and the control ($F_{7, 32}$ = 1.73, P> 0.13). No significant differences were also found in larval mortality after 2 hours observations ($F_{7, 32}$ = 2.87, P< 0.02). From the 3 h to 5 h of observations statistically no significantly different larval mortality was found at the three highest rates which were significantly higher than on the lower rates including the control (Table 18).

Table 18 Mean mortality effect of de-ionized water birbira extract to neonate larvae of *P. opercullela* on potato leaves.

Rate (mg/ml)	Mortality after (mean ± SE)*				
	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
0.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.2	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.20±0.20a
0.4	0.00±0.00a	0.00±0.00a	0.40±0.40a	0.40±0.40a	0.40±0.40a
0.6	0.40±0.40a	0.40±0.40a	0.40±0.40a	0.40±0.40a	0.60±0.40a
0.8	0.00±0.00a	0.00±0.00a	0.60±0.60a	0.80±0.58ab	0.80±0.58a
1.0	1.80±0.91a	2.00±0.83a	2.60±0.87ab	3.20±0.96bc	3.60±0.74b
1.2	1.20±0.96a	2.00±1.04a	2.60±0.92ab	3.60±0.74c	3.60±0.74b
1.4	0.40±0.40a	2.20±1.01a	3.40±0.81b	3.60±0.74bc	3.60±0.74b

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at $P< 0.05$ using Tukey Kramer (HSD).

5.6.1. Toxicity of birbira extract to PTM neonate larvae

The rate dependent mortality values of PTM neonate larvae are presented as dosage mortality curves obtained through probit analysis. The log dose dependent mortality of the larvae supposed to assume linearity (Fig. 9).

Results indicated (Table 19) that acetone extract was more toxic to neonates, where the LC_{50} was 0.06gm birbira seed powder/ml of water. Chloroform and water extracts were the second and third toxic to neonates, where the LC_{50} values were 0.13mg/ml and 0.27mg/ml, respectively. Toluene extract also closely follow water (Table 19). The least toxic extract was hexane with LC_{50}, 1.09.

Table 19 Probit models for the six solvent extracts obtained after parameter estimates converged 32 repeated iterations (dose mortality of neonates).

PTM developmental stage	Birbira seed powder extracted by	LC_{50} of birbira extract	95% confidence limits
Neonate larvae	Water	0.27	0.08436 - 0.48352
	Acetic acid	0.43	0.14828 - 0.74342
	Acetone	0.06	0.00883 - 0.19059
	Chloroform	0.13	0.02817 - 0.28679
	Toluene	0.32	0.07894 - 0.60659
	Hexane	1.09	0.41574 - 2.06329

The dose mortality curve for acetone extract was at the highest peak and did not allowed neonates to settle rather died even at the lowest rate of application (Fig. 9). Water extract dose mortality curve related to the curve chloroform extract but the former showed steppest from all extracts as the rate applied increased (Fig. 9).

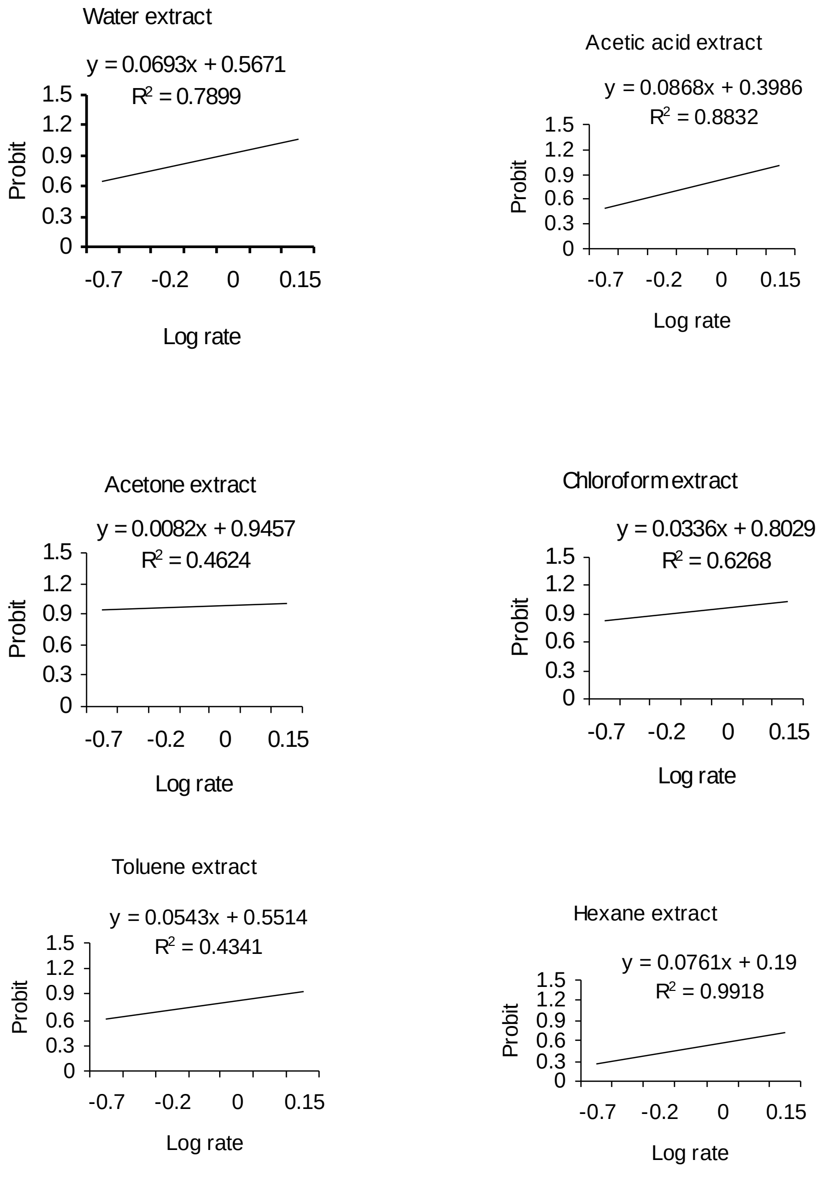


Fig. 9 Toxicity lines of birbira seed powder extracts against PTM neonates (Rate mg/ml).

5.7. Effects of birbira seed powder extracts on deterrence responses of PTM neonate larvae

The number of neonate larvae deterred per potato leaves was not significantly different by treatment type ($F_{5,\ 5}$ = 0.18, P> 0.97). However, it was significantly affected by rate ($F_{7,\ 7}$ = 3.59, P< 0.0012) and by the interaction of extract and rate of application ($F_{35,\ 35}$ = 1.56, P< 0.03).

