Addis Ababa University College of Natural and Computational Sciences

Zoological Department (Insect Science Stream)

Determination of Effective Rates of Concentration and Frequencies of Birbira (*Milletia ferruginea.* Hochst. Baker) application for the Management of Potato Tuber Moth, *Phthorimaea operculella (*Zeller) on Potato (*Solanum tuberosum.* L.) Under Field Condition

By

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Declaration

I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Date of Submission: ______________

Signature: ______________

Dedication

To: Merii Leta (Mami)

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List of acronyms

CFC	Common Fund for Commodities
CIP	International Potato Center
CSA	Central Statistical Agency of Ethiopia
EHDA	Ethiopian Horticultural Development Agency
EIAR	Ethiopian Agricultural Research Institute
FAOSTAT	Food and Agriculture Organization Statistics
HARC	Holetta Agricultural Research Center
IPM	Integrated Pest Management
MOA	Ministry of Agriculture
PTM	Potato Tuber Moth
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis Software
SNNPRS	South Nations Nationalities and Peoples Regional State

Abstract

Globally, Potato is one of the most important crops in terms of production as well as consumption and ranks first among root and tuber crops followed by cassava, sweet potato and yams. After the introduction of potato to Ethiopia higher production and productivity were achieved due to its good climate and edaphic conditions. However, the average production is low when compared to Africa and World potato production. This is due to several biotic and abiotic constraints. Among the biotic constraints, potato tuber moth (*Phthorimaea operculella* (Zeller)) is one of the major constraints under field and storage conditions. To control this pest, farmers had been using chemical pesticide, which can cause problems on human health, environmentally unfriendly, and cost ineffective method.

The current study is conducted to optimize the appropriate rate and frequency of Birbira (*Milletia ferruginea)* against PTM infestation on potato under field condition. Field trial was conducted at Holetta Agricultural Research Center during off-season in 2016. The experimental design was 2x3 RCBD with three replications. The treatment levels were three rates and two frequencies. The treatments include 0.23mg/mlx1, 0.23mg/mlx2, 0.33mg/mlx1, 0.33mg/mlx2, 0.43mg/mlx1, 0.43mg/mlx2, and untreated (check). The twice treatment applications were sprayed in two weeks interval. PTM infestation on different parts of potato was recorded at different potato growth stages. The parameters measured include infested and total number of leaves, petiole and stem. At harvest, total number of tubers and PTM infested tubers were recorded. Before treatment application, there was no significant difference on PTM infestation between all plots. After treatment application of birbira, at the current rate of concentration and time interval, only 0.23 gm/ml single application on leaf showed variation among the different rates of concentration but it did not show variation when compared to the untreated check. However, except 0.23 gm/ml, the rest of the rate of concentration used was not show significant variation ($P<0.05$) among treatments and the untreated check in terms of leaf, petiole, stem, and tuber infestation. In general results obtained from the current experiment showed that birbira is not effective against PTM under field condition.

Key word: Birbira, Field condition, Potato, Potato tuber moth

1. INTRODUCTION

1.1. Background

Potato (*Solanum tuberosum. L.*) belongs to the Solanaceae or nightshade family of flowering plants including tomato and eggplant. Potato is herbaceous annual crop that grows up to 100 cm tall and produces tubers. It is rich in starch, so it ranks as the world's fourth most important food crop, after maize, wheat and rice (FAO, 2008; Pandey *et al*., 2014).

Potato is an important food source globally (FAO and CFC, 2010). Most of the part of the potato tuber that is enlarged to store nutrients is 80% water, so only 20% of the tuber is constitutes the dry matter. Potatoes contain complex carbohydrates which are important for energy supply, but they are low in protein. They contain large amounts of vitamins A, B (B1, B3 and B6) and C as well as minerals, such as ascorbic acid, potassium, phosphorus, magnesium, iron, phenolics, free fat, low energy and density. Freshly harvested potatoes have higher quantities of vitamin C than stored potatoes. The vitamin C of potatoes can prevent the disease known as scurvy if a diet is lacking fruit (Amsel and Bishop, 2008; Gumul *et al.,* 2011). Umadevi *et al*. (2013) suggested that when we cook potatoes it is better to boil them with the skins still on, but washed well. That way you still have the benefits of these needed nutrients. Medium sized potatoes (of 150 g) provide nearly half of the daily adult requirement (100 mg) (FAO, 2008). Potato is considered as good weaning food due to its correct balance between protein and calories (Berga *et al*., 1993).

Potato is the most efficient tuberous crop in terms of time it takes to attain maturity. Potential tubers in potato can be harvested in 60 to 120 days after planting (Naz *et al*., 2011). There are four potato production systems which include: "belg" (short rain), "meher" (long rain), and residual crops and irrigated production. In many areas the "belg" (January to June) crop supplemented with irrigation constituents the bulk of potato production (Adane *et al*., 2010).

In Ethiopia, potato is grown in four major parts of the country: central, eastern, northwestern and southern parts. The central part includes the highland areas surrounding the capital, Addis Ababa. In this part, the major potato growing zones are West Shewa and North Shewa. The eastern part of potato production mainly covers the eastern highlands of Ethiopia, especially the East Harerge zone. The Northwestern part of potato production is situated in the Amhara Regional State such as South Gonder, North Gonder, East Gojam, West Gojam and Agew Awi. The southern part of Ethiopia in which potato is grown, is mainly located in the Southern Nations Nationalities and Peoples Regional State (SNNPRs) and partly in the Oromia Regional State. The major potato producing zones in this part are Gurage, Gamo Goffa, Hadiya, Wolyta, Kambata, Siltie and Sidama in the SNNPRS and West Arsi zone in Oromia. Together, they cover approximately 83% of the potato growers (CSA, 2008/2009).

The Ethiopian potato industry is an important part of the country's agricultural economy. Some serious problems that affect potato production in the country are weather and pests. Too little rain and too much rain can reduce potato yields and quality. Pests such as late blight, bacterial wilt, weeds and potato tuber moth (PTM) were also mentioned by Ethiopian growers as serious risks in potato production (Guenthner, 2006). Potato tuber moth (PTM) is one of the most important constraints to potato production worldwide. In Ethiopia, it causes up to 42% yield loss in storage and on averages 8.7% of the tubers were lost due to field infestation (Sileshi and Teriessa, 2001). PTM was known to damage potato only in the warmer areas, though major production areas mainly cover the highlands. The peak activity months of PTM were January, February, and June. Unlike the field situation, monitoring in the store showed no obvious peak record (Bayeh and Tadesse, 1992).

A variety of broad spectrum chemical insecticides are in use for the management of PTM in the field and storage for the treatment of tubers. Adverse effects of synthetic insecticides on applicators, the food supply and environment, have encouraged the development of biointensive approach for the control of this pest (as cited in Thakur and Chandla, 2013). In order to reduce the impact of insect pest, there should be an integrated pest management approach, which include appropriate cultural practices, pheromone traps, biological control, host plant resistance, botanicals with appropriate rate and time of applications (Binyam Tsedaley, 2015). Among the alternative strategies, the use of plants' insecticidal allelochemicals appears to be promising. Aromatic plants and their essential oils are among the most efficient botanicals (Regnault-Roger, 1997). So, the necessity of botanical agents to control PTM infestation in the field is very mandatory in agro-ecology of Ethiopia. The aim of this study, therefore, is to determine effective rates and frequencies of birbira (*Milletia ferruginea.* Hochst. Baker) seed oil extract for the management of potato tuber moth (*Phthorimaea operculella* (Zeller) on potato (*Solanum tuberosum*, L.) under field condition.

1.2. Objective

1.2.1. General objective

- To optimize the pesticidal effect of Birbira *(Milletia ferruginea.* Hochst. Baker) against potato tuber moth on potato under field condition.

1.2.2. Specific objectives

- To determine the effective rate of Birbira application on PTM.
- To determine the effective frequency of Birbira application on PTM

2. LITRATURE REVIEW

2.1. Global history of potato production and its current status

Globally, potatoes are one of the most important crops in terms of production as well as consumption and ranks first among root and tuber crops followed by Cassava, Sweet potatoes and Yams (FAO, 2008). It was originated in the high Andean mountain of South America about 8000 years ago (Pandey *et al*., 2014) and it was first cultivated in the vicinity of Lake Titicaca near the present day border of Peru and Bolivia. More than 320 million tones of potato are being cultivated annually on 20 million hectares of land (FAO and CFC, 2010). It was first introduced into Europe towards the end of 16^{th} century through Spanish conquerors. There, the potato developed as a temperate crop and was later distributed throughout the world largely as a consequence of the colonial expansion of European countries (Pandey *et al*., 2014) and to Africa in the 19th century (Pliska, 2008). Ethiopia has good climatic and edaphic conditions for higher potato production and productivity. It has the potential to grow in the 70% of the 10 M ha of arable land in Ethiopia. It was introduced to Ethiopia in 1859 by the German botanist Schimper (FAO, 2008). It is the second most important tuber crop grown in Ethiopia next to Enset in terms of area coverage (Solomon, 1985).

In the past 20 years vast differences between developed and developing countries in production of potato were seen. Developed countries like Canada and the common wealth of independent states decreased on average by one percent per year, developing countries increased by five percent per year. So, developing countries are now the world biggest potato producers, importers, and consumers (FAO, 2008). In Ethiopia, potato production also has increased considerably through the twentieth century. In 1975, the area of cultivation was estimated at 30,000 hectares, with an average yield of approximately five tons per hectare. The area of cultivation had reached 50,000 hectares by the mid 1980's and 2001 production area raised up to 160,000 hectares, with average yields around eight tons per hectare. The average yield of potato in Ethiopia is 8- 9 tones/ha which is much lower than the African continent average of 10.8 ton/ha and the world average yield of 16 ton/ha (FAO, 2008; Ferdu *et al*., 2009).

