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Evaluation of Botanical Insecticides for the Management of Potato Tuber Moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) Under Laboratory Condition

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ACCRONOMYS

ANOVA	Analysis of Variance
EEB	Ethanol Extracted Birbera
CRD	Completely Randomized Design
EC	Emulsifiable Concentrate
FAO STAT	Food and Agriculture Organization Statistics
HA	Hectare
HARC	Holleta Agricultural Research Center
IPM	Integrated Pest Management
MASL	Meter Above Sea Level
PFP	Pyrethrum Flower Powder
SE	Standard Error

ABSTRACT

Potato has high yield potential, nutritional quality and a short growing period but, it has major constraints in Ethiopia that include diseases, insect pest such as *Phthorimea operculella*. The objective of this study was to evaluate *Chrysanthemum cinerariafolium* (Anonymous), *Millettia ferruginea* (Hochst) and *Azadirachta indica* (A. Juss) against *P. operculella*. Three sets of experiments were conducted at Holetta Agricultural Research Center under laboratory condition. Five treatments with three replications in completely randomized design were used. In experiment1: the treatments were 0.33mg/ml of birbera seed powder extracted using ethanol, 0.33mg/ml pyrethrum flower powder, 0.33mg/ml combination of birbera seed powder extracted using ethanol and pyrethrum, 0.01ml/ml of ethanol extracted neem oil and water. Then 25 ml of solution were sprayed on potato seedling per pot through dispenser sprayer. After one hour and fifty minutes of treatment application, ten larvae per pot were released. As a result there was no significant difference between botanicals larval mortality, leaf and petiole percent damage. Pyrethrum was a good killer of larva (86.66%) next to the combination (83.33%). There was significant difference in larval mortality between botanicals and water ($F_{4,10}$ at 0.05= 41. 68, $p < 0.0001$). In experiment 2 and 3: similar rates of treatments and procedure were used. 0.33 mg/ml of birbera seed powder extracted using ethanol, 0.33 pyrethrum flower powder, 0.001ml/ml of neem oil extracted using ethanol and 0.33mg/ml combination of birbera seed powder extracted using ethanol with pyrethrum. After preparation of botanicals in solution form five tubers were dipped for one hour and fifty minutes, taken out and air dried for one hour. Tubers received the same treatment were put with enough space between tubers into a transparent plastic cage and 25 larvae were released per cage. There was significance difference between birbera and the remaining treatments. All birbera treated tubers had the least recorded of fecal materials ($F_{4, 10}$ at 0.05= 12.73, $P < 0.0006$), active galleries ($F_{4, 10}$ at 0.05= 11.07, $P < 0.0011$) and larval mortality per cage ($F_{4, 10}$ at 0.05=18.47, $P < 0.0001$). In experiment three; two pairs of adults were released on treated tubers to see the ovipostion response. There was significance difference between botanicals in number of eggs laid (F, 10 at 0.05=4.5, $p < 0.024$) and hatched eggs/ cage ($F_{4, 10}$ at 0.05 =3.44, $p < 0.051$). In terms of all parameters measured birbera either single or mixed with pyrethrum gave the best control of *P. operculella* under storage and can be recommended against the pest.

Key words: Potato, Birbera, Pyrethrum, Neem, Ethanol, *P. operculella*.

1 INTRODUCTION

1.1 Background

Potato (*Solanum tuberosum L.)* is the world′s fourth largest food crop after wheat, rice and maize (Golizadeh and Esmaeili, 2012). Currently, potato is one of the world's prime sources of nutrition which is carbohydrates, fat, protein and mineral. The world average of potato yield was 17.4 tons per hectare in 2010. United States were the most productive in 2010, with a nationwide average of 44.3 tons per hectare (FAOSTAT, 2010). China and India, are the two largest producers of potato, which accounted for over a third of world′s production in 2010, recording yields of 14.7 and 19.9 tons per hectare, respectively (FAOSTAT, 2010). Food and production in the developing countries have almost doubled since 1991, with a corresponding increase in consumption (Hoffler and Ochieng, 2008; FAO, 2008).

Asia and Europe are the world's major potato producing continent, accounting for more than 80 percent of world production in 2007. Africa and Latin America are smaller producer continents compared to Asia and Europe. In Africa, around 20th century potato was arrived. In recent decades, production has been rising from 2 million tons in 1960 to 16.7 million tons in 2007. Potatoes are grown under a wide range of condition through irrigation or rain feed. In Africa, Egypt and South Africa intensively cultivated from irrigated commercial farms. Ethiopia has good climates and soil types for the production of potato (Endale *et al.*, 2008). Potato is a high potential food security crop in Ethiopia due to its high yield potential and nutritional quality tuber. In Ethiopia it is a source of income in the house hold, with short growing period and wide adaptability (Ayalew *et al.*, 2014). The Western, central and eastern Ethiopia are good potato producer areas. Total production coverage in Ethiopia about 160,000 ha wit national average yield of about 9 tons per hectare, which is much lower than the world average of 15 tons per hectare (Ferdu *et al.,* 2009b, Ayalew *et al.*, 2014). In order to get comparative world average production per hectare Ethiopia need to improve on constraints of production.

The constraints which influence production in Ethiopia, include certified seed, price instability, seed potato physical damage, diseases, different insect pests, weeds and lack of awareness to manage the constraints unlike the other crops such as maize, wheat and sorghum (Medhin *et al.,* 2001, Ferdu *et al.,* 2009a, Bezabih and Mengistu, 2011). Mostly in developing countries a great amount of potato is lost in the store mainly by insect pests such as *P. operculella*. Moreover, late blight, bacterial wilt and virus disease are also limiting potato production in the country. In Ethiopia, over 42% of potato tubers are lost due to *P. operculella* infestation (Sileshi and Teriessa, 2001).

P. operculella is a serious destructive insect pest of cultivated potato in the field and storage. Larval feeding cause a serious damage by making tunnels. And moisture release through its moth, consequently, the leaves at the field as well as tuber in storage cause of rotting with bad smelling. Rotting of the tuber in storage, leads to different pathogenic infection like fungi and bacteria. Hence, it is cause of complex yield losses, either directly feeding on the crops or cause of the pathogenic infection (Moawad and Ebadah, 2007). *P. operculella* causes significant crop losses in almost all tropical and sub-tropical potato production systems in Africa, Asia, Central and South America (Lal, 1987; Horgan *et al.,* 2007).