When each rate between treatments was computed (Table 20); statistically no significant difference in the mean number of larvae deterred from the control leaves treated with water ($F_{5,\ 24} = _{0.66}$, P> 0.6523). There was no significant difference in the number of larvae driven away from treated leaves at the lowest rate (0.2mg/ml) applied ($F_{5,\ 24}$ = 2.42, P> 0.0652). Similarly, on potato leaves treated with birbira seed powder extracts of aqueous and organic solvents, no significant difference was found in the number of larvae deterred at rate less than 0..8mg/ml and 1.4mg/ml applied (Table 20). On potato leaves treated with acetic acid and water extracts there was significantly different number of neonate larvae that were rejected form the leaves treated at the rate of 1.0mg/ml ($F_{5,\ 24}$ =7.89, P< 0.0002). There was no significant difference in the number of larvae deterred from leaves treated with acetone, acetic acid, toluene and chloroform birbira extracts at the rate of 1.2mg/ml applied and significantly lower deterrence effect was found from leaves treated with water and hexane birbira seed powder extracts ($F_{5,\ 24}$ = 4.17, P< 0.0072).

Table 20 Effects of birbira seed powder extract obtained using polar and non-polar solvent on the deterrence response of PTM neonate larvae on potato leaves

Rate (mg/ml)	Neonate larvae deterred after 5 hours (mean ± SE)*					
	AABSP	ABSP	CHBSP	HBSP	TBSP	WBSP
0.0	0.20±0.20aA	0.20±0.20aA	0.40±0.40aA	0.00±0.00aA	0.00±0.00aA	0.00±0.00aA
0.2	1.40±0.67aA	1.00±0.31aA	0.40±0.24aA	0.40±0.40aA	0.00±0.00aA	0.00±0.00aA
0.4	1.00±0.31aA	0.40±0.40aA	1.80±0.37aA	0.60±0.40aA	1.20±0.48aA	0.60±0.40aA
0.6	2.00±0.54aA	1.80±0.66aA	0.60±0.24aA	1.40±0.24aA	1.20±0.48aA	1.00±0.63aA
0.8	1.40±0.67aA	1.00±0.63aA	0.80±0.58aA	0.80±0.37aA	0.60±0.24aA	0.00±0.00aA
1.0	0.00±0.00aA	1.60±0.24aB	1.00±0.31aB	0.80±0.20aBA	1.00±0.31aB	0.00±0.00aA
1.2	1.40±0.50aBA	2.00±0.54aB	0.40±0.40aBA	0.00±0.00aA	1.00±0.31aBA	0.20±0.20aA
1.4	0.20±0.20aA	1.40±0.74aA	0.00±0.00aA	1.00±0.44aA	1.00±0.54aA	0.00±0.00aA

* Means of five replicates

Means ± SE followed by the same letter (s) within column (lower case letter) and rows (upper case letters) are not significantly different from each other at P< 0.05 using Tukey Kramer (HSD).

Statistically significant deterrence effect was not found on neonate larvae that were allowed to feed on potato leaves which were treated with acetic acid extract at both lower and higher rates of applications ($F_{7, 32}$= 2.57, P< 0.03) except at 1.0mg/ml rate of application on which significantly lower larvae were settled.

Statistically significant deterrence effect was not found on neonate larvae that were allowed to feed on potato leaves which were treated with acetone extract at both lower and higher rates of applications ($F_{7, 32}$ = 1.61, P> 0.16).

A significant difference in the mean number of neonate larvae deterred was found when chloroform extract at the highest rate (1.4mg/ml) and 0.4mg/ml was applied on potato leaves ($F_{7, 32}$ = 2.32, P< 0.05) and in all other treated leaves there was no significant rejection by neonate larvae ($F_{7, 32}$ = 2.32, P< 0.05).

There was no significantly different deterrence among larvae that were introduced to potato leaves treated with lower and higher rates of hexane extract. Significantly less deterrence was found on leaves treated at rate of 1.2mg/ml ($F_{7, 32} = 2.45$, $P< 0.04$).

Among lower and higher rates of toluene extract there were no significant deterrence responses observed after hour 5 on PTM neonate larvae ($F_{7, 32} = 1.91$, $P> 0.10$)

Statistically significant deterrence effect was not found on neonate larvae that were allowed to feed on potato leaves which were treated with water birbira seed powder extracts at both lower and higher rates of applications ($F_{7, 32} = 1.89$, $P>0.1032$).

6. DISCUSSION

The results of the present study revealed that seed powder extracts of *M. ferruginea* extracted using solvents of varying polarity have a vital potential in protecting potato crop against biological activities (oviposition, ovicidal, feeding and settlement) of PTM. PTM were inhibited from laying eggs on treated potato tubers with *M. ferruginea* seed powder extracts at the highest rates of applications.

The number of eggs oviposited on treated tubers with hexane extract was lower than chloroform and water extract treated tubers at the highest rate (1.4mg/ml) applied. Eggs laid on chloroform extract treated tubers also lower than water extract treated tubers. More number of eggs was deposited on acetic acid; toluene and acetone extract treated tubers with their respective order at the highest rate of application. Therefore, *M. ferruginea* seed powder extracts of hexane, chloroform and water found to show deterrence effect than other solvent extracts. In support of these results though the botanical and the insect pest used were different Maher and Thiery (2004) reported that polar extracts obtained by soaking berries in methanol or water stimulated oviposition, whereas more apolar ones obtained with chloroform or hexane had no significant effect. The polar compounds present on grape berries act as oviposition stimulants for *Lobelia botrana* (Lepidoptera: Tortricidae) (Maher and Thiery, 2004). There were no significant differences on the number of eggs laid on treated and control tubers at each treatments in the study at the lowest rates of application, particularly 0.2mg/ml. In general oviposition by PTM decreased with the increase in the rate of application of aqueous and organic solvent extracts. In agreement with this result Bayeh (2009 unpublished) reported that the number of eggs deposited on treated tubers decreased with the increase in the rate of application of birbira cream in water and water dissolved birbira.

Therefore, the incorporation of oviposition deterrence in insect pest control programs may be an effective alternative for the control of the potato tuber moth in diffused light stores to protect potato tubers stored for seed purpose.

The number of active galleries counted on treated tubers showed a sharp decrease with increased rate of application using birbira extracts of acetic acid, acetone, chloroform and hexane. These extracts may inhibit the PTM larvae from digging deep in the treated tubers or the larvae might died while attempting to feed by acquiring enough of the insecticidal agents that are presented on treated tubers surfaces and failed to produce active galleries. On tubers treated with water and toluene extracts though there was a decrease on the number of active galleries with increasing rate of application, a slight increase of galleries was counted at 1.4mg/ml and 1.2mg/ml, respectively. Particularly for the water extract, the increase at 1.4mg/ml is due to non experimental factor, because the trend at all the rates below was a constant decrease. Thus, by disregarding this, data point one can state that water extract reduces PTM larval feeding significantly.