There has been a dramatic increase in potato production and demand in Asia, Africa and Latin America, where output rose from less than 30 million tons in the early 1960s to more than 165 million tons in 2007. The total world potato production is estimated at 364.808.768 ton in 2013. China is now the biggest potato producer, and almost one third of all potatoes are harvested in China and India (FAOSTAT, 2014). China, India, Russian federation, Ukraine and United States of America are the top five world potato producing countries (FAOSTAT, 2015). Europe and Central Asia produced 144 million ton of roots and tubers. They mostly account 18 percent of the world potatoes output in 2011. The total Indian potato production during 2014-15 has increased by 10.7 per cent from 41.5 million tons in 2013-14 to 45.9 million tons in 2014-15 (FAO STAT, 2014). The area under potato production in Ethiopia in the year 2013/14 was about 66,745 hectares with an average national yield of 11.7 t/ha for the main cropping season (CSA, 2014). The area under potato was 70,132 ha and the total production from potato was 943,233 tons with an average productivity of 13.5 t/ha in the main cropping season of 2015/16 (CSA, 2016).

The main potato exporters are the Netherlands, France and Germany, while Spain and Belgium import most. The Netherlands is responsible for 22 percent of the world's potato exports (FAO

STAT, 2012). Eastern Hararghe (Haramaya and Kombolcha Woredas) is one of the major potato producing areas in Ethiopia (Bezabih and Hadera, 2007). This zone produced an average yield of 19.3 t/ha higher than the average national production in the year 2013/14 of 11.7 t/ha (CSA, 2014). In addition to satisfying domestic demand, the potato produced in the region is being exported to regional markets like Djibouti and Somalia, Middle East countries and the western European countries (EHDA, 2011). For instance, out of the total volume of potato marketed to Somalia, 75 percent is supplied from East Hararghe and about 25 percent from the Central part of Ethiopia (Bezabih, 2008).

2.2. Factors affecting the production of potato in the World

Although potato crops are the most important food crop in the world, there are various biotic and abiotic production constraints that hinder potato production. From those factors: uses of traditional production technology, absence of well adapted varieties, absence of sufficient and high quality seed potatoes, diseases (like late blight, early blight, bacterial wilt, tuber rots), insect pests, lack of improved storage techniques, poor market, low price and high temperature or drought are the most important ones (Tewodros Ayalew, 2014; Gastelo *et al*., 2014). Low soil fertility is another main limiting factor for potato production in eastern Africa (Muriithi *et al*., 2004). Nitrogen and phosphorus are scarce in most. Ethiopian soils and thus application of these nutrients could significantly increase crop yields (Nitrogen has greater influence on growth and yield of crop plants than any other essential plant nutrient (MOA, 2004).

2.3. Insect Pests of potato

Potato crops are damaged by a wide variety of insect pests that can completely destroy the crop if left uncontrolled. We can divide potato pests into two group namely indirect (above ground by feeding on leaves or stems), and direct (below ground by feeding on tuber) (Vincent *et al.,* 2013). From a huge number of potato insect pests a few include Colorado potato beetle, Aphids, Leafhopper, Psyllid, Wireworms and Potato Tuber Moths are the most important ones (Figure 1). From these pests Aphids and Potato Tuber Moth (PTM) are the major pests (Weber, 2013). PTM is the most common and worldwide pest of potato. The larvae of the moth both mine leaves and bore in tubers. Host plants like tomato, tobacco, eggplant and beets may be also infested by PTM (FAO, 2008).

2.4. Potato tuber moth

2.4.1. History and Distribution of potato tuber moth

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), (Lepidoptera: Gelechiidae), probably originated in tropical mountainous regions of South America (Sporleder, 2007). It has been reported to occur in tropical and subtropical areas in South, Central, and North America; Africa; Australia; and Asia. Specifically in the United States, PTM is distributed over many states such as California, Arizona, Maryland, Virginia, Colorado, North Carolina, South Carolina, New York, North Dakota, Oregon, Washington, and Idaho (Rondon, 2010). It is also observed as widely distributed in East Africa including Ethiopia (CABI/EPPO, 2017). In Ethiopia, PTM was recognized as an important pest, primarily, in the warmer areas where potato is grown. However, now a day PTM has become established in all 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 Mulatu *et al*., 2008).

2.4.2. Ecology and host of potato tuber moth

PTM attacks the members of the family Solanaceae mainly potato (*Solanium tuberosum*), tomato (*Lycopersicon esculentum*), tobacco (*Ivicolina tabacum*), eggplant (*Solanum melongena*) and pepper (*Capsicum* spp.) (Bin-Cheng, 1994).

Temperature is reported to be critical for rapid increase of PTM. Average daily temperatures between 20-25^{o}C are optimum for PTM development (Raman, 1980). During the hot season, PTM can complete a generation in about 3 weeks. Larval growth and development slow down at cooler temperatures, but continue as long as temperatures are above 11^{o}C. During the winter, generation time may be as long as 7 months. During the cool season PTM populations remain low and do not cause significant damage to potato crops (Hanafi, 1999).

2.4.3. Identification of potato tuber moth

Larvae of PTM mine leaves, stems, and petioles and excavate tunnels through potato tubers (Plate 1A-D) (Rondon, 2010). Mines are the typical symptoms of leaf damage caused by the larvae eating the mesophyll without damaging the upper and lower epidermis. Larvae also enter into leaf axils and growing points of young plants. Larvae enter tubers through the potato eyes. Inside the tubers, the larvae bore irregular galleries that may run into the interior or remain directly under the skin. Larval excrement is pushed out through the holes, which can be noted immediately after the larvae start mining. When heavily infested, tubers are often rotten making them unsuitable for human consumption. At harvest, tubers do not always show signs of damage, but may harbor eggs and early instar larvae. This may result in severe post-harvest losses of stored potato in the absence of adequate control measures. Mining results in weight losses of tubers, which is exacerbated by increased transpiration through the wounds and causing them to shrink. The wounds provide entry points for microorganisms and cause secondary infection, particularly by species of *Penicillium* (Alvarez *et al.,* 2005).

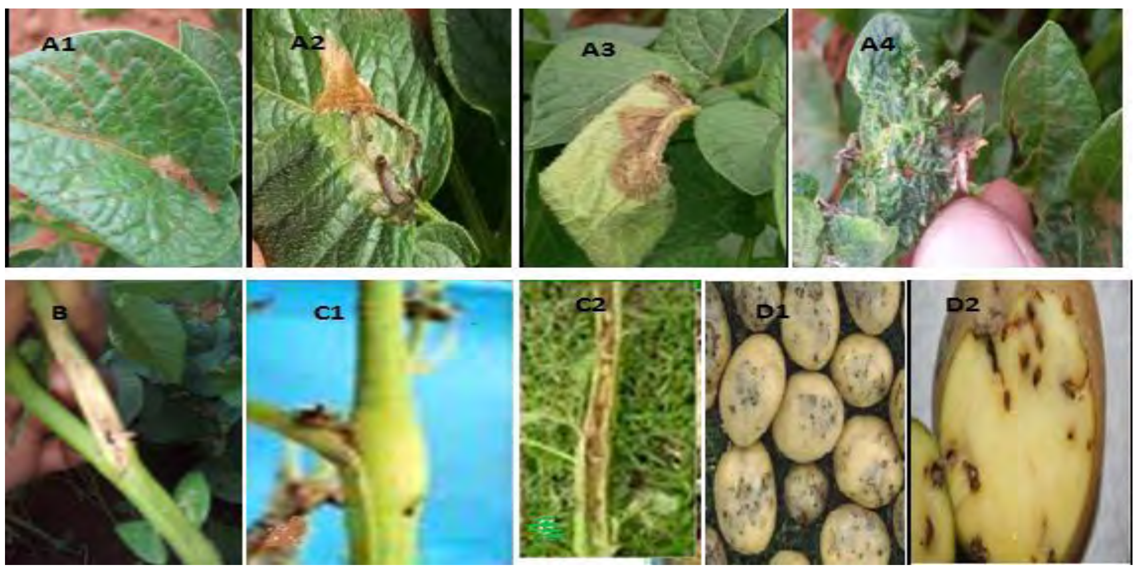

Plate 1. PTM infestation symptoms on (A) leaf, (B) petiole, (C) stem and (D) tuber
Source: (Current field work)

2.4.4. Life cycle and biology of potato tuber moth

Potato tuber moth passes through complete metamorphosis (egg, larva, pupa, and adult) (Plate 2)

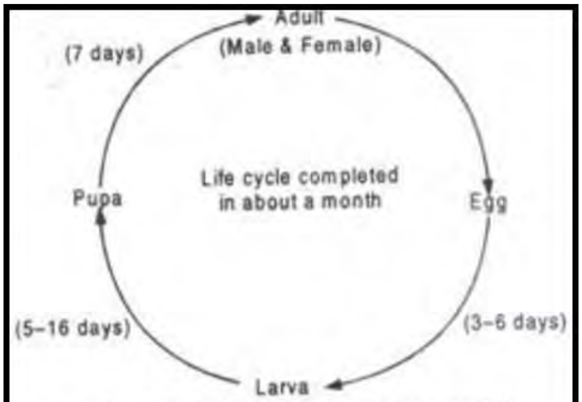

Fig. 96. Life cycle of *Phthorimaea operculella*.

Plate 2. Life cycle of PTM

Egg

The PTM Egg is small (0.5 mm) white to yellow in color and deposited singly. The eggs are invisible to the naked eye on potato leaves and tubers. Of all the host plants, the females prefer to lay eggs on potato. The females lay between 100 and 300 eggs depending on temperature and food availability. Egg sometimes may put on underside of leaves, stems, tuber, sacks and containers used for storage (Raman, 1980).

Plate 3. PTM egg
Source: (Rondon *et al*., 2007)

Larva

The newly emerged larva is about 1 mm long and very active that soon commences to mine leaf. It develops through four larval stages. When fully fed, it reaches about 12 mm long, with a dark brown head and a body which is greenish if the larva developed in foliage or pinkish grey if developed in a tuber (Plate 3). The larval period lasts about 14 days in summer, but is much longer during cool weather. When fully grown the larvae leave the plants or the tubers and spin flimsy cocoons amongst clods or plant debris on the ground (Plate 3). If they are in stored potatoes they make the cocoons between tubers, in the entrances to tunnels in the tubers or on the sides of bags or containers (Hamilton, 2003).