There are different management options of *P. opercullella* including cultural, botanical insecticides, biological and synthetic chemicals. Treatment with chemical insecticides may be possible, but it is not recommended because of food safety and environmental concern. Problems of food safety and environmental concern can be minimized using of botanical plant extracts, such as birbera (*M. ferruginea* (Hochst)), pyrethrum flower powder (*Chrysanthemum cinerarii folium*) and Neem oil (*Azadirachta indica* A. Juss**)**. Different authors confirmed and determined that toxicity of birbera extracts against stored insect pests such as *Calloso bruchus chinensis* (L.) (Bayeh and Tadesse, 2000). Both aqueous and organic birbera seed powder extract, were found to be effective against a number of pests through contact and as a stomach poison (Bayeh, 2007).

Pyrethrum is a natural insecticide produced from Dalmatian. Application of pyrethrum flowers as insecticide in home gardens and organic farming become dominant for pest management practice (Glynne - Jones, 2001). Therefore, there are enough evidences justifying that extracts from birbera seed and pyrethrum flower powder could kill *P. operculella*. The effectiveness of pyrethrum and birbera in aqueous and organic solvent extracts, both by contact and stomach poison affect oviposition, feeding on leaves, stems, tubers and delays development. Neem oils were also found effective against soft-bodied arthropods including *P. operculella. P. operculella*, with its hidden feeding habits is preferable to bioassay ovipositon. And feeding deterrence effects as well as ovicidal effects of *M. ferruginea* seed powder extracted with both polar and non-polar solvents. Different types of extracted birbera seed powder applied on host plant leaf inhibit settling of *P. operculella* larvae and its lethal effect to increase with increased rates of application and hours of exposure. Moreover, *M. ferruginea* has a potential to kill many insect pests and it used as a natural insecticide (Ayalew Tadesse, 2010).

Decline in the efficacy of the commonly used insecticides, resistance development, environmental hazard and high cost forced the scientists to look for alternative methods of pest control. One of the alternatives is the use of botanicals which is relatively safe interms of the negative side effects of synthetic chemicals. Hence, the current study was conducted to look botanical alternatives for management of *P. operculella*. Apparent and the population of *P. operculella* pest resistant to insecticides and most of the chemical has environmental hazardous within its cost. Now a day due to environmental concern the chemical production and its distribution is very much cost, because of taking time and needs clear standardization to produce environmental friendly pesticides. In Ethiopia impact of pesticides to a given locality or on the environment has not been clearly identified, assessed and compiled, there is no system for risk monitoring and communication (Federal environmental protection authority of Ethiopia, 2004).

P. operculella infestation as a major problem for potato production in Ethiopia the producers are using synthetic chemicals. Therefore, there is probability to increased chemical bio concentration day today due to inappropriate usage of chemicals. Therefore, by modifying indigenous as well as exotic botanical insecticide can be reduced chemical risk to the environment. This study was planned to evaluate, ethanol extracted birbera seed, pyrethrum flower powder, and ethanol dissolved neem oil and the combination of ethanol extracted birbera seed and pyrethrum for management of *P. operculella* under laboratory condition on seedling and tubers of potato. For determination of the efficacy of botanical insecticides at selected rate against *P. operculella.* To see the larval feeding response on treated potato leaf, petiole, tubers and adult ovipositon on treated tubers.

1.2 Objective of the study

General Objective:

To evaluate the selected botanical insecticides efficacy for management of *P. operculella* under laboratory condition.

Specific Objective

1. To determine the efficacy of Pyrethrum flower *(Chrysanthemum cineraria folium,* Birbera *(Millettia ferruginea (*Hochst*))* and Neem *(Azadirachta indica)* on *P. operculella* larvae feeding in potato seedling.

2. To determine the efficacy of Pyrethrum flower *(Chrysanthemum cineraria folium,* Birbera *(Millettia ferruginea* (Hochst)) and Neem *(Azadirachta indica)* on *P. operculella* larvae feeding and adults ovipostion on potato tubers.

3. To determine the combination effect of birbera seed powder extracted using ethanol and pyrethrum flower powder against *P. operculella*.

2 LITERATURE REVIEW

2.1 GEOGRAPHIC Distribution and Hosts of *Phthorimaea operculella (Zeller)*

P. operculella is found in tropical, subtropical, and Mediterranean areas (Westedt *et al.,* 1998; Flanders and Radcliffe *et al*., 1999; Visser 2005; Golizadeh and Esmaeili, 2012). It has appeared and spread in the United States in the past century and the most insect pest in the world which consist several species. Three major species with different geographical distributions attack foliage, tubers (Scrobipalpula absoluta, Scrobipalpopsis solanivora and *P. operculella*. Among these, *P. operculella*, is the most common and widely distributed in the world. *P. operculella* has been reported in more than 90 countries (Anonymous, 2013).

The countries majorly affected by *P. operculella* are from Africa, Asia, Europe, Americas and Oceania (Golizadeh and Esmaeili, 2012; Golizadeh and Zalucki, 2012; Ahmed *et al*., 2013; Kroschel *et al*., 2013, Gills *et al*, 2014). It is usually found in warm climates for overwinter survival and considered a subtropical pest which is considered as the most serious pest of potato in tropical and subtropical regions. According to the research done at Alemaya by Sileshi and Teriesa (2001), *P. operculella* infestation varies with potato cultivar where the level of infestation was 62% for susceptible variety and about 6% for resistant variety.

P. operculella is an oligophages insect pest which is feeding on a restricted range of plants in the family Solanaceae mainly potatoes (*Solanum tuberosu*m L.) and tomatoes (*Solanum lycopersicum* L). *P. operculella* has different host plants such as eggplants (*Solanu melongena L.*), peppers (*Capsicum* spp.), tobacco and wild Solanaceous plants like Jimson weed or datura (*Datura stramonium* L.) (Alvarez *et al*., 2005).

2.2. Life History and Ecology of *P. operculella*

P. operculella is a complete metamorphosis insect (Rondon *et al*., 2007). Eggs, larva and pupa of *P. operculella* can survive on potatoes or in the soil after harvest. When the temperature below is 50°F, larval development is interrupted. *P. operculella* larva, pupate in the soil, discarded piles of potatoes, dead leaves, on storage walls, or on eyes of stored tubers (Raman, 1980). They can also pupate in crevices in walls and floors. *P. operculella* has four larval instars. These instars may occur together in overlapping generations. There are 2-8 generations per year, depending upon climatic conditions. In temperate climatic conditions in North America it has 2 generations per year (Alvarez *et al*., 2005). Total life cycle of *P. opreculella* from egg to adult emergency takes about 21-44 days (Raman, 1980; Alvarez *et al.,* 2005 ; Chandel *et al*., 2005).