The number of terminated galleries counted on treated tubers showed similar trend on tubers treated with acetic acid and acetone extracts. For these extracts there was no significant difference on the mean number of terminated galleries carved by the PTM larvae at each rates applied. Therefore the efficacy of acetic acid and acetone extracts may not inhibit, in rate dependent manner the larvae from creating feeding sites. Similar result was shown on tubers treated with toluene extracts where there was no difference on the number of terminated galleries counted at rate 0.2mg/ml and 1.4mg/ml. On the other hand the number of terminated galleries counted on water, chloroform, and hexane extract treated tubers indicated negative relationship with increasing rate of application. A terminated gallery in these extracts decreased with the increase in the rate of application of extracts. This decrease is synchronized well with the decrease in the active galleries that occurred with increased rate of application of the extracts. Thus increased rates of application of the extracts reduced the establishment of PTM on potato tubers. Similarly with other insect pest and botanicals preparation Moawad and Ebadah (2007) reported that cardamom and rosemary oils of 1.5%conc.dust (1.5% oils mixed with talcum powder) and then dusting potato tuber on potato tuber obtained the lowest percentage of PTM larval penetration. This result confirmed the previous works done using water extracts to determine feeding responses of PTM larvae on treated potato tubers (Bayeh, 2009 unpublished). Deterrent activity of botanicals may drive the insect's larvae away after exposure to the plant without necessarily feeding (Morallo-Rejesus, 1986) or gives the larvae the opportunity to feed on plant part, but the food intake is reduced until the insects die from starvation (reviewed by Morallo-Rejesus, 1986).

The emergence of healthy PTM adults from treated potato tubers in general did not show a clear pattern as was the case with the larvae. The physical appearance of PTM adults emerged from larvae that fed from tubers treated with *M. ferruginea* seed powder extracts of polar and non-polar solvents was normal as emerging adults from tubers treated with water (control) except in acetic acid and chloroform extracts treated tubers. The emergence of deformed adults from treated tubers at all rates was found to be similar for all the treatments and was very low or did not occur. This might indicate that the insecticidal agent contained in birbira seed powder extracts did not go under the potato tubers' skin to interfere with the activity of larvae that managed to establish most before or while trying to settle under tuber skin due to killing effect of birbira extracts.

The oviposition response on potato leaves was tested using crude aqueous and organic solvent extracts from birbira seed powder. Application of these extracts in general at varying concentration on potato leaves prevented PTM females from laying eggs at the higher rates compared to the control and the lowest rate (0.2mg/ml). In this study of oviposition response, particularly rates greater than 1.0mg/ml were found to consistently to prevent oviposition by PTM on leaves treated with acetic acid, acetone, chloroform and water extracts of birbira seed powder. The reduction in oviposition on treated potato leaves might be attributed to olfactory input after contact with the treated tuber surface which is caused by chemical stimuli and contributed to the avoidance of the suitable oviposition substrate, potato leaves or might be because of the contact toxicity of birbira extracts to PTM gravid female. The results of oviposition responses of PTM to treated leaves may underline the bases to practically apply crude birbira extracts to reduce the infestation level to a degree that prevents foliage damage due to PTM larvae in the field which consequently might reduce infestation level on tubers before harvest. The use of plant allelochemicals for the suppression of insect pests is considered as having potential for providing sustainable pest control methods (Isaman, 2000 cited in Koschier and Sedy,

2003) and the oviposition deterrence effects of the extracts are due to allelochemicals in birbira that should be identified and confirmed for toxicity.

The highest numbers of eggs were oviposited away from treated potato tubers, on the cage inner surfaces with acetic acid, water and chloroform extracts, indicating that females avoided treated tubers for oviposition. This was not shown on the water treated tubers (control). The least number of eggs were laid on inner chambers surfaces with toluene and hexane extracts, away from treated leaves. On the other hand, oviposition deterrence effect on treated potato leaves have resulted that higher numbers of eggs were laid away treated leaves, on inner chambers surfaces with extracts of acetic acid, acetone, water and chloroform in their respective orders. This result has indicated that females avoided treated leaves for oviposition which was not shown on the water treated leaves (control). This result confirmed the previous work on tubers by Bayeh (2009 unpublished). In agreement with this result using botanicals (Lemon grass, *Cymbopogen citratus*) against Lesser Cotton Leaf cutworm, *Spodoptera exigua* (Hbn) Sharaby (1987) reported that most of the eggs were laid on the glass surfaces of jars, indicating that females avoided treated leaves for oviposition. The least numbers of eggs oviposited away from treated leaves was found with toluene and hexane birbira seed powder extracts. Thus counting the eggs that may be deposited away from suitable host when treated with extracts from plants with botanical property is an important parameter that needs to be considered. Therefore, the increase in eggs oviposited away from the treated tubers/leaves with increased rate of application of the extracts implies that birbira indeed has oviposition deterrence effect.

The highest percentage of unhatched eggs was recorded with ranges of 60% to 100% by all solvent extracts of *M. ferruginea* seed powder in this study except hexane extract which was below 40% in all rates of applications. Similarly, PTM eggs treated with other botanical oils as reported by Moawad and Ebadah (2007) showed that 0.02 and 0.05%conc. of cardamon oils exhibited the best reduction in percentage of eggs hatchability (67.47 and 86.74%). The concentration of birbira seed powder extracts in water that caused 50% mortality was more toxic to the *P. opercullela* eggs, where the

LC_{50} was 0.23. Acetone extract was also more toxic at the LC_{50} of 0.24 than the remaining extracts. The third toxic to the eggs was acetic acid birbira seed powder extract at LC_{50} value of 0.41. The least toxic was hexane extract. From the different solvents (aqueous and organic) used water was found to be most effective as compared to all other solvents (Bekele *et al.,* 2002). Contact toxicity of birbira extracts adults of on three Aphid species was confirmed in the previous study by Bayeh (2007). The present study adds on to the confirmation of the contact toxicity of birbira extract. Hence, expanding the spectrum of use of this natural insecticide as a contact botanical.

Botanicals generally act in one of two ways: either as a contact poison when sprayed on the insect, or as a stomach poison when applied to the plant and eaten by the insect. Sharaby (1988) account that the percentage of lesser cotton leaf worm, *Spodoptera exigua* (Hbn) egg hatchability decreased as the essential oil of lemon grass, *Cymbopogen citratus* concentration increased. According to Sharaby (1988) there was no significant difference in percentage of egg hatchability between untreated and treated egg masses with 0.25% of oil concentration. This was found to be not because of the reduced oviposition on untreated potato leaves but because of reduced efficiency due to lower rate of use. Therefore the ovicidal effect of the birbira seed powder extracts on PTM eggs reported here may be attributed to one or more of factors such as fumigation effect through membrane pores, contact effect resulting toxicity after it enter through porous surfaces of the egg membrane. With the other botanical similar result had been reported by Moawad and Ebadan (2007) indicated that dusting Neemerich oil (*Azadirachta indica*) on potato tuber caused ovicidal action against PTM. Unhatched eggs and hatched larvae count revealed that majority of eggs were killed (or failed to hatch) by the extracts of water, acetone and acetic acid with their respective order.