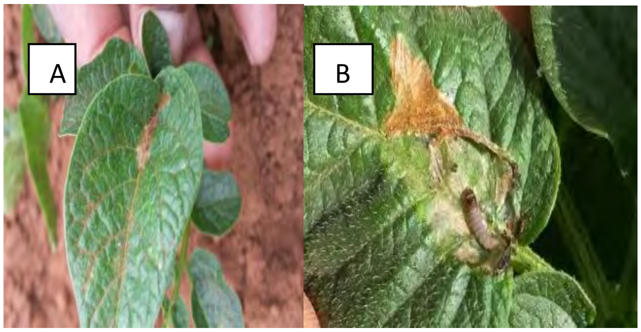

Plate 4. PTM Larvae within leaf (A) and exposed leaf (B)
Source: (current field work)

Pupa

A pupa is brownish 6 mm long and enclosed in a white silk like cocoon (Plate 4). They may be in various places, old dry leaves on the plant, soil litter, eyes of tubers, storage walls, etc (Raman, 1980).

Plate 5. PTM Pupa
Source: (Rondon et al., 2007)

Adult

Adult moths have silvery and brownish gray colored body (7-9 mm). Adults are usually fly at night on the posterior edge of the forewings and on both posterior and inner edges of the hind wings. 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*., 2007) (Plate 5). At rest the wings are folded to form a roof-like shape. The wingspan is between 12 and 16mm. The tip of the female's abdomen is cone-shaped, whereas the males possess two claspers at the hairy tip of their abdomen. The male's sexual organs are situated in the middle of the ninth abdominal segment, the females in the middle of the eight. The sexual pheromones by which females attract males have been isolated and identified as trans-4, cis-7-tridecadien-1-ol acetate (PTM1) and trans-4, cis-7, cis-10-tridecatrien-1-ol acetate (PTM2). A mixture of the two is far more effective that one component used by itself (Raman, 1980).

Plate 6. Adult PTM (A) Female PTM (B) Male PTM
Source: (Rondon *et al.,* 2007)

2.5. Management of potato tuber moth

2.5.1. Cultural control

Cultural control involves several activities such as pre harvest measures: weed elimination from fields and surrounding areas; good coverage of potato seeds with soil to protect damage by adults and larvae; discard infested tuber seeds and use only healthy seed tubers for planting; prompt harvesting soon after crop maturity (Rondon *et al*., 2007).

Moth populations are maintained in plant and tuber debris in the field in the absence of main crop. Therefore, timely field cleanliness is an important preventive post harvest measure. Avoid leaving harvested tubers overnight in the field as these potatoes could act as egg laying sites for potato tuber moth (Raman 1980; Alvarez *et al.,* 2005). Ahmed Ibrahim (2015) concluded that mud blocket storage, mat storage, simple silo of land (investigated by Holetta Agricultural Research Center) can be used to protect consumption potatoes from potato tuber moth and increases three months and ten days. Field as well as storage sanitation is the best and most effective way of reducing the damage of potato tuber moth and can improve the quality of our products (Binyam Tsedaley, 2015).

2.5.2. Biological control

2.5.2.1. Predators and Parasitoids

The roles of common predators such as lady beetles, big-eyed bugs, and ground beetles have been proved in controlling PTM (CABI/EPPO, 2017). El-sawi and Momen (2005) suggested *Typhlodromips swirskii* as a potential biological control agent against PTM**.** Parasitoid wasps such as *Copidosoma* spp. and *Apanteles* spp are important in PTM control (CABI/EPPO, 2017).

2.5.2.2. Microbial control

Tekalign Zeleke *et al*. (2015) recommended for the effective management of PTM for potato tuber protection using higher concentration of enthomopathogenic fumgi in Ethiopia. Sabbour and Abdel-Raheem (2014) observed that using entomopathogenic fungi such as *Beauveria* *brongniartii and Nomuraea rileyi* reduced the number of eggs laid per female and the emerged adults. The yield weight of potatoes also increased after being treated with fungi as compared to the control. The nano entomopathogenic fungi, nano-I. fumosorosea and nano-M. flavoviride were significantly decreased PTM infestation and increased yield of potato field (Sabbour, 2015). Fumigation of tubers with *Muscodor albus* volatiles also used to control PTM (Lacey *et al.,* 2008). *Nomuraea rileyi* were observed to decrease significantly the number of PTM egg laid and increased yield (Sabbourand and Nayera, 2015).

The PTM granulovirus (PhopGV) has been developed as a liquid suspension and resulted in successful control of PTM in the stores (Abd-Alla *et al.,* 2015).*Bacillus thuringiensis* (*Bt*) formulations have proved effective for potato tuber moth control in various parts of world (Alvarez *et al.*, 2005).The work of Kepeneci *et al*. (2013) indicated that *P. operculella* larvae are quite susceptible to entomopathogenic nematode infection and, in particular, *Steinernema carpocapsae* blacksea strain has a high level of potential to control this pest. *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* caused the highest mortality in both larval and pre-pupal stages of PTM (Hassani-Kakhki *et al*., 2013).

2.5.2.3. Pheromone traps

Using baited PTM sex pheromone and water detergent resulting in a significant reduction ($P<0.05$) of tuber damage of PTM and reduces PTM reproduction (Larraín *et al*., 2009). Mehdi, (2015) investigated pheromone traps, rubber dispensers containing a mixture of the compounds E4-Z7 Tridecadienil acetate, and E4-Z7-Z10 Tridecatrienil acetate were placed on wires and suspended above water traps (water + detergent) to kill the captured males by drowning. Finally a green water-pan trap has been effective to capture male *P. operculella*.

2.5.3. Host resistance

Transgenic line developed using *CrylAc9* gene has been proved to inhibit the larvae growth of PTM and prevent pupation of larvae on field grown leaves. It also maintains common phenotypic appearance and yield comparable with non transgenic control (Wratten and conner, 2002). Another study proved that transforming potato with *CryV-Bt* gene showed 96% PTM larvae control on host plant (Douches *et al.,* 1998). Douches, *et al.* (2010) demonstrated that *Crylal* gene transformed potatoes were completely resistant (100% mortality) potato tuber moth.

2.5.4. Chemical control

Chemical control of PTM is challenging because of the protected tunneling behavior of larvae in foliage and tubers. Because of this, the pest has developed resistance to many traditional organophosphate, carbamate, and pyrethroid insecticides (Binyam Tsedaley, 2015). Treatment application should also be established based on field-specific information. This is because *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 insecticides. In areas under threat by PTM, growers are encouraged to monitor the insect numbers using pheromone traps (Rondon *et al.,* 2007). In Ethiopia the synthetic insecticide diazionon 60% EC used effectively (as cited in, Ayalew Tadesse, 2010). To control PTM in the field Vaneva-Gancheva and Dimitrov, (2013) concluded as Acetamiprid and Imidacloprid can be applied together to control the attacks of the potato tuber moth on tobacco in the initial stages after planting, i.e. as the period of protection of the tobacco plants is extended, the extent of damages increases. Rafiee-Dastjerdi *et al.* (2013) showed that deltamethrin had the higher toxicity to egg and adult stages of PTM and abamectin had the higher toxicity to its first instar larvae. Since deltamethrin increased the stable population parameters of PTM specially its intrinsic rate of increase, thus it might not be recommended for control of PTM. However, based on lethal and sublethal effects, abamectin could be suitable for management of this pest.

2.5.5. Botanical control

Aschalew Sisay and Ahmed Ibrahim, (2012) concluded in their study that *Lantana camara, E. globulus* and Pyrethrum flowers can be used to protect seed potatoes from potato tuber moth damage in storage. Other study demonstrated that *Thymus syriacus* essential oil is promising as a fumigant against the different developmental stages of PTM (Tayoub *et al.,* 2016). Study on *Marrubium vulgare* and *Achillea millefolium* indicated that their crude extracts had toxic effects against egg, 1st larva instars, and adult stages of PTM (Allahverdizadeh and Mohammadi, 2016). Dusting potato tubers with bulb powder of *Allium cepa* (50% con. mixed with talcum powder) displayed a highly effective role in the reduction of deposited eggs as well as adult emergence and *Cymbopogon citratus* caused high reduction in larval penetration into treated tubers (Sharaby *et al*., 2014).

2.5.5.1. Description and Ethinobotanical use of birbira

Milletia ferruginea (Birbira) belongs to the family Leguminaceae and subfamily Papilionoideae (Thulin, 1983). Birbira is a large shady tree which grows up to 35m and performs well in moist lowland as well as in the dry, moist and wet semi-highland climate of the country up to an elevation of 1000-2500 m above the mean sea level (Azene Bekele, 2007; MacLachlan, 2001). The genus *Milletia* constitutes about 200 species in tropical and subtropical Africa, Asia and Australia (Thulin, 1983). Ethiopian indigenous tree (Birbira) is widely distributed in many parts of the country known to occur in two sub-species. These are: - *Milletia f. ferruginea,* which is restricted to the northern part of the country and *Milletia f. darasana,* which occurs in southern region, particularly Sidama. Bibira trees from central and western Ethiopia consisted of both sub-species (Azene Bekele, 2007; Jembere *et al.,* 2005).

M. ferruiginea is used for nitrogen fixation (Berhanu and Amare, 2012; MacLachlan, 2001), since it belongs to Leguminaceae family. *M. ferruginea* utilized as a forage (Berhanu and Amare, 2012) and can be integrated in the diets of sheep (Banerjee *et al*., 2013), goat (Sisay Tadesse *et al.,* 2015) and honey bee (Teklu Gebretsadik, 2016). The seeds, leaves, stem barks and roots containing the toxic chemical rotenone were widely used as insecticides and piscicides (to kill fishes) through contact and stomach poisoning (Jembere *et al.,* 2005; MacLachlan, 2001). *M. ferruginea* had much better efficacy against ticks, *Rhipicephalus decoloratus* (Askale Gizaw, 2015). Adem Nega, (2015) indicated that *M. ferruginea* has possibility in controlling *Diamondback moth, (Plutella xylostella L.).*Therefore the above research works are motivating positive results to try *M. ferruginea* against PTM at field condition.