Eggs: Freshly laid egg of *P. operculella* is opaque, shining white in color, smooth and oval. As the egg becomes older, it looks like yellow. The eggs are deposited on the underside of leaves, stem or petiole (Raman, 1980; Alvareze *et al*., 2005). Most of the time, adult *P. operculella* prefer lamina along with mid rib to deposit eggs on it. When the foliage is not available for *P. operculella*, it moves slowly a short distance through cracks in loose soil to find a tuber and use as an oviposition site and also they can deposit in storage walls. Within four days, *P. operculella* female deposit 60 to 200 eggs singly directly on host plant parts (stem, underside of leaves, tuber eye crack and usually hatch after five days.

Larva: Larva of *P. operculella* is white or yellow with a brown head and 0.5 to 0.6 inches in its length (Raman, 1980; Alvarez *et al.,* 2005, Rondon., *et al* 2010). The thorax has small black points and bristles on each segment. Color of *P. operculella* larva changes from white or yellow to pink or green as they mature. This pest, larva can feed its host plant up to two weeks before pupation. The newly hatched larva is very difficult to detect on the leaf surface. The newly hatched larva of *P. operculella* is quite active and starts feeding within few hours after emerging. The larva after hatching moves away from its hatching site. It begins to mine the leaves. Different larval instars stage can be distinguished on the basis of their head capsule in which they fall in four distinct stages.

First instar larva: Larva is light dirty white in color with a dark brownish head, and it is very thin worms. The newly hatched larva measures 0.62-0.74 mm in length. The first larval instars take 2-4 days under favorable conditions (Chandel *et al*., 2005).

Second instar larva: The body color, little pale white with dark brown head capsule. Body length varies from 2.8-3.3 mm and its duration is 2-6 days during summer months. The leaf mining by second instars becomes broader in size with length extending up to 15 mm.

Third instar larvae: Third instar larva has no much difference in morphological characters and appearance compared to the second instars and its body length 5-6 mm, its duration period 2.8-5.35 days during a warmer period, however, in winter it can be extended up to 25 days (Chandel *et al*., 2005).

Fourth instar larva: This is the final stage of *P. operculella* larval instar. It turns to pink in color with dark coloration, but the color of head capsule remains dark brown, 9.8-11.3 mm. Its body length slightly contracted (fusiform). Full fed larva of the fourth instar leave the host substrate and moves around in search of the favorable pupation site and make dirty silken cocoons with loose strands. After these posses it spends 2-3 days in the silken cocoons before changing into pupa.

Pupae: are white, narrow shapes and 0.5 inches long and formed in dry and hidden places. They are frequently found near on the eyes in tuber along with excreta, on the dry leaf of the host, between different layers of potato tubers, in cracks in storage and within its excreta on the surface of rotten tubers. Female and male of white, narrow shapes and 0.5 inches long pupae are different morphologically. Body length and weight of female pupa is greater than the male. Female genital opening and last spiracle are found on the 8th abdominal segment, but the male genital opening found on the 9th abdominal segment. Pupae are more tolerant to temperature at 33.5-34.1^{0}C. Depending upon climatic conditions, pupae take 10 to 30 days to emerge to adult

(Raman, 1980; Alvarez *et al*., 2005, Rondon., *et al* 2010). The silken cocoon rolled around pupae can become covered with soil and debris. The pupae have shown three different colors: these are, at the beginning pupation time it looks like green, in middle stage it looks like a black brown or brown. At the end it looks like black which is a good indicator of the pupa soon emerging to adult.

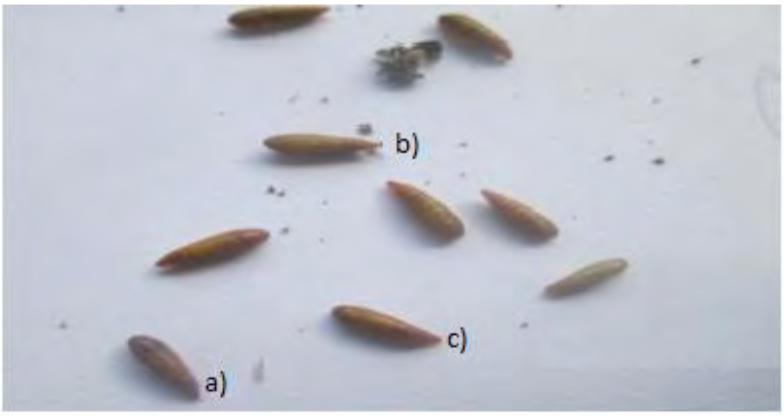

Plate 1: Variation of *P. operculella* pupae color at different stages (photo by Kidist Teferra)

a) black b) green c) black brown

Adults: The moths are narrow bodied, silver gray in color, 0.4 inches long with a wing span of 0.5 inches. Wings of *P. operculella* are grayish-brown, fringed and extend with small brown or black patterns. Both pairs of wings have fringed edges. During resting time, the wings are held close to the body, which makes that the moth has been a slenderical appearance. Forewings are yellowish gray with dark spots (2-3 dots on males and characteristic "X" pattern of females). The hind wings are gray (Raman, 1980; Alvarez *et al.,* 2005, Rondon *et al* 2007, Rondon., *et al* 2010,). The adults are fast fliers and like most other moths require an insect-collecting net for capturing. These moths live for one to two weeks and feed on nectar. Females are slightly larger than males. Mating begins 24 hours after emergence.

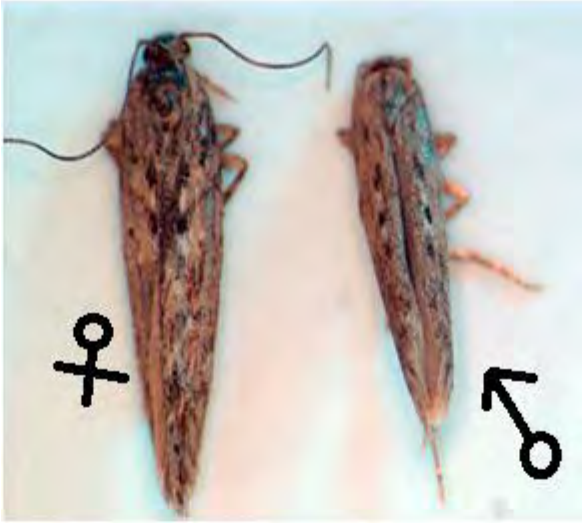

Plate 2. *P. operculella* adults forewings have 2-3 dark spots on males (right), and "X" pattern on females Source: (Rondon, *et al.,* 2007)

Economic damage mostly occurs towards the end of the growing season when tubers become exposed and are infested by *P. operculella* during harvest (Lacey *et al*., 2008). The peak months in Ethiopia were January, February, and June unlike the field situation. Monitoring in the store showed no obvious peak record (Bayeh and Tadesse, 1992).