The results obtained using neonates indicate that seed powder extracts of *M. ferruginea* obtained using solvents with varying polarities brought about consistent negative effect. There was significantly reduced settlement of larvae on treated potato leaf within 5 h periods and with increased rate of applications. This indicates that the extracts have the potential to prevent colonization of potato plants by PTM. Therefore making it possible to study for how long birbira extracts remain potent after applied on potato plants/tubers. With the other botanical similar result had been reported by Moawad and Ebadan (2007) in which they indicated that dusting Neemerich oil (*Azadirachta indica*) on potato tuber caused larvicidal action against PTM.

The different extracts have caused significant neonate larvae mortality at the three highest rates tested and in the last hours of exposure (3, 4 and 5 hrs after treatment application). The dead neonates were banana shaped and turned light brown in their color. This was confirmed by failure of larvae to respond to the gentle touch with fine brush. Among the extracts acetone and chloroform extracts caused significant mortality even in the first two exposure periods. In agreement with these results, with another insect pest Bekele *et al.* (2007) reported that acetone extracts of *M. ferruginnea* and *Tephrosia vogellii* seeds were the most active resulting 100% mortality of the bean bruchid, *Zabrotes subfaciatus*. On the other hand, hexane extract acted slowly to cause significant larval mortality. This might have been due to the low concentration of the active botanicals in hexane than the others.

The LC_{50} values for the different extracts on neonates of PTM larvae were different and the most toxic that acted at the very low rate was acetone extract. The second most potent was chloroform extract and the third toxic was water extract. The least toxic was obtained with hexane birbira seed powder extract. However, all extracts were found to be toxic with varying degree of toxicity. These results show the complex nature of the toxic in birbira that act as insecticide on PTM neonate larvae. This is because the application solvent was water and the potent botanicals that were extracted by solvents of different polarity dissolved in water and acted on PTM.

7. CONCLUTION AND RECOMMENDATION

7.1. Conclusion

- Birbira seed powder extracts were found effective against PTM activities on host plant potato with significant oviposition responses (deterrence), inhibiting neonate larval settling on treated leaves, killing the larvae and with highest percentage of ovicidal action.
- PTM females were inhibited from laying eggs on treated tubers with birbira seed powder extracts of chloroform, hexane and water considerably more than that on tubers treated with acetic acid, acetone and toluene extracts of similar concentrations.
- In oviposition response study particularly rates greater than 1.0mg/ml found consistently to prevent oviposition by PTM on leaves treated with acetic acid, acetone, chloroform and water extracts.
- Generally, the number of active and terminated galleries carved by PTM larvae tend to decreased (in different proportion) with increasing rate of application at each treatments.
- The physical appearance of PTM adults emerged from larvae that fed from tubers treated with *M. ferruginea* seed powder extracts of polar and non-polar solvents was normal as adults emerged from tubers treated with water (control).
- The highest numbers of eggs were oviposited away from treated potato tubers, on the cage inner surfaces more with acetic acid, water and chloroform extracts, indicating that females avoided treated tubers for oviposition.
- Similarly, oviposition deterrence effect on treated potato leaves have resulted that higher numbers of eggs were laid away from treated leaves, on inner chambers surfaces more with extracts of acetic acid, acetone, water and chloroform in their respective orders. The percent of treated eggs hatched in to larvae decreased compared to the control group for all the test extracts.
- *M. ferruginea* seed powder extracted by acetone has been shown better insecticidal property to cause neonate larval mortality at the lowest rate and during early hours of exposures (1 hr. to 2 hr.). No larvae were found settled on

potato leaf treated with acetone extract. This was followed by water and chloroform extracts.

- Ø The water extract was found to cause the highest egg mortality followed by acetone and acetic acid extracts. In both situations the least toxic was obtained with hexane birbira seed powder extract. It is the water and acetone extracts that showed consistently in their effect.

To sum up, *M.ferruginea* demonstrated enormously satisfying results as a native botanical insecticide.

7.2. Recommendations

- Ø Extraction procedures, times, solvent chemicals and equipments might limit the efficacy of birbira extracts so with this area further information is required.
- Ø Further research should target chemical isolation of the active ingredient contained in birbira seed powder extracts and formulation of optimum dosages to enhance efficacy of this botanical insecticide.
- Ø Birbira extracts has shown highest efficiency against potato tuber moth, therefore water, acetone, chloroform extracts could be selected for future purification and identification of the active insecticidal substances by chromatography or other methods.
- Ø Extracts obtained using solvents of varying polarities are effective as contact and stomach poison that is effective against leaf-feeding insect (PTM); therefore its broad spectrum might be further confirmed by researches on major insect pests.
- Ø Besides its insecticidal potential M. *ferruginea* is a multi purpose tree and farming practices in Ethiopia should incorporate plantation of birbira for ecological and economic uses.
- Ø Awareness should be created about botanicals use concerning crop protection among crop growers on the bases of scientific approach and environmentally sound alternative insect pest control methods could be implemented.

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ANNEXES

Annex 1 Summery table for analysis of variance (ANOVA) for mean oviposition responses of PTM on potato tubers.

a. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the oviposition response of PTM on Potato tubers (analyzed by each extracts).

Acetic acid birbira extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	242.70000	34.6714	2.0761	0.0754
Among treatment	32	534.40000	16.7000		
Total	39	777.10000			

Effects of Acetone extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	177.10000	25.3000	3.4305	0.0075
Among treatment	32	236.00000	7.3750		
Total	39	413.10000			

Chloroform extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	54.40000	7.77143	2.8260	0.0207
Among treatment	32	88.00000	2.75000		
Total	39	142.40000			

Hexane extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	522.57500	74.6536	10.1743	<.0001
Among treatment	32	234.80000	7.3375		
Total	39	757.37500			

Toluene extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	71.10000	10.1571	0.6880	0.6813
Among treatment	32	472.40000	14.7625		
Total	39	543.50000			

Water extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	116.17500	16.5964	3.1094	0.0128
Among treatment	32	170.80000	5.3375		
Total	39	286.97500			

b. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the oviposition response of PTM on Potato tubers (analyzed at each rate).

Rate 0.0mg/ml (control).

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	2.566667	0.51333	0.2095	0.9552
Among treatment	24	58.800000	2.45000		
Total	29	61.366667			

Rate 0.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	254.70000	50.9400	2.6123	0.0506
Among treatment	24	468.00000	19.5000		
Total	29	722.70000			

Rate 0.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	71.46667	14.2933	1.0030	0.4372
Among treatment	24	342.00000	14.2500		
Total	29	413.46667			

Rate 0.6mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	185.90000	37.1800	5.7643	0.0012
Among treatment	24	154.80000	6.4500		
Total	29	340.70000			

Rate 0.8mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	93.76667	18.7533	1.4352	0.2477
Among treatment	24	313.60000	13.0667		
Total	29	407.36667			

Rate 1.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	126.40000	25.2800	3.2902	0.0211
Among treatment	24	184.40000	7.6833		
Total	29	310.80000			

Rate 1.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	59.90000	11.9800	2.2675	0.0801
Among treatment	24	126.80000	5.2833		
Total	29	186.70000			

Rate 1.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	105.86667	21.1733	7.0578	0.0003
Among treatment	24	72.00000	3.0000		
Total	29	177.86667			

Annex 2. Summery table for analysis of variance (ANOVA) for mean active and terminated galleries carved by neonates on Potato tubers

a. Active galleries carved by PTM larvae on potato tubers treated with birbira seed powder extracts obtained using polar and non-polar solvents (analyzed by each extracts).