2.5.6. Integrated pest management

There is a wide variety of techniques that can be applied under IPM approaches. Applicability of individual techniques depends on various factors, including the crop, the cropping system, the pest problems, the climate, the agro-ecological conditions, etc. Generally, IPM involves a combination of techniques (IPM, 2014). The most effective management program combines cultural, biological, and chemical approaches. A number of insecticides have proven effective in controlling PTM. Since this pest prefers foliage rather than tubers, and tuber infestation is reduced when a full or partial canopy is present, early use of insecticides may not be acceptable (Rondon *et al.,* 2007).

PTM control in stores was found to be more effective if infestation in fields was kept to minimum. Low infestation at harvest and rapid handling of the potatoes going into store established good initial storage conditions and decreased the likelihood of post-harvest losses. Integrated (cultural, biological and chemical) means of reducing PTM infestation in potato fields and 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 (Hanafi, 1999). An integrated control approach comprising *Bacillus thuringiensis* subsp, *Kurstaki* (*Btk*) applied at the beginning of the storage period in combination with early harvest has been effective (Lacey and Kroschel, 2009). Cooper *et al*., (2009) pointed out that combining avidin with a stronger natural host plant resistance factor from *Solanum berthaultii, Solanum commersonii, Solanum sparsipilum, and Solanum sucrense* provide superior protection against insect pests. Abdel-Razek *et al*. (2014) showed that Nimbecidine and Bio-power could be incorporated into an integrated pest management program of potato tuber moth. Binyam Tsedaley, (2015) recommended that integrating of many management options helps in reducing the risk of pesticide resistance development, reduce the impacts of the insecticide to environment, non-targeted organisms, beneficial insects such as natural enemies and human hazards. It is very important to store our products at appropriate storage conditions in order to reduce the damage from insect pests as well as other organisms. Omar *et al.,* (2011) Thus early planting of potato summer plantation in January, Sex pheromone–baited water traps and bioinsecticides, Azadirachtin (Achock 15% EC) and Soybean oil (Naturals 96% EC) were a promising components of IPM strategies for reducing PTM population in potato fields.

3. MATERIAL AND METHODS

3.1. Description of the study area

The field experiment was conducted at Holetta Agricultural Research Center (HARC) that is located 28 Km west of Addis Ababa, between 8^o 59' 35.53" N latitude and 38^o 29'16.12" E longitude. The altitudinal range is 2060 m to 3380 m above sea level. The average annual temperature is 21^o C and rain fall is 900 mm-1100 mm. The soil type is Eutric Nitosol (http://www.eiar.gov.et/index.php/holeta-agricultural-research-center).

"Gudene" potato variety was used for this experiment. Several researches conducted research on agronomic, diversity and varietal performance of the crop at HARC. "Gudane" is among the newly released nine potato varieties with an average yield of 2.91 t/ha and it is commonly grown at an altitudinal range between 1600 m-2800 m a.s.l. "Guidene" (CIP-386423.13) variety was developed and released by Ethiopian Institute of Agricultural Research (EIAR) and Holetta Agricultural Research Center in 2006 with a wide-range of environmental adaptation in Ethiopia (Agajie Tesfaye *et.al.* 2013).

3.2. Experimental design

The experiment was laid out in a randomized complete block design (RCBD) with three replications and twenty one plots. The experiment was done under irrigated condition. Each plot consisted of four rows, with 3m length and 3m width with the spacing of 75 cm between rows and 30 cm between plants. The sprouted and healthy potato seed tubers of Gudene variety were sown at a depth of 15 cm in a row. Each row has 10 plants so a plot has 40 plants. Inorganic fertilizer was applied at the rate of 165 kg/ha of UREA and 195 kg/ha of DAP. For each plot

0.1485 Kg/ha of UREA and 0.1755 Kg/ha of DAP were mixed together and applied during planting time.

3.3. Botanical plants Collection and Extraction

Matured Birbira pods were collected from Addis Ababa (AAU-Arat-Kilo campus), Holetta and partly the seeds were obtained from HARC Entomology laboratory. The pods (pale yellow in color) had been sun dried until all the seeds were being released, for three week. The released seeds were collected from the sac and the seed coat was removed to avoid moisture and again left for sun dried to coarsely powdered. After the seed coat removed, it was ground into fine powder, to pass through 0.5 mm mesh, manually using a mortar and pestle and the powder was kept in polythene bags at 4^{o} C until use.

About 5.84 kg *M. ferruginea* of seed powder was soaked into plastic container of 3L Ethanol (99%). Birbira seed powder weighing 1.38 kg, 1.98 kg, and 2.48 kg were separately added into three Pyrex glass beakers, containing 500 ml of distilled water. Then after, all the three mixtures were stirred with glass rod and further dissolved in distilled water using magnetic stirrer (Stuart Scientific UK) for 1hr and the hot plates were set at an appropriate temperature range in order to evaporate the Ethanol. The well resolved crude oil of *M. ferruginea* was discharged into separate Pyrex glass beakers and filtered through fine permeable muslin cloth. The residual was separated and then finally the creamy part was collected. The crude oil, extracted from the above three amounts, following this recipe was each dissolved in water, making a total volume of 6 L, which finally made three rates (levels) of concentration, namely 0.23 gm/ml, 0.33 gm/ml, and 0.43 gm/ml.

Plate 7. Birbira crude oil extraction process and application
Source: (Current field work)

3.4. Treatment application

To evaluate the optimum rate of concentration and frequencies of Birbira extract for the control of potato tuber moth, three different rates and two levels of frequencies were tested. Accordingly the tested treatments were:

i. 0.23 gm/ml x 1 time
ii. 0.23 gm/ml x 2 times
iii. 0.33 gm/ml x 1 time
iv. 0.33 gm/ml x 2 times
v. 0.43 gm/ml x 1 time
vi. 0.43 gm/ml x 2 times
vii. untreated (check)

Treatments were applied in a manner that all the plots (18) were treated with their respective rates during the first spray, except the control, whereas for the second spray only one half of the total plots (9) treated twice, i.e., nine plots received the treatment once and the other nine plot twice. The treatments were applied two months after planting and two weeks after the first treatment application. Three plots were also used as a control.

3.5. Data collection and analyses

3.5.1. Leaf, Petiole and Stem infestation

Five plants from each row within each plot were randomly selected to count the infestation of leaf, petiole and stem by PTM. The parameters taken were: the ratio of infested leaf to the total leaf, the ratio of infested petiole to the total petiole, and the ratio of infested stem to the total stem before and after treatment application.

3.5.2. Tuber infestation

Five plants were randomly selected from the middle two rows within a plot and dugout to score the presence or absence of PTM infestation on tuber. The total number of infested and the total number of tubers were recorded and weighed separately for each treatment and for the control, in order to determine the yield, at time of harvest.

3.6. Data analysis

Data collection was done starting from Vegetative stage to harvesting time. Each data was recorded for infestation level of PTM to potato leaf, stem, petiole and tuber. The collected data were subjected to different analyses using Statistical Analysis Software (SAS) ver. 9.1. These include descriptive statistics, separate and combined ANOVAs, multiple mean comparisons (Duncan's Multiple Range test) and treatment means versus control mean comparison (Dunnet's procedure) were done.

4. RESULT

4.1. Effect of birbira seed powder on PTM infestation

4.1.1. Potato leaf infestation

Results of potato leaf infestation by PTM are shown in Tables 1 and 2. Potato leaf infestation by PTM ranged from 22% to 48% before treatment application (Table 1). Treatment application did not stop or minimize PTM infestation on leaf as mean percent infestation at 1st, 2nd, 3rd and 4th records after first treatment application, which ranged from 31% to 63% (Table 1). Except in the 1st records shown variation between treatment 0.23gm/mlx1 and 0.23gm/ml x2, there was no significant difference shown among the treatments and the untreated check.

After second treatment application, the mean percent PTM infestation on potato leaf at 1st, 2nd, 3rd and 4th records ranged from 47% to 68% (Table 2). Only record four show significant variation between treatment 0.43gm/ml x1 and 0.43gm/mlx2.In spite of these variations, there was no significant difference between treatments and untreated check. In general, treating potato leaf by Birbira seed powder extract in rate of 0.23mg/ml, 0.33mg/ml and 0.43mg/ml with one time or two times application did not show any PTM infestation reduction at field of potato.

Table 1. Mean (± SE) Percent PTM infestation on potato leaf before and after first treatment application

	Percent PTM infestation before treatment application		Percent PTM infestation after treatment application			
			After first application			
	Vegetative stage		Vegetative stage			
Treatments*	1st Record (1st week)	2nd Record (2nd week)	1st Record (1st day)	2nd Record (3rd day)	3rd Record (5th day)	4th Record (7th day)
0.23 gm/mlx1	22.88± 4.41^{a}	42.16±5.44^{a}	**45.17±3.97^{a}**	**49.56±5.68^{a}**	**43.89±4.35^{a}**	**31.42±15.9^{a}**
0.23 gm/mlx2	26.76±5.19^{a}	47.62±4.33^{a}	**62.40±4.43^{b}**	56.73±5.69^{a}	51.32±3.75^{a}	**63.34±1.61^{b}**
0.33 gm/mlx1	**21.89±4.23^{a}**	40.41±4.59^{a}	**50.30±6.49^{ab}**	54.84±6.12^{a}	47.41+4.96^{a}	**53.24±7.46^{ab}**
0.33gm/mlx2	29.05±6.36^{a}	43.03±5.58^{a}	**57.47±4.67^{ab}**	56.10±7.49^{a}	51.36±7.61^{a}	**60.22±1.33^{b}**
0.43 gm/mlx1	22.81±4.44^{a}	42.68±1.39^{a}	**53.96±1.26^{ab}**	51.54±2.68^{a}	49.48±4.18^{a}	**60.77±5.69^{b}**
0.43 gm/mlx2	22.58±7.62^{a}	**39.92±1.92^{a}**	**49.28±3.08^{ab}**	53.46±0.61^{a}	44.02±1.59^{a}	**52.86±4.24^{ab}**
Untreated check	26.24±6.92^{a}	45.51±2.02^{a}	**48.43±4.26^{ab}**	51.35±1.83^{a}	49.08±4.17^{a}	59.16±5.11^{b}