The field results showed that *P. operculella* activity peaked up during January to February and in June. The two peaks in January and June were mainly attributed to the population that had been multiplying on the left over tubers in the field from the main season and irrigated potato, respectively. The catches in February were more important because of off-season planted potato is young in the field and liable for white, narrow shapes and 0.5 inches attack long. On the other hand, the populations of *P. operculella* in the seed tuber stores never showed obvious peaks whereby the number of adults caught remained low all year round. The usual high population in fields had not been contributing much for infestations that occurred in seed tuber stores (Bayeh and Tadesse, 1992).

Optimum conditions for *P. operculella* motivate to develop are temperatures of 22-26°C and the air humidity levels of 70-80%. When the temperatures are below -4 and above 36°C all stages of white, narrow shapes and 0.5 inches long dead. Caterpillars can tolerate sharp temperature fluctuations, remaining alive even when tubers freeze. Moth viability is shown in a wide range of positive temperatures from 8-35°C (Chumakov, and Kuznetsova 2009).

P. operculella has no diapauses phenomena in unfavorable environmental condition, which allows its continuous development under suitable temperature conditions and presence in the presence of enough food sources (in potatoes storehouses). *P. operculella* can adapt to in temperate climates with a sum of active temperatures. Caterpillars of the pest are selective (oligophages), specializing in feeding on plants of the family Solanaceae only. In all developmental stages, it spreads mainly by transportation of potato tubers, fresh fruits of tomato and eggplant, and on tobacco packages and boxes taken from infected farms and areas.

Antennal cleaning, quiescence, walking, wing fanning, contact with female, hair brush display are major components to copulation of *P. operculella* before mating (Tomohiroono, 1985). During mating time the male and female attached their abdominal parts being together over six hours without separation (Plate 3). Most of the literature review reported that the complete life cycle of *P. operculella* is between 30 to 35 days. However, according to my observation in some circumstance it takes over two months.

Plate 3: Mating position of *P. operculella* (Photo by Kidist Teferra)

2.3 *P. operculella* Damage and Symptoms on Potato

Larvae of *P. operculella* feed on potato leaves, petioles, stems, and more importantly potato tubers in the field and in the storage. The newly hatched larvae create mines on leaves by feeding on leaf tissue, while, leaving the upper and lower epidermis of the leaf intact, damage of potato leaf cause of significantly economic damage (Rondon *et al*., 2007). They prefer feeding on young foliage. Typical damage results from larvae boring tunnels in tubers. Larvae depositing their excreta make tubers not for consumption. Potato tuber eyes become pink due to deposition of silk and excrement by *P. operculella* infestation. Harsh infestations result in yield and quality losses during storage where previously infested tubers are stored with healthy potato tubers (Malakar and Tingey, 2006; Rondon, 2010). This generally destroys the entire stored potatoes.

Economic damage mostly occurs to potato tubers in storage conditions in developing countries and is caused by larval feeding (Gill *et al*., 2014). Presence of even one larva is sufficient to destroy a tuber. Rapidly moving caterpillars penetrate the tubers, form galleries coated with silken threads and eject frass outside the tuber. Caterpillars form galleries on the leaves and then penetrate other plant parts. After two to three weeks, the caterpillars leave the plant and it moves through cracks in the soil and pupate on walls of potato bags lying in potato fields. Fungi, bacteria, and mites can develop inside the tunnels, which made by the larva, those pathogenic organisms are causes of tubers rot and emit unpleasant smell (Alvarez *et al*., 2005)

Stored crop losses in potatoes ranging from 50% in Yemen and Peru, 86% in Tunisia, Algeria, and Turkey, 90% in Kenya, and 100% in India (Alvarez *et al*., 2005). In Egypt, *P. operculella* has caused up to 100% losses to potato plants in fields as well as in storage (Ahmed *et al*., 2013). In Ethiopia, potatoes at storage and in the field encounters challenges that cause considerable yield loss by *P. operculella* (Ayalew *et al*., 2014) and over 42% of potato tubers exposed to *P. operculella* infestation (Sileshi and Teriessa, 2001).

The adult laid the egg underside of the leaf or the upper part of the leaf, then by using the vein of the leaf goes to the main stem through the nodes of the leaf. The infestation level is very sensitive on the central tissue of the potato leaf. *P. operculella* can destroy the whole parts of the potato at the field as well as at the storage. The larvae hidden and occupied the whole internal parts of potato stem. The physical damage is observed and look like cause of disease when the larva goes to the stem through petiole and veins, if the person who have no understanding about the exact *P. operculella* damage or symptom, it is difficult to make a decision. On the below (Plate 4) on the left side stems wilted and look like because of disease, but the right side picture tells us, the reality is because of *P. operculella* when we dissect of the stem.

a b

Plate 4 a) Damage of potato stems by larvae b) larvae inside the stem (photo by Kidist Teferra)

a) c)

Plate 5 a) Symptom of *P. operculella* On potato leaf . c) Infestation of *P. operculella* at central part of potato seedling .(photo by Kidist Teferra)

2.4 Control Methods of *P. operculella*

Like any other agricultural insect pest *P. operculella* has different options to control it. These options include chemicals, cultural practice, and biological. The most effective control method are, combines cultural, biological, and chemical approaches (Guenthner *et al.,* 2003), *P. operculella* management consists of practices that reduce adult populations on plant foliage (host plant) and limit the larvae movement to reach the tubers.