Acetic acid extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	49.775000	7.11071	6.3206	0.0001
Among treatment	32	36.000000	1.12500		
Total	39	85.775000			

Acetone extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	62.575000	8.93929	7.6897	<.0001
Among treatment	32	37.200000	1.16250		
Total	39	99.775000			

Chloroform extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	89.77500	12.8250	7.7143	<.0001
Among treatment	32	53.20000	1.6625		
Total	39	142.97500			

Hexane extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	106.70000	15.2429	13.8571	<.0001
Among treatment	32	35.20000	1.1000		
Total	39	141.90000			

Toluene extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	51.10000	7.30000	3.6049	0.0057
Among treatment	32	64.80000	2.02500		
Total	39	115.90000			

Water extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	26.300000	3.75714	4.3561	0.0017
Among treatment	32	27.600000	0.86250		
Total	39	53.900000			

b. Effects of birbira seed powder extracts obtained using polar and non-polar solvents on the active galleries carved by neonates on Potato tubers (analyzed by each rates).

Rate 0.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	8.300000	1.66000	1.0945	0.3890
Among treatment	24	36.400000	1.51667		
Total	29	44.700000			

Rate 0.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	27.766667	5.55333	3.5828	0.0146
Among treatment	24	37.200000	1.55000		
Total	29	64.966667			

Rate 0.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	30.400000	6.08000	2.2658	0.0803
Among treatment	24	64.400000	2.68333		
Total	29	94.800000			

Rate 0.6mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	15.366667	3.07333	1.9411	0.1246
Among treatment	24	38.000000	1.58333		
Total	29	53.366667			

Rate 0.8mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	0.566667	0.11333	0.0667	0.9966
Among treatment	24	40.800000	1.70000		
Total	29	41.366667			

Rate 1.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	8.700000	1.74000	2.8216	0.0384
Among treatment	24	14.800000	0.61667		
Total	29	23.500000			

Rate 1.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	10.000000	2.00000	5.2174	0.0022
Among treatment	24	9.200000	0.38333		
Total	29	19.200000			

Rate 1.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	10.000000	2.00000	3.6364	0.0137
Among treatment	24	13.200000	0.55000		
Total	29	23.200000			

c. Effects of birbira seed powder extracts obtained using polar and non-polar solvents on the terminated galleries carved by neonates on Potato tubers (analyzed by each extracts).

Acetic acid extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	89.97500	12.8536	0.7257	0.6514
Among treatment	32	566.80000	17.7125		
Total	39	656.77500			

Acetone extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	101.77500	14.5393	1.2740	0.2942
Among treatment	32	365.20000	11.4125		
Total	39	466.97500			

Chloroform extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	200.40000	28.6286	2.3857	0.0441
Among treatment	32	384.00000	12.0000		
Total	39	584.40000			

Hexane extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	252.77500	36.1107	8.6235	<.0001
Among treatment	32	134.00000	4.1875		
Total	39	386.77500			

Toluene extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	103.97500	14.8536	3.8332	0.0039
Among treatment	32	124.00000	3.8750		
Total	39	227.97500			

Water extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	234.80000	33.5429	11.0885	<.0001
Among treatment	32	96.80000	3.0250		
Total	39	331.60000			

d. Effects of birbira seed powder extracts obtained using polar and non-polar solvents on the terminated galleries carved by neonates on Potato tubers (analyzed at each rates).

Rate 0.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	27.76667	5.55333	1.4875	0.2309
Among treatment	24	89.60000	3.73333		
Total	29	117.36667			

Rate 0.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	243.06667	48.6133	4.0737	0.0081
Among treatment	24	286.40000	11.9333		
Total	29	529.46667			

Rate 0.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	134.26667	26.8533	3.2615	0.0218
Among treatment	24	197.60000	8.2333		
Total	29	331.86667			

Rate 0.6mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	183.50000	36.7000	2.8486	0.0371
Among treatment	24	309.20000	12.8833		
Total	29	492.70000			

Rate 0.8mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	87.76667	17.5533	2.1896	0.0890
Among treatment	24	192.40000	8.0167		
Total	29	280.16667			

Rate 1.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	196.26667	39.2533	4.3374	0.0059
Among treatment	24	217.20000	9.0500		
Total	29	413.46667			

Rate 1.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	241.06667	48.2133	6.4716	0.0006
Among treatment	24	178.80000	7.4500		
Total	29	419.86667			

Rate 1.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	117.10000	23.4200	2.8160	0.0387
Among treatment	24	199.60000	8.3167		
Total	29	316.70000			

Annex 3. Summery table for analysis of variance (ANOVA) for mean oviposition responses of PTM on potato leaves.

a. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the oviposition response of PTM on Potato leaves (analyzed by each extracts).

Acetic acid extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	379.57500	54.2250	14.0844	<.0001
Among treatment	32	123.20000	3.8500		
Total	39	502.77500			

Acetone extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	339.50000	48.5000	11.6168	<.0001
Among treatment	32	133.60000	4.1750		
Total	39	473.10000			

Chloroform extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	437.10000	62.4429	7.0857	<.0001
Among treatment	32	282.00000	8.8125		
Total	39	719.10000			

Hexane extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	302.00000	43.1429	16.5140	<.0001
Among treatment	32	83.60000	2.6125		
Total	39	385.60000			

Toluene extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	608.97500	86.9964	47.3450	<.0001
Among treatment	32	58.80000	1.8375		
Total	39	667.77500			

Water extracts

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	334.30000	47.7571	53.0635	<.0001
Among treatment	32	28.80000	0.9000		
Total	39	363.10000			

b. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the oviposition response of PTM on Potato leaves (analyzed by each rates).

Rate 0.0mg/ml (control).

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	15.46667	3.09333	0.6270	0.6808
Among treatment	24	118.40000	4.93333		
Total	29	133.86667			

Rate 0.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	24.56667	4.91333	0.8906	0.5028
Among treatment	24	132.40000	5.51667		
Total	29	156.96667			

Rate 0.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	551.10000	110.220	16.2887	<.0001
Among treatment	24	162.40000	6.767		
Total	29	713.50000			

Rate 0.6mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	258.56667	51.7133	7.9355	0.0002
Among treatment	24	156.40000	6.5167		
Total	29	414.96667			

Rate 0.8mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	88.70000	17.7400	2.5104	0.0579
Among treatment	24	169.60000	7.0667		
Total	29	258.30000			

Rate 1.0mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	40.16667	8.03333	1.0783	0.3972
Among treatment	24	178.80000	7.45000		
Total	29	218.96667			

Rate 1.2mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	32.96667	6.59333	1.7740	0.1564
Among treatment	24	89.20000	3.71667		
Total	29	122.16667			

Rate 1.4mg/ml

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	21.06667	4.21333	1.0276	0.4238
Among treatment	24	98.40000	4.10000		
Total	29	119.46667			

Annex 4. Summery table for analysis of variance (ANOVA) for mean settling responses of PTM neonate larvae on potato leaves.

a. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on settling responses of PTM neonate larvae on potato leaves (analyzed by each extracts exposure periods).