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test
*x1=one time application; x2=two times application

Table 2. Mean (± SE) Percent PTM infestation on potato leaf after second treatment application

Treatments*	Percent PTM infestation after treatment application			
	After second application			
	Vegetative stage			
	1st Record (1st day)	2nd Record (3rd day)	3rd Record (5th day)	4th Record (7th day)
0.23 gm/mlx1	**52.98±7.8[a]**	**52.54±7.98[a]**	**49.80±8.86[a]**	**51.92±2.58[ab]**
0.23 gm/mlx2	66.78±5.59[a]	64.75±3.32[a]	55.64±11.08[a]	**54.41±3.53[ab]**
0.33 gm/mlx1	58.90±6.46[a]	55.36±6.01[a]	56.33±5.69[a]	**56.18±6.71[ab]**
0.33gm/mlx2	62.86±8.47[a]	61.51±9.24[a]	59.16±5.08[a]	**64.42±6.48[ab]**
0.43 gm/mlx1	62.18±5.67[a]	65.69±4.51[a]	52.15±11.30[a]	**47.37±4.99[a]**
0.43 gm/mlx2	59.72±3.97[a]	64.10±4.73[a]	65.06±5.03[a]	**68.26±9.16[b]**
Untreated check	59.49±4.59[a]	62.47±4.76[a]	58.21±0.94[a]	**64.21±5.29[ab]**

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test
*x1=one time application; x2=two times application

4.1.2. Potato petiole infestation

The results of potato petiole infestation by PTM are summarized in Table 3 and 4. Before treatment application, there was no sign of petiole infestation by PTM. Therefore, data were taken after the treatment application. Mean percent PTM infestation on potato petiole in 1st and 2nd records ranges from 3% to 10% after first treatment application. There was no significant difference among the treatments and the untreated check (Table 3). ANOVA and mean separation by Dunnet's test also showed no significant variation between rates and frequencies and the untreated check (Annex 1 table 11 through 16, annex 2 and Annex 3).

Percentages of PTM infestation on potato petiole after second treatment application were calculated (Table 4). The percent infestation ranges from 1% to 6%. In the second treatment application gradual PTM infestation minimization was shown in descending order from1st, 2nd, 3rd and 4th records but it was not significant. There was also no significant variation between treatments and untreated check (Table 4 and Annex 3).

Table 3. Mean (±SE) Percent PTM infestation on potato petiole at Vegetative stage after treatment application

	Percent PTM infestation after treatment application	
	After first application	
	Vegetative stage	
Treatments*	1st Record (5th day)	2nd Record (7th day)
0.23 gm/mlx1	7.12+5.42a	2.61+1.78 a
0.23 gm/mlx2	9.80+6.15 a	5.72+0.77 a
0.33 gm/mlx1	6.79+2.31a	2.99+0.39 a
0.33gm/mlx2	10.42+3.26b	4.12+0.34 a
0.43 gm/mlx1	8.55+5.92 a	4.18+0.13 a
0.43 gm/mlx2	9.30+5.29 a	2.72+0.65 a
Untreated check	10.20+4.07a	3.06+1.69 a

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test

*x1=one time application; x2=two times application

Table 4. Mean (±SE) Percent PTM infestation on potato petiole after second treatment application

Treatments*	Percent PTM infestation after treatment application			
	After second application			
	Vegetative stage			
	1st Record (1st day)	2nd Record (3rd day)	3rd Record (5th day)	4th Record (7th day
0.23 gm/mlx1	3.47+0.55[a]	3.26+1.25 [a]	2.09+0.35 [a]	0.93+0.38 [a]
0.23 gm/mlx2	4.50+1.11[a]	4.80+1.47 [a]	2.46+0.55 [a]	1.49+1.31 [a]
0.33 gm/mlx1	3.11+0.52 [a]	2.35+0.77 [a]	3.01+0.71 [a]	1.59+0.37 [a]
0.33gm/mlx2	5.66+2.30 [a]	4.18+1.21 [a]	2.36+1.23 [a]	2.04+0.92 [a]
0.43 gm/mlx1	4.31+1.38 [a]	2.82+0.56 a	2.90+0.94 [a]	1.26+0.64 [a]
0.43 gm/mlx2	4.02+0.170 [a]	3.53+0.34 [a]	4.61+1.63 [a]	1.81+0.77 [a]
Untreated check	3.65+1.13 [a]	1.95+0.27 [a]	3.78+1.12 [a]	1.66+0.42 [a]

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test

*x1=one time application; x2=two times application

4.1.3. Potato stem infestation

Table 5 demonstrates pair wise treatments mean percent comparison of potato stem infestation by PTM. Mean percent PTM infestation on stem ranges from 27% to 85% according to 1st, 2nd, 3rd and 4th records. The highest PTM infestation during the 4th record might be the indication of foliage damage and the peak stage of stem infestation. Duncan's comparisons show no significant difference between treatments. Dunnet's test also show there was no significant infestation difference between treated and untreated check (Annex 3).

4.1.4. Potato tuber infestation and Yield (t/ha)

Potato tuber infestations by PTM and yield estimation are shown in table 6. Mean percent PTM infestation on potato tuber ranges from 37% to 43%, according to records taken at time of harvest. The result of potato yield ranges from 3 t/h to 4 t/ha depending on their weight per plot. Although there are large differences, no significant variation was shown between treatment and untreated check in both parameters (Table 6 and Annex 3).

Table 5. Mean (±SE) Percent PTM infestation on potato stem at Vegetative stage after treatment application

Treatments *	Percent infestation after treatment application			
	After second application			
	Vegetative stage			
	1st Record	2nd Record	3rd Record (5th	4th Record (7th
0.23 gm/mlx1	51.53+15.09^{a}	50.39+17.08^{a}	35.50+11.65^{a}	85.61+4.46a
0.23 gm/mlx2	30.96±7.06^{a}	40.88±5.72^{a}	**26.51±10.08^{a}**	75.79±5.75^{a}
0.33 gm/mlx1	33.08±10.58^{a}	42.04±14.48^{a}	32.01±1.25^{a}	75.13±8.16^{a}
0.33gm/mlx2	38.33±3.53^{a}	52.93±793^{a}	37.24±16.89^{a}	**61.73±8.33^{a}**
0.43 gm/mlx1	**30.95±3.40^{a}**	42.22±8.01^{a}	41.51±7.72^{a}	82.09±5.24^{a}
0.43 gm/mlx2	37.45±8.73^{a}	**33.22±6.42^{a}**	30.98±10.85^{a}	62.59±13.82^{a}
Untreated check	44.36±8.97^{a}	35.71±7.14^{a}	30.34±5.51^{a}	70.83±4.17^{a}

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test
*x1=one time application; x2=two times application

Table 6. Mean (±SE) Percent PTM tuber infestation and yield of potato (t/ha)

Treatments *	Percent infestation after treatment application	Yield (t/ha)
	After second application	
	Maturity stage	
	1st record	
0.23 gm/mlx1	41.27±5.23 [a]	4.33±0.88 [a]
0.23 gm/mlx2	43.26±4.26 [a]	4.0±0.58 [a]
0.33 gm/mlx1	41.26±2.48 [a]	4.0±00 [a]
0.33gm/mlx2	40.49±3.08 [a]	3.33± 0.67 [a]
0.43 gm/mlx1	41.03±3.52 [a]	3.33±0.33 [a]
0.43 gm/mlx2	39.33±2.3 [a]	4.33±0.33 [a]
Untreated	**37.52±1.09 [a]**	**4.0±.058** [a]

Means followed by different letters, in the same column, are significantly different at 5% level using Duncan's Multiple Range Test
*x1=one time application; x2=two times application

4.1.5. ANOVA and mean separation for PTM infestation

All the combined analyses of variances (ANOVA) calculated based on the mean infestation rates showed no significant variation both among rates, frequencies and rate interaction with frequency (Annex 1 Table 1-22). Separate ANOVA between treatments also showed no significant variation (Annex 2 Table 1).

In addition to the above pair wise mean comparisons by Dunca's Multiple Range Test (Table 1-6), Dunnet's procedure was also used to evaluate the infestation rates with untreated check

(Annex 3 Table 1-22). As a result no significant difference was observed.

5. DISCUSSION

Results of the current study revealed that application of seed powder extract of birbira did not show PTM control on potato. Hence, birbira under field condition is not fast effective against PTM, at the tested rates and frequencies. These results agree with Foot (1976) who reported that a number of foliar botanicals used were not effective against PTM.

The natural behavior of larvae feeding in protected tunneling and hard covering on leaves may make birbira treatment ineffective to control PTM under field condition. Related work by Thakur and Chandla, (2013) stated that neem, *Lantana camara*, *Tegetes erectus* and *Ruta graveolens* were 100 per cent effective up to 5 days of application. All the treatments were quite effective in keeping the damage below Economic Threshold Level up to two weeks, but thereafter the PTM incidence started rising and even a second spray of biopesticides (on 15^{th} day) could not keep the damage below ETL Under polyhouse conditions. Botanicals are fast degradable in sunlight, air and moisture (rain fall), therefore repeated application may improve birbira efficacy at field condition. For example, Abdel-Razek *et al*. (2014) reported that Nimbecidine® treatments after 3 applications reduced the larval mine of PTM by 79.6 and 43.8% when applied at 5.0 and 2.5 ml/l at field condition, respectively.