2.4.1 Cultural Control Methods

Most of the time different cultural practices were best options for *P. operculella* management. For example, covering hills with soil provides good protection and these are preventive way of disrupting the egg laying by the pest before harvest (Clough *et al*., 2010). Planting tubers in appropriate time prevent soil cracking through using sprinkle irrigation, which can reduce economical damage of *P. operculella*. Host plants can be prevented from *P. operculella* damage through resistance together with good appropriate biological and cultural practices such as covering hills or well earth up, good land preparation before planting, timing of planting, use resistant varieties, keep infested tuber translocation from infested to an infested area, plant tubers deeply, clear the field after harvest (remove infested tubers from the field, remove host plants from the field) (Rondon *et al*., 2009). The farmers culturally leave their potatoes in the ground until the skin of the tuber hardened which is good to improve tuber storage quality and reduces spoilage but on the other hand increase tuber exposure to moth infestation.

2.4.2 Botanical Insecticides

Prevention of attack is also assisted by the application of vegetable oils. Sharaby (1988) showed that orange peel oil reduced the fecundity of *P. operculella*. Reproduction was significantly reduced when either males or females were exposed to the oil vapor. Pyrethrum flower, Lantana camara, Eucalyptus globules and Birbera have a potential effect to against *P. operculella* (Ayalew Tadesse, 2010, Aschalew

Sisay and Ahmed Ibrahim, 2012). Moawad and Ebadah (2007), the higher concentration of the cardamom oil, resulted in the lower percentage of egg hatchability of 6.47% and 86.74%. The use of attractant or repellent volatile plant compounds offers a promising alternative two ways and sustainable control measure to *P. operculella* (Das *et al*., 2007).

2.4.2.1 Description and use of *Millettia ferruginea* (Hochst)

There are two different types' subspecies of *M. ferruginea* (MacLachlan, 2002). The range of sub species *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; the hairs on the flowers are lighter in color than the other subspecies, a golden brown. Subspecies darassanal is found more in southern Ethiopia, but is found North to Shewa. This subspecies has only a few hairs on the leaflets. The hairs on the flowers in this subspecies are darker, blackish brown (MacLachlan, 2002 as reviewed in Bekele *et al.,* 2007).

Birbera is available in most part of the country traditionally to control insect pests for agricultural practice, such as, to control bean bruchids, *Chaloso brochures chinensis* (L.) (Bayeh and Tadesse, 2000). Birbera tests on insects which can suck plant saps/ solution such like Diamond back moth aphids feeding on external foliage and *P. operculella* feeding habits on tuber and the leaves were treated to their different developmental stages and their host plants to suppress their population below economic damage level (Bayeh, 2007).

2.4.2.2 Description of *Chrysanthemum cineraria folium* (Anonymous)

Synthetic pyrethroids derived from natural products, pyrethrum, which refers to the oleoresin extracted from the dried flowers of *Tanacetum cineraria folium* (*Asteraceae*), formerly of the genus *Chrysanthemum*, and is the source of the pyrethrins, *chrysanthemates* and pyrethrate (Isman, 2006). Products containing pyrethrum contain a mixture of at least six brethren esters (Casida and Quistad 1995). This natural pyrethrum mixture of esters has highly unusual insecticidal properties and has been used safely and effectively for the past 160 years as a botanical insecticide around the world. It is fast acting and toxic to insects at very low doses, as well as degrading quickly in the environment due to its instability toward heat, light, and air.

2.4.2.3 Description of Neem Oil (*Azadirachta indica* A . Juss)

Azadirachta indica is well known and popular large, evergreen tree. It is used for different purpose like agricultural industry to manufacture organic pesticides includes insecticides. Insecticides which, extracts from neem plants have a wide range of effects for management of different insect pests in different mode of action which include repellence, feeding, ovipostion deterrence, toxicity, sterility and growth regulatory activity (Van Randen and Roitberg, 1996). In agriculture, neem oil, fruit and the different by products such as seed cake are used as bio pesticides, fungicides and organic manures (Subbalakshmi *et al*., 2012). Neem pesticides have a role in pest management, to produce bio pesticide and it is very crucial to possess germicidal and anti bacterial insecticide, which are very important to against the plant pests (Anis Josph *et al*., 2010).

The insect pests which are difficult to control through conventional insecticides due to insecticide resistance, can manage by using neem (Subbalakshmi, *et al*., 2012). Neem can minimize insect pest infestation through different modes of action; these are acting as growth regulators, feeding deterrence, ovipositional deterrent (Jeff Schalau, 2003; Anis Joseph *et al*., 2010; Subbalakshmi *et al*., 2012). Natural product of neem is very effective to control different crop insect pest in the field as well as at the storage. It is ecofriendly, because has no environmental residual and low-cost alternatives to agrochemicals (Mondal and Mondal 2012).

2.4.3 Biological Control

P. operculella has different natural enemies. These are include:
Predators: invertebrate or vertebrate

Parasitoids: Egg (Trichogrammatidae), larval (Eulophidae, Eneyrtidae, Braconidae, Ichneumonidae)/ parasitoid

Pathogens: candidate microbial

Virus: *P. operculella* granulovirus and bacteria (Bacillus thuringiensis)

Fungi: Biofunigant-Muscodoralbus and insect specific Nematodes are major biological control of *P. operculella*. The biological control agents, Bacillus thuringiensis (Bt) granulosis (Gv), used in different regions of the world to control *P. operculella*. Today variety of Bt are developed and currently being used to genetically transform potato to develop *P. operculella* resistant line (Mohammed *et al*., 2000).

2. 4. 4 Chemical Control Methods

Synthetic chemical insecticides: Chemicals against leafhoppers and aphids are adequate against *P. operculella* during the early part of the season, but best applications are towards the end of the season when vines are dying and tubers are maturing (University of Nebraska-Lincoln, 2014). There are main registered to control *P. operculella*. These include synthetic granules applied at planting and foliar sprays. Susceptibility of potatoes to *P. operculella* attracts large scale use of chemical pesticides, making it the second highest consumer of agricultural pesticides worldwide, next to cotton (Das *et al*., 2007). Application of insecticides for control of *P. operculella* because it needs supporting pheromone traps. The insecticide should be directed towards the newly hatched larvae and sprays are properly calibrated with the correct nozzles to ensure coverage during spray the time. The utility of insecticides, however, is limited by high cost, persistence of residue in tubers, hazarded to the environment, and development of insecticide - resistant pests (Douches *et al*., 2002).