Effects of Acetic acid extract after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	5.100000	0.728571	2.3314	0.0484
Among treatment	32	10.000000	0.312500		
Total	39	15.100000			

Effects of Acetic acid extract after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	18.975000	2.71071	3.2367	0.0104
Among treatment	32	26.800000	0.83750		
Total	39	45.775000			

Effects of acetic acid extract after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	70.37500	10.0536	8.3780	<.0001
Among treatment	32	38.40000	1.2000		
Total	39	108.77500			

Effects of acetic acid extract after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	76.57500	10.9393	9.4101	<.0001
Among treatment	32	37.20000	1.1625		
Total	39	113.77500			

Effects of acetic acid extract treatment after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	76.57500	10.9393	9.4101	<.0001
Among treatment	32	37.20000	1.1625		
Total	39	113.77500			

Effects of acetone extract after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.5750000	1.22500	32.6667	<.0001
Among treatment	32	1.2000000	0.03750		
Total	39	9.7750000			

Effects of acetone extract after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	50.575000	7.22500	72.2500	<.0001
Among treatment	32	3.200000	0.10000		
Total	39	53.775000			

Effects of acetone extract after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	63.175000	9.02500	42.4706	<.0001
Among treatment	32	6.800000	0.21250		
Total	39	69.975000			

Effects of acetone extract after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	77.175000	11.0250	126.0000	<.0001
Among treatment	32	2.800000	0.0875		
Total	39	79.975000			

Effects of acetone extract after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	84.700000	12.1000	322.6667	<.0001
Among treatment	32	1.200000	0.0375		
Total	39	85.900000			

Effects of chloroform extract on settling of neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	4.375000	0.625000	2.5000	0.0362
Among treatment	32	8.000000	0.250000		
Total	39	12.375000			

Effects of chloroform extract on settling of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	11.100000	1.58571	2.0796	0.0749
Among treatment	32	24.400000	0.76250		
Total	39	35.500000			

Effects of chloroform extract on settling of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	70.300000	10.0429	21.7143	<.0001
Among treatment	32	14.800000	0.4625		
Total	39	85.100000			

Effects of chloroform extract on settling of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	84.400000	12.0571	38.5829	<.0001
Among treatment	32	10.000000	0.3125		
Total	39	94.400000			

Effects of chloroform extract on settling of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	84.400000	12.0571	38.5829	<.0001
Among treatment	32	10.000000	0.3125		
Total	39	94.400000			

Effects of hexane extract on settling of PTM neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	9.600000	1.37143	2.3343	0.0482
Among treatment	32	18.800000	0.58750		
Total	39	28.400000			

Effects of hexane extract on settling of PTM neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	54.400000	7.77143	7.0649	<.0001
Among treatment	32	35.200000	1.10000		
Total	39	89.600000			

Effects of hexane extract on settling of PTM neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	77.97500	11.1393	9.7928	<.0001
Among treatment	32	36.40000	1.1375		
Total	39	114.37500			

Effects of hexane extract on settling of PTM neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	89.97500	12.8536	13.7105	<.0001
Among treatment	32	30.00000	0.9375		
Total	39	119.97500			

Effects of hexane extract on settling of PTM neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	89.97500	12.8536	13.7105	<.0001
Among treatment	32	30.00000	0.9375		
Total	39	119.97500			

Effects of toluene extract on settling of PTM neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	3.175000	0.453571	1.6494	0.1574
Among treatment	32	8.800000	0.275000		
Total	39	11.975000			

Effects of toluene extract on settling of PTM neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.000000	1.14286	2.3443	0.0473
Among treatment	32	15.600000	0.48750		
Total	39	23.600000			

Effects of toluene extract on settling of PTM neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	29.500000	4.21429	8.6447	<.0001
Among treatment	32	15.600000	0.48750		
Total	39	45.100000			

Effects of toluene extract on settling of PTM neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	59.100000	8.44286	12.9890	<.0001
Among treatment	32	20.800000	0.65000		
Total	39	79.900000			

Effects of toluene extract on settling of PTM neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	59.100000	8.44286	12.9890	<.0001
Among treatment	32	20.800000	0.65000		
Total	39	79.900000			

Effects of de-ionized water extract on settling of PTM neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.775000	1.25357	4.0114	0.0030
Among treatment	32	10.000000	0.31250		
Total	39	18.775000			

Effects of de-ionized water extract on settling of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	11.600000	1.65714	4.4190	0.0016
Among treatment	32	12.000000	0.37500		
Total	39	23.600000			

Effects of de-ionized water extract on settling of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	49.375000	7.05357	12.8247	<.0001
Among treatment	32	17.600000	0.55000		
Total	39	66.975000			

Effects of de-ionized water extract on settling of PTM neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	63.175000	9.02500	16.4091	<.0001
Among treatment	32	17.600000	0.55000		
Total	39	80.775000			

Effects of de-ionized water extract on settling of PTM neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	68.975000	9.85357	17.5175	<.0001
Among treatment	32	18.000000	0.56250		
Total	39	86.975000			

Annex 5 Summery table for analysis of variance (ANOVA) for mean mortality responses of PTM neonate larvae on potato leaves.

a. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on mortality responses of PTM neonate larvae on potato leaves (analyzed by each extracts exposure periods).