In the current study birbira treatment may show loss in its efficacy due to low application rates (0.23 gm/ml, 0.33 gm/ml and 0.43 gm/'ml) for field trial. However, birbira treatment is effective at the rate of 0.33 gm/ml to control PTM under laboratory condition (Kidiest Tefera, 2016). Ripen fruit of *Lindera neesiana* and rhizomes of *Acorus calamus* with 0.005%, 0.5% and 5%

were found effective against PTM in the laboratory (Niroula and Vaidya, 2004). Visser (2004) conducted study by extracts from fresh syringa leaves using 40g/l were effective on the larvae of the potato tuber moth under field conditions. The essential oils from the leaves of *Rosmarinus officinalis* could be exploited as alternative means against *P. operculella* during storage of potato tubers (Hannour *et al*., 2017). Botanical preparations from 35 plant species are effective against PTM either in the storage or in the laboratory according to a literature published from 1915 to 1993 and surveyed by Das (1995).

As this study was practiced at irrigation condition, during which high shortage of water encountered that favored the PTM proliferation, consequently become uncontrollable by applying botanicals. The shortage of water creates condition for soil cracking and entrance of PTM to infest potato. This may be a good factor for prevalence of PTM infestation in the field. Different research works recommended regular irrigation, as one cultural method of insect pest control. Because continuous irrigation keeps soil moist and the surface conserved enable the control of PTM (Alvarez *et al.,* 2005).

Unusual climatic change (Elnino) which happened in Ethiopia during our field work in 2016, may also have its own contribution to enhance the PTM infestation. Some reports stated that months from January to June is a peak time of PTM reproduction and hot summers allow a rapid PTM population increase which surpasses control thresholds in response to climate changes (Bayeh and Tadesse, 1992; Sporleder, 2007; Rondon *et al.,* 2007).

In the present study the majority of the harvested tuber became rotten due to PTM infestation and only few were left healthy. In similar way, Sileshi and Teriessa, (2001) categorized the infested tubers, which show rotting symptoms, as non-marketable. According to Rondon *et al.* (2007) and

Hanafi (1999) most economic damage occurs when PTM infests potato tubers, but heavy foliage infestation may also cause yield losses and reduce market value.

Other researchers showed effective outcome in controlling insect pests using botanical insecticides under storage, greenhouse and field condition. For example, *Ocimum sanctum* was effective significantly at 0.6% concentration under laboratory condition. It was applied in a field trial and was resulted lower adult mortality and a higher nymphal mortality (Prishanthini and Vinobaba, 2014). Foliar sprays of *Mexican marigold* at rate of 4kg/ha was the most effective insecticide against pea aphids (Dagne and Ermias, 2016). Appling 2ml of castor bean oil was found sufficient to destroy 50% of the weevels within one hour (Melaku and Hatamu, 2015). Garlic bulb (*Allium sativum*), bitter melon leaf (*Momordica balsamina*) and neem seed oil (*Azadirachta indica*) are effective in controlling Diamondback Moth (*Plutella xylostella*) of cabbage at 5% concentration under field condition (Degri and Zakaria, 2015). Emana Getu (2014) investigated that water and acetone extracts of *M. ferruginea* gave 100% mortality of adult *Zabrotes subfasciatus* with in 24h after treatment application at the rates of 2 and 3ml. The seed extracts of *M. ferruginea* showed high mosquitocidal activity against larvae, pupae and adult stages of both the laboratory strains and field population of *A. arabiensis* (Abiy *et al*., 2014). Abaynesh and Abebe (2015) reported that petroleum ether crude extract of *Lagera tomentossa* and *Ocimum lamiifolium* result in highest mortality of 96% and 97.5% respectively against the larvae of Mediterranean fruit fly. Ahmadi *et al.,* (2012) investigated that tondexir (pepper extract), palizin (eucalyptus extract) and sirinol (garlic extract) are effective control against citrus mealybug. Wild mushroom are a potential source of bio-insecticides for commercial mosquito vector management for example, Lactarius densifolius chloroform extract was effective against *Anophles gambiae* and moderate effective against *Culex quinquefasciatus* respectively in aquatic ecosystems (Chelela *et al*., 2014).

Eyob *et al*., (2010) indicated that the seed water extract of 10% concentration of *M. ferruginea* are highly toxic to enset root Mealybug. Dipping young enset seedling in a Milletia solution before field establishment and multiple drenching the root zone of infested plant with the solution can be used for the management of enset root mealybug. Ibrahim *et al*., (2015) reported that 30-37% onion bulb yield were obtained in tree tobacco treatment and it had the best performance in controlling onion thrips compared to control. In another study Solangi *et al.* (2014) also showed that the efficacy of tobacco based biopesticides against whitefly was 70.88%, against thrips 57.27% and against aphid 60.40%. Water extract the Birbira seed caused 45-60% mortality on sorghum chaffer within 24-48 hrs and this was significantly higher than mortality caused by the standard insecticide, carbaryl, applied at recommended rate of 1.5 kg/ 400 L of water (Bekele *et al*., (2002). Gedeon Yohannes, (2006) indicated that 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.

Table 7. Summary of effective botanicals on non-PTM insects pests at different condition

Name of botanical plant	Part of botanical used	Rate of application	Treated insect pest	Effective environment	Authors
Tree tobacco (*Nicotinia glauca*)	leaf	50g/l	Thrips on Onion	Field	Ibrahim et al., (2015)
Garlic (*Allium sativum*), bitter melon (*Momordica balsamina*) and neem oil (*Azadirachta indica*)	Bulb, leaf and seed, respectiely	0.05	Diamondback moth on cabbage	Field	Degri and Zakaria, (2015)
Birbira (*Milletia ferruginea*)	seed	10- 40% w/v	Termites (*Macrotermes*) and Sorghum chaffer (*Pachnoda interrupta*)	Laboratory	Bekele *et al*.,(2002)
Birbira (*M. ferruginea*)	seed	10% w/v	Mealybug on Enset	Laboratory and Field	Eyob et al., (2010)
Mexican marigold (*Tagetus minuta* L.)	leaf	4 kg/ha	Green pea aphids on field pea	Field	Dagne and Ermias (2016)
Birbira (*M. ferruginea*)	seed	5, 10 and 15w/w	Bean bruchids on haricot bean seed	Labratory and storage	Emana Getu (2014)
Tulasi (*Ocimum sanctum*)	leaf	0.01	Mealybug on cotton	Labratory and Field	Prishanthini and Vinobaba (2014).
Birbira (*M. ferruginea*)	seed	14.7 mg/l_ 74.54 mg/l	On mosquito, *A. arabiensis*	Laboratory and field	Abiy *et al*., (2014).
Castor bean oil(*Ricinus communis* L.)	seed	2ml	Weevil on maize		Melaku and Hatamu, (2015).

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

High percent of PTM infestations were observed in all parts of potato plant under the current field experimental condition. The application of birbira was not shown remarkable reduction on PTM infestation throughout the potato developmental stages on all of its parts. This may be due to low rate of concentration used and high degradability nature of birbira seed extract. Therefore, the current study concludes that birbira seed powder extract by the current rate of concentration and time interval did not show PTM control under field condition.

6.2. Recommendation

Based on the result obtained from field experiment on PTM control using birbira seed oil extract the following can be recommended

- Further study should be conducted using higher rates and frequencies of birbira.
- Integrating birbira with cultural, host plant resistant, pheromone trap or some other techniques must be conducted to formulate IPM for better control of PTM under field condition.

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Annexes

Annex 1. Results of Combined ANOVA based on GLM procedure RCBD

Table 1. ANOVA table for potato leaf infested by PTM before treatment application record 1(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	1061.91	530.9551	11.78	0.0084
Rate	3	34.73024	11.57675	0.26	0.8541
Bl*Rate	6	49.49651	8.249418	1.4	0.33
Freq	2	67.82526	33.91263	0.75	0.5111
Rate*Freq	1	31.72589	31.72589	0.7	0.4337
Error	6	270.5383	45.08972		
Total	20	1516.226457			

Table 2. ANOVA table for potato leaf infested by PTM before treatment application record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	168.0212	84.01059	1.38	0.3203
Rate	3	67.54277	22.51426	0.37	0.7771
Bl*Rate	6	129.8728	21.64547	1.04	0.44
Freq	2	35.40437	17.70218	0.29	0.7569
Rate*Freq	1	30.98145	30.98145	0.51	0.5017
Error	6	363.9781	60.66302		
Total	20	795.8007238			

Table 3. ANOVA table for potato leaf infested by PTM after first treatment application record 1(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	45.59361	22.7968	0.28	0.7668
Rate	3	75.69491	25.23164	0.31	0.8197
Bl*Rate	6	233.0631	38.84384	0.65	0.61
Freq	2	250.4135	125.2067	1.53	0.2914
Rate*Freq	1	305.0103	305.0103	3.72	0.1022
Error	6	492.5524	82.09207		

Total	20	1402.327724			

Table 4. ANOVA table for potato leaf infested by PTM after first treatment application record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	521.8084	260.9042	4.8	0.0568
Rate	3	43.60408	14.53469	0.27	0.8467
Bl*Rate	6	166.4133	27.73555	0.52	0.68
Freq	2	67.75267	33.87633	0.62	0.5674
Rate*Freq	1	17.22736	17.22736	0.32	0.5937
Error	6	325.916	54.31933		
Total	20	1142.721781			

Table 5. ANOVA table for potato leaf infested by PTM after first treatment application record 3(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	114.0534	57.0267	0.7	0.5351
Rate	3	25.20142	8.400475	0.1	0.9557
Bl*Rate	6	310.3171	51.71952	0.16	0.92
Freq	2	20.93103	10.46552	0.13	0.8826
Rate*Freq	1	129.8989	129.8989	1.58	0.255
Error	6	492.199	82.03317		
Total	20	1092.600857			

Table 6. ANOVA table for potato leaf infested by PTM after first treatment record 4(%)

Source	DF	SS	MS	F	P
Bl	2	420.4069	210.2034	8.22	0.0263
Rate	3	14.33462	4.778206	0.19	0.901
Bl*Rate	6	307.2619	51.21032	0.09	0.96
Freq	2	91.53409	45.76705	1.79	0.2593
Rate*Freq	1	390.9294	390.9294	15.28	0.0113
Error	5	127.8837	25.57674		
Total	19	1352.351			