Abamectin and fenitrothion are good example of chemicals to control *P. operculella* under storage conditions (Abdel-Megeed *et al.,* 1998). Management of *P. operculella* with chemical is challenging because of the protected tunneling behavior of larvae in foliage and tubers, consequently pests developed resistance to many traditional organophosphate, carbamate, and pyrethroid insecticides (Collantes *et al*., 1986). Chloranrhaniliprole, Chlorpyifosethyl + Cypermethrin and deltamethrin have fast and the strongest effect on the young larvae of *P. operculella* on the tobaccos. Organic phosphate and Carbamate are good example of synthetic chemicals to apply at planting foliar sprays for management of *P. operculella*. Early out use of insecticides may not be warranted (Rondon *et al*., 2007).

2. 4. 5 Integrated Pest Management of *P. operculella*

Many cultural practices that are used by farmers to improve the yield and quality of potato can also limit the development of the *P. operculella* and minimize damage to the tubers. Use new material such as new sack during harvesting time one of preventive way of *P. operculella* infestation (Anonymous, 2013). 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 manage *P. operculella*. Effective integrate pest management in potato fields and stores can minimize economical damage of *P. operculella.*

Effective IPM strategy in the field can minimize storage damage of potato tubers (Binyam Tsedaley, 2015). 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. 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).

Chemical management of *P. operculella* is challenging because of the protected tunneling behavior of larvae in foliage and tubers. Repeated sprayed of chemicals cause of resistance developed to many traditional organophosphate, carbamate, and pyrethroid insecticides. In order to reduce the impact of this key insect pest need to develop an integrated pest management approach: including appropriate cultural practices, using pheromone traps, using biological control, host plant resistant, using botanicals and appropriate rate and time of chemical applications. Integrating of many management options helps in reducing the risk of pesticide resistance development, reduce the impacts of the insecticide to the environment, non-targeted organisms, beneficial insects such as natural enemies and human hazards (Binyam Tsedaley, 2015). Generally combination of cultural, biological and chemical approaches are effective management system for *P. operculella.*

3 MATERIALS AND METHODS

3.1 Description of the Study Area

This research was done at Holetta Agricultural Research Center (HARC), under laboratory condition. HARC is about 30 km away from Addis Ababa to the west. The research activities focused on Cereal crops, oil and legumes, potato, temperate fruits, Crop protection, Dairy and Nutrition. The center is located at the highland in sub-humid agro ecology (M_2-5), with the relative humidity of 60.6% and an elevation range of 2000-3000 m. a. s. l. The total annual rain foil of the area 1040 mm and the average maximum and minimum daily temperature are 21 ^{0}c and 6 ^{0}c.

3.2 Insect Rearing

Potato tubers were obtained from HARC for rearing of *P. operculella* and for experimental setting. The experiments were done in the Entomology Laboratory at HARC, at room (ambient) temperature and relative humidity (22 ^{0}c and 63.8% respectively). *P. operculella* instars larvae collected from non-sprayed potato field of HARC, were placed into a mica chambers (25 x15 x10 cm), and fed with fresh potato leaf. The emerging *P. operculella* adult was transferred to plastic bag containing potato seedlings. The adult mate and lay eggs on the potato seedlings. The hatched larvae continue feeding on the seedling. When the seedlings were became too old, the larvae were transferred to another plastic bag containing potato seedlings which continued until 3rd larval instars, which were used for the experimental purpose to test feeding response of *P. operculella* larvae on the sprayed potato leaf and petiole in the pot.

To test *P. operculella* larval feeding response and adult ovipostion on treated potato tubers, the emerging adults were enclosed in mica chambers to allow them to mate and lay eggs. The third instars larva was selected from the cage and released on treated tubers. for experiment one 150 3rd instars larvae and for experiment two 375 3rd instars larvae of *P. operculella* were used, while for third experiment total number of 60 adults were used, with the proportion of 4 adults per cage. In all cases cotton soaked with honey solution was used as adult feed, while all larvae were either provided with leaf or tuber as a source of food.

3. 3 Material Preparation and Treatment Application

3.3.1 Botanical Plants Material Source, Collection And Extraction

Physiologically matured Birbera, *M. ferruginea* pods containing seeds, were collected from and around Holetta Agricultural Research Center, while Neem oil and Pyrethrum (*Chrysanthemum cineraria folium*) were obtained from HARC Entomology laboratory. The seed coat of birbera was removed, ground into fine powder manually using a mortar and Pestle. The powder of birbera seed and the pyrethrum flower were kept in polythene bags, and then kept at optimum temperature until use (4^0c). 400g of birbera was extracted using 600ml of ethanol (98%) in the Pyrex glass. Mixture of birbera and ethanol were stirred using magnetic stirrer, and was filtered through fine porous muslin cloth. After filtrations, crude oil of birbera, was placed in the open air in order to evaporate the remaining ethanol. The residue was separated and dried at room temperature (22 ^{0}c) for 48 hours. Which means the more concentrated the alcohol, the more rapidly evaporation should occur furthermore ethanol evaporates quite rapidly with respect to water due to its relatively low specific heat capacity and high vapor pressure (Ted Cook, Susan English and Katie Lanier, http// people.uncw.edu/../ alevap.htm).The upper creamy part was collected and ready for use.

The concentration of treatment for each of three experiment are: 0.33mg/ml, of birbera seed powder extracted by ethanol , 0.33mg/ml of pyrethrum flower powder, 0.33mg/ml combination of birbera seed powder extracted using ethanol and pyrethrum flower powder, and 0.001ml/ml ethanol dissolved neem oil.

3.4 Experiment 1: Effect of Botanical Insecticide on Larval Feeding Habits and Percent leaf and Petiole Damage

Prepare pots and fill with soil for each replication and planting the sprouted potato tuber, preventing it from any infestation by covering cage with air tied. The treatments were 200 mg of birbera seed powder extracted using ethanol and 200 mg of Pyrethrum flower powder separately dissolved with 600 ml of water, combination of birbera seed powder extracted using ethanol and Pyrethrum flower powder in one to one ratio (100 mg +100mg) dissolved with 600 ml of water, 0.18 ml of ethanol dissolved Neem diluted with 180 ml of water and pure water was used as a control. Finally 25 ml of solution from each of dissolved treatment were sprayed on the potato seedling on the pots with good cage coverage during vegetative stage. After one hour and fifty minutes treatments application release ten 3rd larvae of *P. operculella* per cage and closed it. Day today follow-up was needed what look like its feeding motivation. The treatments that were five with three replication

From this experiment the following data were recorded:

- Number of dead larvae
- Number of leaves per pot
- Damage of leaf per pot in percentage
- Number of petiole of leaf per pot
- Damage of petiole per pot in percentage