Acetic acid extract on mortality responses of PTM neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	10.300000	1.47143	1.0799	0.3988
Among treatment	32	43.600000	1.36250		
Total	39	53.900000			

Acetic acid extract on mortality responses of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	24.400000	3.48571	2.2309	0.0576
Among treatment	32	50.000000	1.56250		
Total	39	74.400000			

Acetic acid extract on mortality responses of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	36.175000	5.16786	2.7199	0.0248
Among treatment	32	60.800000	1.90000		
Total	39	96.975000			

Acetic acid extract on mortality responses of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	64.40000	9.20000	7.5102	<.0001
Among treatment	32	39.20000	1.22500		
Total	39	103.60000			

Acetic acid extract on mortality responses of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	93.10000	13.3000	11.2000	<.0001
Among treatment	32	38.00000	1.1875		
Total	39	131.10000			

Acetone extract on mortality responses of neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	62.975	8.996	2.66	0.027
Among treatment	32	108.000	3.375		
Total	39	170.975			

Acetone extract on mortality responses of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	68.77500	9.82500	4.8221	0.0009
Among treatment	32	65.20000	2.03750		
Total	39	133.97500			

Acetone extract on mortality responses of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	65.37500	9.33929	4.6990	0.0010
Among treatment	32	63.60000	1.98750		
Total	39	128.97500			

Acetone extract on mortality responses of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	66.57500	9.51071	6.7332	<.0001
Among treatment	32	45.20000	1.41250		
Total	39	111.77500			

Acetone extract on mortality responses of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	66.57500	9.51071	6.7332	<.0001
Among treatment	32	45.20000	1.41250		
Total	39	111.77500			

Chloroform extract on mortality responses of neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	73.57500	10.5107	3.7707	0.0043
Among treatment	32	89.20000	2.7875		
Total	39	162.77500			

Chloroform extract on mortality responses of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	92.97500	13.2821	11.5497	<.0001
Among treatment	32	36.80000	1.1500		
Total	39	129.77500			

Chloroform extract on mortality responses of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	72.575000	10.3679	13.3779	<.0001
Among treatment	32	24.800000	0.7750		
Total	39	97.375000			

Chloroform extract on mortality responses of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	74.300000	10.6143	14.3923	<.0001
Among treatment	32	23.600000	0.7375		
Total	39	97.900000			

Chloroform extract on mortality responses of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	74.300000	10.6143	14.3923	<.0001
Among treatment	32	23.600000	0.7375		
Total	39	97.900000			

Hexane extract on mortality responses of neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	0.3000000	0.042857	0.8571	0.5499
Among treatment	32	1.6000000	0.050000		
Total	39	1.9000000			

Hexane extract on mortality responses of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.800000	1.25714	2.9580	0.0165
Among treatment	32	13.600000	0.42500		
Total	39	22.400000			

Hexane extract on mortality responses of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	26.775000	3.82500	3.1224	0.0125
Among treatment	32	39.200000	1.22500		
Total	39	65.975000			

Hexane extract on mortality responses of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	47.175000	6.73929	4.9463	0.0007
Among treatment	32	43.600000	1.36250		
Total	39	90.775000			

Hexane extract on mortality responses of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	47.175000	6.73929	4.9463	0.0007
Among treatment	32	43.600000	1.36250		
Total	39	90.775000			

Toluene extract on mortality responses of neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	22.800000	3.25714	5.5441	0.0003
Among treatment	32	18.800000	0.58750		
Total	39	41.600000			

Toluene extract on mortality responses of neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	23.175000	3.31071	2.2257	0.0581
Among treatment	32	47.600000	1.48750		
Total	39	70.775000			

Toluene extract on mortality responses of neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	35.175000	5.02500	2.8921	0.0185
Among treatment	32	55.600000	1.73750		
Total	39	90.775000			

Toluene extract on mortality responses of neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	47.600000	6.80000	4.4590	0.0015
Among treatment	32	48.800000	1.52500		
Total	39	96.400000			

Toluene extract on mortality responses of neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	55.600000	7.94286	7.0603	<.0001
Among treatment	32	36.000000	1.12500		
Total	39	91.600000			

Water extract on mortality responses of PTM neonate larvae after hour 1

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	15.975000	2.28214	1.7388	0.1351
Among treatment	32	42.000000	1.31250		
Total	39	57.975000			

Water extract on mortality responses of PTM neonate larvae after hour 2

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	37.775000	5.39643	2.8781	0.0189
Among treatment	32	60.000000	1.87500		
Total	39	97.775000			

Water extract on mortality responses of PTM neonate larvae after hour 3

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	66.30000	9.47143	5.1197	0.0006
Among treatment	32	59.20000	1.85000		
Total	39	125.50000			

Water extract on mortality responses of PTM neonate larvae after hour 4

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	95.60000	13.6571	8.0336	<.0001
Among treatment	32	54.40000	1.7000		
Total	39	150.00000			

Water extract on mortality responses of PTM neonate larvae after hour 5

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	98.00000	14.0000	9.4118	<.0001
Among treatment	32	47.60000	1.4875		
Total	39	145.60000			

Annex 6 Summery tables for analysis of variance (ANOVA) for mean deterrence response of PTM neonate larvae on potato leaves

a. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the deterrence response of PTM neonate larvae on potato leaves (analyzed by each extracts).

Mean deterrence effect of acetic acid extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	18.700000	2.67143	2.5749	0.0318
Among treatment	32	33.200000	1.03750		
Total	39	51.900000			

Mean deterrence effect of acetone extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	14.575000	2.08214	1.6172	0.1663
Among treatment	32	41.200000	1.28750		
Total	39	55.775000			

Mean mortality deterrence of chloroform extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	10.375000	1.48214	2.3249	0.0490
Among treatment	32	20.400000	0.63750		
Total	39	30.775000			

Mean deterrence effect of hexane extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.175000	1.16786	2.4586	0.0388
Among treatment	32	15.200000	0.47500		
Total	39	23.375000			

Mean deterrence effect of toluene extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	8.700000	1.24286	1.9121	0.1002
Among treatment	32	20.800000	0.65000		
Total	39	29.500000			

Mean deterrence effect of water extract

SOV	DF	SS	MS	F ratio	P value
With in treatment	7	4.975000	0.710714	1.8952	0.1032
Among treatment	32	12.000000	0.375000		
Total	39	16.975000			

b. Effects of birbira seed powder extracts obtained using polar and non-polar solvent on the deterrence response of PTM neonate larvae on potato leaves (analyzed at each rate) after 5 hrs.

Mean deterrence effect of birbira extracts at rate 0.0mg/ml (control).

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	0.6666667	0.133333	0.6667	0.6523
Among treatment	24	4.8000000	0.200000		
Total	29	5.4666667			

Mean deterrence effect of birbira extracts at rate 0.2mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	7.866667	1.57333	2.4205	0.0652
Among treatment	24	15.600000	0.65000		
Total	29	23.466667			

Mean deterrence effect of birbira extracts at rate 0.4mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	6.666667	1.33333	1.6667	0.1810
Among treatment	24	19.200000	0.80000		
Total	29	25.866667			

Mean deterrence effect of birbira extracts at rate 0.6mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	6.666667	1.33333	1.0667	0.4032
Among treatment	24	30.000000	1.25000		
Total	29	36.666667			

Mean deterrence effect of birbira extracts at rate 0.8mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	5.366667	1.07333	0.9200	0.4850
Among treatment	24	28.000000	1.16667		
Total	29	33.366667			

Mean deterrence effect of birbira extracts at rate 1.0mg/ml.