Table 7. ANOVA table for potato leaf infested by PTM after second treatment application record 1(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2.00	550.57	275.28	1.97	0.22
Rate	3.00	7.30	2.43	0.02	1.00
Bl*Rate	6.00	258.12	43.02	0.06	0.98
Freq	2.00	119.95	59.97	0.43	0.67
Rate*Freq	1.00	198.20	198.20	1.42	0.28
Error	6.00	837.86	139.64		
Total	20.00	1971.99			

Table 8. ANOVA table for potato leaf infested by PTM after second treatment application record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	666.3902	333.1951	2.97	0.1269
Rate	3	170.1845	56.72815	0.51	0.6926
Bl*Rate	6	229.2766	38.21277	1.48	0.31
Freq	2	149.0355	74.51777	0.66	0.5489
Rate*Freq	1	135.1165	135.1165	1.2	0.3146
Error	6	673.2316	112.2053		
Total	20	2023.234981			

Table 9. ANOVA table for potato leaf infested by PTM after second treatment application record 3(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	1074.888	537.4441	4.35	0.0681
Rate	3	129.9539	43.31796	0.35	0.7909
Bl*Rate	6	662.0723	110.3454	0.39	0.76
Freq	2	241.6458	120.8229	0.98	0.4293
Rate*Freq	1	71.52995	71.52995	0.58	0.4758
Error	6	742.0381	123.673		
Total	20	2922.128			

Table10. ANOVA table for potato leaf infested by PTM after second treatment application record 4(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	276	138	0.86	0.4697

Rate	3	287.5308	95.84361	0.6	0.64
Bl*Rate	6	217.2206	36.20343	2.65	0.14
Freq	2	630.3618	315.1809	1.96	0.2209
Rate*Freq	1	135.6317	135.6317	0.84	0.3935
Error	6	963.4551	160.5758		
Total	20	2510.2			

Table 11. ANOVA table for potato petiole infested by PTM after first treatment record 1(%)

Source	DF	SS	MS	F	P
Bl	2	550.7243	275.3621	7.69*	0.0221
Rate	3	6.759798	2.253266	0.06	0.9775
Bl*Rate	6	210.7778	35.12964	0.06	0.98
Freq	2	31.02176	15.51088	0.43	0.6671
Rate*Freq	1	0.387758	0.387758	0.01	0.9205
Error	6	214.7077	35.78462		
Total	20	1014.379			

Table 12. ANOVA table for potato petiole infested by PTM after first treatment record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	3.19683358	1.59841679	0.85	0.4804
Rate	3	8.68581930	2.89527310	1.54	0.3127
Bl*Rate	5	7.04839975	1.40967995	2.05	0.23
Freq	2	1.88880958	0.94440479	0.50	0.6321
Rate*Freq	1	7.11090709	7.11090709	3.79	0.1091
Error	5	9.37948333	1.87589667		
Total	18	37.31025263			

Table 13. ANOVA table for potato petiole infested by PTM after second treatment application record 1(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	28.74678	14.37339	3.49	0.0987
Rate	3	1.208464	0.402821	0.1	0.9584

Bl*Rate	6	8.364686	1.394114	0.29	0.83
Freq	2	6.138914	3.069457	0.75	0.5138
Rate*Freq	1	5.332336	5.332336	1.3	0.2985
Error	6	24.7014	4.1169		
Total	20	74.49258095			

Table14. ANOVA table for potato petiole infested by PTM after second treatment application record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	16.41444	8.207219	6.54*	0.0311
Rate	3	8.777548	2.925849	2.33	0.1738
Bl*Rate	6	13.6929	2.282149	1.28	0.36
Freq	2	14.48489	7.242446	5.77*	0.04
Rate*Freq	1	0	0	0	1
Error	6	7.531667	1.255278		
Total	20	55.80438			

Table15. ANOVA table for potato petiole infested by PTM after second treatment application record 3(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	12.09063	6.045314	1.56	0.2851
Rate	3	9.018967	3.006322	0.77	0.5492
Bl*Rate	6	8.072105	1.345351	2.33	0.185
Freq	2	3.013511	1.506756	0.39	0.6941
Rate*Freq	1	2.228856	2.228856	0.57	0.4772
Error	6	23.28073	3.880122		
Total	20	57.7048			

Table16. ANOVA table for potato petiole infested by PTM after second treatment application record 4(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	3.746924	1.873462	1.2	0.3632
Rate	3	1.154062	0.384687	0.25	0.8605

Bl*Rate	6	11.13681	1.856135	0.21	0.89
Freq	2	1.260073	0.630037	0.41	0.6838
Rate*Freq	1	0	0	0	1
Error	6	9.3294	1.5549		
Total	20	26.5853			

Table17. ANOVA table for potato stem infested by PTM after second treatment application record 1 (%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	1842.407	921.2033	8.55*	0.0175
Rate	3	415.0018	138.3339	1.28	0.3622
Bl*Rate	6	1391.688	231.9479	0.60	0.64
Freq	2	247.1169	123.5584	1.15	0.3787
Rate*Freq	1	437.6948	437.6948	4.06	0.0905
Error	6	646.4794	107.7466		
Total	20	4980.386981			

Table18. ANOVA table for potato stem infested by PTM after second treatment application record 2(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	1968.333	984.1663	5.99*	0.0372
Rate	3	483.1792	161.0597	0.98	0.4623
Bl*Rate	6	1567.418	261.2363	0.62	0.63
Freq	2	189.5602	94.7801	0.58	0.5901
Rate*Freq	1	245.6921	245.6921	1.49	0.2673
Error	6	986.1539	164.359		
Total	20	5440.336			

Table19. ANOVA table for potato stem infested by PTM after second treatment application record 3(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	2263.318	1131.659	4.87	0.0555
Rate	3	120.1259	40.04197	0.17	0.9114
Bl*Rate	6	733.5052	122.2509	0.33	0.81
Freq	2	135.8094	67.90471	0.29	0.7568
Rate*Freq	1	192.9445	192.9445	0.83	0.3975

Error	6	1395.148	232.5246		
Total	20	4840.851			

Table 20. ANOVA table for potato stem infested by PTM after second treatment application record 4(%)

Source	DF	SS	MS	F-Value	Pr>F
Bl	2	1247.438	623.7189	3.43	0.1016
Rate	3	494.304	164.768	0.91	0.4918
Bl*Rate	6	209.4843	34.91405	4.72	0.0508
Freq	2	935.0523	467.5262	2.57	0.1563
Rate*Freq	1	48.75161	48.75161	0.27	0.6232
Error	6	1091.615	181.9358		
Total	20	4026.645			

Table 21. ANOVA table for potato tuber infested by PTM (%)

Source	DF	SS	MS	F	P
Bl	2	181.2686	90.6343	3.31	0.1075
Rate	3	46.55941	15.5198	0.57	0.6569
Bl*Rate	6	133.9227	22.32044	0.7	0.588
Freq	2	33.16863	16.58431	0.61	0.5761
Rate*Freq	1	0	0	0	1
Error	6	164.3487	27.39144	0.70	0.58
Total	20	537.278			

Table 22. ANOVA table for potato tuber yld (t/h)

Source	DF	SS	MS	F	P
Bl	2	0.38	0.19	0.13	0.88
rate	3	0.81	0.27	0.45	0.73
Bl*rate	6	3.62	0.60	0.42	0.84
freq	2	0.03	0.02	0.01	0.99
rate*freq	1	2.30	2.30	1.59	0.25
Error	6	8.67	1.44		
Total	20	15.81			

Annex 2. Separate ANOVA to compare treatments as only effect for PTM infestation on different parts of potato

Table 1. Separate ANOVA

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
leaf infested by PTM BT R1(%) * treat	Between Groups	(Combined)	134.281	6	22.380	.227	.961
	Within Groups		1381.945	14	98.710		
	Total		1516.226	20			
leaf infested by PTM BT R2(%) * treat	Between Groups	(Combined)	133.929	6	22.321	.472	.818
	Within Groups		661.872	14	47.277		
	Total		795.801	20			
leaf infested by PTM AT1 R1(%) * treat	Between Groups	(Combined)	631.119	6	105.186	1.909	.149
	Within Groups		771.209	14	55.086		
	Total		1402.328	20			
leaf infested by PTM AT1 R2(%) * treat	Between Groups	(Combined)	128.584	6	21.431	.296	.929
	Within Groups		1014.138	14	72.438		
	Total		1142.722	20			
leaf infested by PTM AT1 R3(%) * treat	Between Groups	(Combined)	176.031	6	29.339	.448	.835
	Within Groups		916.570	14	65.469		
	Total		1092.601	20			
petiole infested by PTM AT1 R1(%) * treat	Between Groups	(Combined)	38.169	6	6.362	.091	.996
	Within Groups		976.210	14	69.729		
	Total		1014.379	20			

leaf infested by PTM AT1 R4(%) * treat	Between (Combined) Groups	2126.989	6	354.498	2.125	.115
	Within Groups	2336.063	14	166.862		
	Total	4463.052	20			
petiole infested by PTM AT1 R2(%) * treat	Between (Combined) Groups	22.542	6	3.757	1.198	.363
	Within Groups	43.888	14	3.135		
	Total	66.430	20			
stem infested by PTM AT1 R4(%) * treat	Between (Combined) Groups	1041.509	6	173.585	.710	.647
	Within Groups	3422.260	14	244.447		
	Total	4463.769	20			
leaf infested by PTM AT2 R1(%) * treat	Between (Combined) Groups	325.443	6	54.240	.461	.826
	Within Groups	1646.548	14	117.611		
	Total	1971.990	20			
petiole infested by PTM AT2 R1(%) * treat	Between (Combined) Groups	12.680	6	2.113	.479	.813
	Within Groups	61.813	14	4.415		
	Total	74.493	20			
stem infested by PTM AT2 R1(%) * treat	Between (Combined) Groups	1099.813	6	183.302	.661	.682
	Within Groups	3880.574	14	277.184		
	Total	4980.387	20			
leaf infested by PTM AT2 R2(%) * treat	Between (Combined) Groups	454.337	6	75.723	.676	.672
	Within Groups	1568.898	14	112.064		
	Total	2023.235	20			
petiole infested by PTM AT2 R2(%) * treat	Between (Combined) Groups	18.165	6	3.028	1.126	.397
	Within Groups	37.639	14	2.689		
	Total	55.804	20			