3.5 Experiment 2: Effect of Botanical Insecticide on *P. operculella* Larvae Feeding Response on Treated Tubers

Insect pest free tubers were stored under room temperature and relative humidity and prevent from any *P.operculella* infestation by binding with sack and laid down in to the ground under the laboratory. During experiment setting the tubers was clean up with clothe, for feeding assay. The treatments were 400mg of birbera seed powder extracted using ethanol, 400mg of pyrethrum flower powder, and combination of birbera seed powder extracted using ethanol and pyrethrum flower powder in one to one ratio (200mg +200mg) each of these three treatments were dissolved using 1200ml of water separately in Pyrex, 1ml ethanol dissolved neem oil were dissolved with 1000 ml of water and water was use as a control. The five comparable sized tubers were dipped into bowls which was containing the prepared treatments separately, for 1hr and fifty minutes. Then, the dipped tubers were taken out and air dried for one hour and after one hour the five tubers that were received the same treatment were put with enough space between tubers into a transparent mica chamber on each replication and release twenty five 3rd larvae of *P. operculella* on each 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 within a tuber often remain concealed feeding in the tuber that first successfully tunnels until they matured and enter pupation.

Data recorded from this experiment were:

- Larval mortality per cage
- feeding condition, number of fecal material per tuber
- Active galleries per tuber in each cage and terminated galleries.
- Furthermore, simple observation of potato tuber damage on each setting was done

3.6 Experiment 3: Effect of Botanical Insecticides on Adult Ovipostion

Two pairs of newly male and female emerged *P. operculella* adult were introduced into each cage. Ovipositional response of adult *P. operculella* on the treated potato tuber, this adult's oviposition experiment was measured in no choice setting (meaning the experiment was under control, adults were no option to lay egg without that plastic cage). Adults of *P. operculella* were allowed to contact on treated tubers. Therefore, this assay setting was a no choice arena and four *P. operculella*, newly emerged male and female (2:2) were introduced into cage on each replication. Before the adult emerged select the full grow up (black) pupae and put into vials and covered by cotton every pupa in single form under vials. This was to prevent direct mating until to meet the objective and to get the exact total number of adult's females and males. Honey solutions were feed for emerging adults until release on treated tubers in the cage. The treatment rate and preparation was similar to experiment two. Female were allowed to oviposit on treated tubers with different five treatments and the assessment was started after twenty four hours and data recorded began and then their ovipositon responses for each treatment was compared on the base of the number of eggs laid. The number of eggs laid per tuber , away from tuber on the inner surfaces of each cages and total eggs per cage were counted and recorded. On a total of 90 potato tubers on the experiment was keep until hatching out the eggs in to adult on each replication setting and comparing the total number of emerged adults from total number of egg laid in each cage. The female *P. operculella* was allowed to lay eggs on treated tubers. The assessment and data recorded were started after forty eight hours.

The data recorded from this experiment were:

* Total number of eggs per tuber
* Total number of eggs per cage
* Total number of hatching eggs per cage
* Percentage of hatched eggs

3.7 Experimental design

A complete random design (CRD) with three replications for each experiment was used one way, ANOVA ($P \leq 0.05$).Five treatments, within three replications, the total number of observations was 15 (5x3).

3.8. Data Analysis

Data analysis was done using SAS soft ware 9.1 versions. One way ANOVA was run in GLM model. Significant means ($\alpha = 0.05$) were separated using a Tukey studentized Range test.

4 RESULTS AND DISSCUSSIONS

4.1 The Effect of Botanicals Insecticide on Leaf and Petiole Damage, Larval Mortality and Adult Emergency

The effect of botanical treatments on leaf and petiole damage, larval mortality and adult emergency shown in (Table 1). According to the Table, there was no significant difference between the botanical treatments and water in terms of percent leaf and petiole damage, but the botanical treatments provided similar effects on percent larval mortality and percent adult emergence. The water treatment gave significantly the lowest mean percent larval mortality and the highest mean adult emergence. There was highly significant difference between botanical treatments and water (F4,10 at 0.05=41.68, p <0.0001) interms of larval mortality and adult emergence. Least percent of larval mortality and high adult emergence were recorded on pure water.

TABLE 1. Percent damage of leaf, petiole, larval mortality and emerged adult (Means ± SE)*

Treatments	**Concentration**	**%leaf damage**	**%petiole damage**	**%larval mortality**	**% emerged adult**
Birbera	0.33mg/ml	27.75 ± 6.99 a	4.73 ± 1.05 a	80.00 ± 5.77 a	20.00 ± 5.77b
Pyrethrum	0.33mg/ml	27.49 ± 4.09a	23.08 ± 7.85 a	86.66 ± 3.33 a	13.33 ± 3.33b
Neem	0.001ml/ml	35.12 ± 4.68 a	28.80 ± 7.07 a	70.00 ± 5.77 a	30.00 ± 5.77b
Birbera & Pyrethrum	0.33mg/ml	28.35 ± 2.92 a	16.39 ± 6.55 a	83.33 ± 3.33 a	16.66 ± 3.33 b
Water		35.42 ± 5.20a	10.41 ±6.44 a	20 .00 ± 0.77 b	80.00 ± 0.77a
F- value		0.67	2.35	41.68	41.68
P- value		> 0.625	> 0.1251	< .0001	<.0001
%CV		27.88	28.19	12.97	25.18

Means ± SE followed by the same letter (s) within a column are not significantly different from each other at $\alpha = 0.05$ using a Tukey Studntized range test (HSD).

Higher larval mortality was recorded with Pyrethrum flower powder followed by a combination of ethanol extracted birbera and pyrethrum flower powder (86.66%, 83.33 %, respectively) (Table 1). A number of authors demonstrated the efficacy of these botanicals on different stage of *P. operculella*. Isman (2006) reported that botanicals such as pyrethrum flower powder is fast acting and toxic to insects at very low doses.

These botanicals are effective for management of *P. operculella* no difference between at the selected rate. At selected rats (0.33mg/ml of birbera, pyrethrum , combination of birbera and pyrethrum and 0.001ml/ ml of ethanol dissolved neem oill) have no difference, for management of *P. operculella*, however, a little bet Pyrethrum flower powder has a potential to kill larvae. This finding agrees with the research results reported by Isman (2006) it is fast acting and toxic to insects at very low doses, as well as degrading quickly in the environment due to its instability towards heat, light, and air.