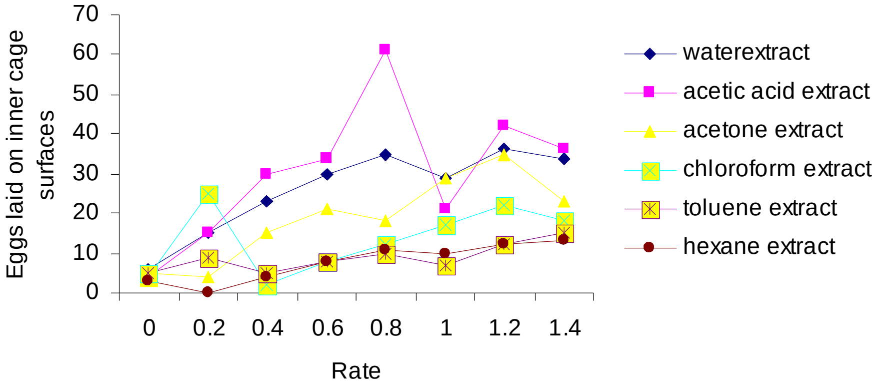
SOV	DF	SS	MS	F ratio	P value
With in treatment	5	9.866667	1.97333	7.8933	0.0002
Among treatment	24	6.000000	0.25000		
Total	29	15.866667			

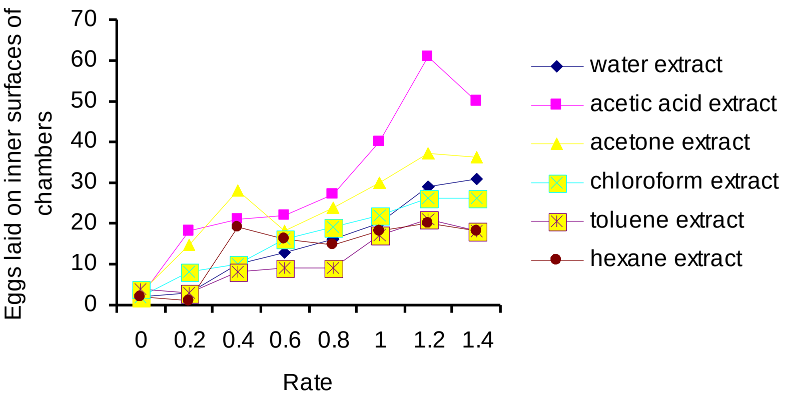
Mean deterrence effect of birbira extracts at rate 1.2mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	14.966667	2.99333	4.1767	0.0072
Among treatment	24	17.200000	0.71667		
Total	29	32.166667			

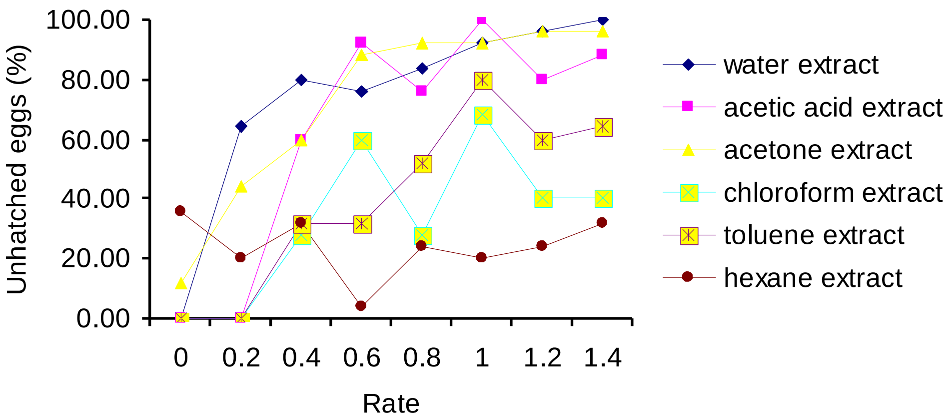
Mean deterrence effect of birbira extracts at rate 1.4mg/ml.

SOV	DF	SS	MS	F ratio	P value
With in treatment	5	9.200000	1.84000	2.0073	0.1139
Among treatment	24	22.000000	0.91667		
Total	29	31.200000			





Annex 7 Oviposition deterrence effect of birbira seed powder extract on potato tuber (top) and leaves (bottom).



Annex 8 Percentage of unhatched *P. oprculella* eggs after dipped in six solvent extracts of birbira seed powder.

Annex 9. Number of adults emerged from treated potato tubers per cage

Rate (mg/ml)	Birbira extracts of												Total	
	Water		Acetic acid		acetone		chloroform		toluene		hexane			
	HA*	DA*	HA	DA	HA	DA	HA	DA	HA	DA	HA	DA	HA	DA
0.0	7	0	8	0	28	0	15	0	6	0	14	0	78	0
0.2	9	0	39	1	18	0	71	1	0	0	3	0	140	2
0.4	7	0	47	1	8	0	3	0	11	0	4	0	80	1
0.6	3	0	18	0	34	0	0	0	5	0	5	0	65	0
0.8	13	0	42	0	33	0	2	1	6	0	1	0	97	1
1.0	1	0	15	0	20	0	23	0	1	0	1	0	61	0
1.2	4	0	35	0	22	0	35	0	8	0	0	0	104	0
1.4	13	0	21	0	19	0	2	0	6	0	0	0	61	0
Total	57	0	225	2	182	0	151	2	43	0	28	0	686	4

HA*- Healthy adult, DA*- Deformed adult

Annex 10 Infested potato tubers in sacks (Top) and in cages (bottom) for PTM rearing.

Annex 11 Experimental setups to determine oviposition response

Annex 12 potatoes planted in the green house used for rearing and get leaves for experiments of oviposition, ovocidal and larval settlements responses.

Annex 13 Experimental setups to study neonate larval settling response on treated potato leaves.

Annex 14 Experimental setups for oviposition deterrence on leaves and ovicidal.

Annex 15 Data collection sheets.

Oviposition and Feeding responses test results of ---------------extract applied on potato tubers

Conc. (mg/ml)	Replicate	Adult PTM Per cage	Oviposited eggs per tuber	Oviposited eggs away tubers	Deep galleries per tuber	Terminated Galleries Per tuber	Total galleries	Adults emerged		Total adults
								Healthy	Deformed	

Oviposition responses test results of polar and non-polar extracts of birbira seed powder applied on tubers

Treatment	Conc. (gm/ml)	Eggs laid per tuber	Tot.	Eggs laid away tubers	Number of tuber eyes

Oviposition responses test results of polar and non-polar extracts of birbira seed powder applied on potato leaves

Treatment	Rate (mg/ml)	Eggs laid per leaf	Tot.	Eggs laid away leaves

Ovicidal effects of water extracts of Birbira seed powder on leaves

conc. (mg/ml)	Number Of eggs dipped	Number of Hatched neonate larvae	Number of Un-hatched eggs	Condition of neonate larvae

Declaration

I hereby declare to have independently prepared this work with no other than the indicated sources and support. This thesis is my original work and its composition has never been submitted elsewhere. All sources of materials used for the thesis have been duly acknowledged.

Name: ___________________________ Date________________

Signature_______________

Place: Addis Ababa University, Ethiopia

The thesis has been presented under my supervision

Name: ____________________________ Date________________

Signature__________________