stem infested by PTM AT2 R2(%) * treat	Between Groups	(Combined)	918.432	6	153.072	.474	.817
	Within Groups		4521.905	14	322.993		
	Total		5440.336	20			
leaf infested by PTM AT2 R3(%) * treat	Between Groups	(Combined)	443.130	6	73.855	.417	.856
	Within Groups		2478.999	14	177.071		
	Total		2922.128	20			
petiole infested by PTM AT2 R3(%) * treat	Between Groups	(Combined)	14.261	6	2.377	.766	.609
	Within Groups		43.443	14	3.103		
	Total		57.705	20			
stem infested by PTM AT2 R3(%) * treat	Between Groups	(Combined)	448.880	6	74.813	.238	.956
	Within Groups		4391.971	14	313.712		
	Total		4840.851	20			
leaf infested by PTM AT2 R4(%) * treat	Between Groups	(Combined)	1053.524	6	175.587	1.688	.197
	Within Groups		1456.676	14	104.048		
	Total		2510.200	20			
petiole infested by PTM AT2 R4(%) * treat	Between Groups	(Combined)	2.372	6	.395	.229	.960
	Within Groups		24.213	14	1.730		
	Total		26.585	20			
stem infested by PTM AT2 R4(%) * treat	Between Groups	(Combined)	1478.108	6	246.351	1.353	.299
	Within Groups		2548.537	14	182.038		
	Total		4026.645	20			
tubr % infested * treat	Between Groups	(Combined)	57.738	6	9.623	.281	.937
	Within Groups		479.540	14	34.253		
	Total		537.278	20			

Yield * treat	Between (Combined) Groups	2.809	6	0.468	0.50	0.795
	Within Groups	13	14	0.9286		
	Total	15.809	20			

Annex 3. Mean comparisons of all treatments against control (Check) using Dunnet's procedure

Significant Comparisons at the 0.05 level are indicated by ***.

Table 1. Mean comparisons for leaf infested before treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	9.160	-4.528	22.848
2 - 7	0.517	-11.726	12.759
1 - 7	-3.357	-15.599	8.886
5 - 7	-3.433	-15.676	8.809
6 - 7	-3.660	-15.903	8.583
3 - 7	-5.732	-17.184	5.719

Table 2. Mean comparisons for leaf infested before treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	2.255	-13.727	18.237
2 - 7	2.107	-12.188	16.401
5 - 7	-2.833	-17.128	11.461
1 - 7	-3.350	-17.645	10.945
6 - 7	-5.593	-19.888	8.701
3 - 7	-6.817	-20.189	6.554

Table 3. Mean comparisons for leaf infested after first treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2 - 7	13.973	-4.169	32.116
4 - 7	12.573	-7.711	32.857
5 - 7	5.533	-12.609	23.676
3 - 7	1.898	-15.073	18.869
6 - 7	0.850	-17.293	18.993
1 - 7	-3.260	-21.403	14.883

Table 4. Mean comparisons for leaf infested after first treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	11.178	-4.352	26.708
2 - 7	5.383	-8.507	19.274
6 - 7	2.110	-11.780	16.000
3 - 7	0.596	-12.397	13.589
5 - 7	0.193	-13.697	14.084
1 - 7	-1.787	-15.677	12.104

Table 5. Mean comparisons for leaf infested after first treatment application record three

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	9.138	-10.383	28.659
2 - 7	2.240	-15.220	19.700
5 - 7	0.403	-17.057	17.863
3 - 7	-4.107	-20.439	12.226
6 - 7	-5.060	-22.520	12.400
1 - 7	-5.187	-22.647	12.273

Table 6. Mean comparisons for leaf infested after first treatment application record four

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2 - 7	4.173	-12.915	21.262
4 - 7	2.347	-16.759	21.452
5 - 7	1.607	-15.482	18.695
3 - 7	-4.821	-20.806	11.164
6 - 7	-6.307	-23.395	10.782
1 - 7	-12.038	-31.144	7.067

Table 7. Mean comparisons for petiole infested after first treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	3.420	-12.910	19.750
2 - 7	-0.400	-15.006	14.206
6 - 7	-0.900	-15.506	13.706
5 - 7	-1.650	-16.256	12.956
1 - 7	-3.083	-17.690	11.523
3 - 7	-4.102	-17.766	9.561

Table 8. Mean comparisons for petiole infested after first treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2 - 7	1.1333	-2.6013	4.8680
5 - 7	-0.4100	-4.1446	3.3246
1 - 7	-0.6750	-4.7661	3.4161
4 - 7	-0.6950	-4.7861	3.3961
3 - 7	-1.2050	-4.7480	2.3380
6 - 7	-1.8667	-5.6013	1.8680

Table 9. Mean comparisons for leaf infested after second treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	11.630	-12.493	35.753
2 - 7	7.290	-14.287	28.867
5 - 7	2.690	-18.887	24.267
6 - 7	0.227	-21.350	21.803
3 - 7	-3.725	-23.908	16.458
1 - 7	-6.507	-28.083	15.070

Table10. Mean comparisons for leaf infested after second treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	6.730	-15.578	29.038
5 - 7	3.223	-16.730	23.176
2 - 7	2.283	-17.670	22.236
6 - 7	1.633	-18.320	21.586
3 - 7	-9.418	-28.082	9.247
1 - 7	-9.927	-29.880	10.026

Table11. Mean comparisons for leaf infested after second treatment application record three

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
6 - 7	6.843	-18.982	32.668
4 - 7	5.432	-23.442	34.305
2 - 7	-2.570	-28.395	23.255
3 - 7	-3.418	-27.575	20.739
5 - 7	-6.067	-31.892	19.758
1 - 7	-8.413	-34.238	17.412
3 - 7	0.3100	-2.5216	3.1416

Table12. Mean comparisons for leaf infested after second treatment application record four

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
6 - 7	4.053	-20.184	28.290
4 - 7	3.953	-23.145	31.051
3 - 7	-7.837	-30.508	14.835
2 - 7	-9.793	-34.030	14.444
1 - 7	-12.290	-36.527	11.947
5 - 7	-16.840	-41.077	7.397

Table 13. Mean comparisons for petiole infested after second treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
4 - 7	4.0483	0.8459	7.2508	***
2 - 7	0.8533	-2.0110	3.7177	
5 - 7	0.6633	-2.2010	3.5277	
6 - 7	0.3733	-2.4910	3.2377	
1 - 7	-0.1767	-3.0410	2.6877	
3 - 7	-0.9167	-3.5960	1.7627	

Table14. Mean comparisons for petiole infested after second treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	3.3250	-0.0595	6.7095
2 - 7	2.8533	-0.1738	5.8805
6 - 7	1.5833	-1.4438	4.6105
1 - 7	1.3133	-1.7138	4.3405
5 - 7	0.8667	-2.1605	3.8938
3 - 7	0.3100	-2.5216	3.1416
1 - 7	-9.927	-29.880	10.026

Table15. Mean comparisons for petiole infested after second treatment application record three

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
6 - 7	0.830	-3.136	4.796
4 - 7	-0.738	-5.173	3.696
5 - 7	-0.883	-4.849	3.083
3 - 7	-1.283	-4.993	2.427
2 - 7	-1.323	-5.289	2.643
1 - 7	-1.693	-5.659	2.273

Table16. Mean comparisons for petiole infested after second treatment application record four

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
4 - 7	0.5417	-3.0327	4.1161
6 - 7	0.1433	-3.0537	3.3404
3 - 7	-0.0458	-3.0364	2.9447
2 - 7	-0.1733	-3.3704	3.0237
5 - 7	-0.4000	-3.5970	2.7970
1 - 7	-0.7367	-3.9337	2.4604

Table 17. Mean comparisons for stem infested after second treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
1 - 7	7.170	-24.171	38.511
3 - 7	-8.607	-37.923	20.710
4 - 7	-8.737	-43.777	26.303
6 - 7	-10.977	-42.317	20.364
2 - 7	-13.400	-44.741	17.941
5 - 7	-13.403	-44.744	17.937

Table18. Mean comparisons for stem infested after second treatment application record two

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
1 - 7	14.68	-21.69	51.05
3 - 7	13.00	-21.02	47.03
4 - 7	9.31	-31.35	49.98
5 - 7	6.51	-29.86	42.88
2 - 7	5.16	-31.21	41.53
6 - 7	-2.49	-38.86	33.88
3 - 7	0.3100	-2.5216	3.1416

Table19. Mean comparisons for stem infested after second treatment application record three

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
3 - 7	11.411	-17.805	40.627
5 - 7	11.177	-20.056	42.410
1 - 7	5.160	-26.073	36.393
6 - 7	0.640	-30.593	31.873
2 - 7	-3.827	-35.060	27.406
4 - 7	-9.962	-44.881	24.958

Table 20. Mean comparisons for stem infested after second treatment application record four

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
1 - 7	14.777	-12.175	41.728
5 - 7	11.253	-15.698	38.205
2 - 7	4.953	-21.998	31.905
3 - 7	0.789	-24.421	26.000
6 - 7	-8.240	-35.191	18.711
4 - 7	-8.788	-38.921	21.344

Table 21. Mean comparisons for tuber infested after second treatment application record one

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2 - 7	5.737	-6.346	17.819
4 - 7	3.880	-9.629	17.389
1 - 7	3.750	-8.332	15.832
5 - 7	3.513	-8.569	15.596
3 - 7	3.087	-8.215	14.390
6 - 7	1.807	-10.276	13.889

Table 22. Mean comparisons for yield (t/ha)

treat Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2 - 7	1.133	-2.885	5.151
5 - 7	-0.410	-4.428	3.608
1 - 7	-0.675	-5.076	3.726
4 - 7	-0.695	-5.096	3.706
3 - 7	-1.205	-5.017	2.607
6 - 7	-1.867	-5.885	2.151