According to different authors (Jeff, 2003, Isman , 2006, Ayalew, 2010) reported that neem oil, pyrethrum flower powder, and birbera seed extracts act as feeding deterrence, when larval feel hunger and wants to feed on the treated leaf, it will affect. This experimental result indicated that, by making continuous assessment and further studies on those botanicals at the field, we able to manage potato crop from *P. operculella* damage, by spraying those selected botanicals insecticide. Pyrethrins, is insect toxine repellents and deterrents (Misra, 2014). PFP used as natural insecticide for years in traditional farming system has no toxicity to human being and animals and it possesses ecological benefits that have led to increasing worldwide production (Ban *et al*, 2010). Due to feeding behavior of *P. operculella* which hides in the leaf, consequently, it needs repeated spray of synthetic insecticides cause of environmental pollution and insecticide resistance. Thus, by using botanical insecticide we can minimize environmental risk. Botanical insecticides have potential to kill the insect by contact or stomach poisoning.

4.2 The Effect of Botanical Insecticide on *P. operculella* Larval Feeding Response on Treated Tubers

The response of *P. operculella* larval feeding on treated tubers is shown in (Tables 2 and 3). There was significant difference between birbera and the rest of the botanicals with respect to larval feeding. Least recorded of fecal materials per tuber ($F4, 10$ at $0.05 = 12.73$, $P< 0.0006$),least recorded of active galliers pertuber ($F4, 10$ at $0.05 =11.07$, $P< 0.0011$), and total percentage of larval mortality per cage ($F4, 10$ at $0.05= 18.47$, $P < 0.0001$) .The mean number of fecal materials and active galleries per tuber was recorded 1.28, and 0.41, respectively. The average dead larvae on each counting date per day on bibera and on the combination of birbera and pyrethrum were recorded 14.20, 9.25 respectively. Its total percentage of larval mortality was 96%, followed by the combination of birbera and pyrethrum, 88% as a result of (Table 2).

TABLE 2. The effect of botanical treatments on *P. operculella* larval feeding response on treated tubers (Means ±SE)*

Treatments	Concentration	Number of fecal/ tuber	Terminated galleries/ tuber	Active galleries / tuber	Total % of dead larvae per cage
Birbera	0.33mg/ml	1.28 ± 0.49 c	0.06 ± 0.04 b	0.41± 0.16 a	96.00 ± 2.30a
Pyrethrum	0.33mg/ml	3.91 ± 0.55 ab	0.06 ± 0.04 b	1.34± 0.10 a	84.00 ± 0.06a
Neem	1ml/ml	3.85 ± 0.18 ab	0.12 ± 0.11 b	1.38 ±0.08 a	85.33 ± 3.52a
Birbera +Pyrethrum	0.33mg/ml	3.12 ± 0.30 bc	0.05 ± 0.00 b	1.25± 0.24 a	88.00 ± 0.33a
Water		5.15 ± 0.33 a	0.04 ± 0.04 a	2.37 ± 0.34 b	21.33 ± 9.61b
F-value		12.73	0.25	11.07	18.47
P-value		< 0.0006	>0.9040	< 0.0011	< 0.0001
%CV		19.91	13.5	26.72	16.30

Means ± SE followed by the same letter (s) within a column are not significantly different from each other at $\alpha = 0.05$using a Tukey studntized range test (HSD).

There was significant difference between ethanol extracted birbera and among remaining botanicals on larval mortality on each counting date. The result shown in the (Table 3) indicated that at the first ($F_{4,10}$ at 0.05=1.6, pr>0.24) and second ($F_{4,10}$ at 0.05=1.69, pr > 0.22 counting date, there was no a significant difference between the botanicals. At count three ($F_{4, 10}$ at0.05=6.89, P< 0.0062), four ($F_{4, 10}$ at 0.05=10.25, P< 0.0015), five ($F_{4, 10}$ at 0.05=6.72, P<0.0068)and six ($F_{4, 10}$ at 0.05=5.58, P< 0.0126) there was a significant difference between birbera and remaining botanicals. At counting seven, eight and nine there was no significant difference. Experimental study shown that, birbera seed powder extracted by ethanol at the rate of 0.33mg/ ml was effectively reduced number of fecal material, terminated, non terminated galleries per tuber and high percentage of larval mortality followed by the combination of birbera seed powder extracted by ethanol and pyrethrum flower powder compare to the rest botanical treatments. This result agree with Ayalew Tadesse (2010) reported that the number of active and terminated galleries carved by *P. operculella* larvae tend to decrease with increasing rate of birbera. During the data collection time, the mortality of larva increase day today in each replication which was treated by birbera seed powder extracted using ethanol.

On each counting date the result shown that birbera was superior in killing of larvae of *P. operculella in* time sequence within compared to the other botanical treatments, followed by the combination of birbera and pyrethrum. During the nine counting days the daily larval mortality of *P. operculella* by ethanol extracted birbera out of twenty five larvae within nine counting dates are 2, 9, 12, 17, 18,19,20,24, and 24 respectively. Ayalew Tadesse (2010) stated that *M. ferruginea* seed powder extracted by acetone had showed better insecticidal properly to cause neonate larval mortality at the lost rate and during early hours of exposures with in 1 hour to 2 hours. The result of this experiment also shown that, birbra seed powder extracted by ethanol was a good killer against *P. operculella* larvae within three to five days as compared to the remains botanical treatments (with in short time of period). Pyrethrum flower powder and neem oil extracted using ethanol were not significantly effective compared to birbera seed powder extracted using ethanol.

TABLE 3. Mean number of dead larvae on each counting date (Means ±SE)*

	Counting day	Count 1	Count 2	Count 3	Count 4	Count 5	Count 6	Count 7	Count 8	count 9
Treatments	Birbera	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a	2.33 ± 1.2 a
	Pyrethrum	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57 a	1.00 ± 0.57a	1.00 ± 0.57 a
	Neem oil	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a	0.66 ± 0.33a
	Birbera + Pyrethrum	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a	0.66 ± 0.66 a
	Water	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	F-value	1.6	1.69	6.89	10.25	6.72	5.58	5.19	18.47	18.47
	P-value	O.24	0.22	< 0.0062	< 0.0015	< 0.0068	< 0.016	< 0.0159	< 0.0001	< 0.0001
	%CV	20.5	25.55	22.21	37.81	38.88	40.89	30.90	32.37	32.37

Counting date Means ± SE followed by the same letter (s) within a column are not significantly different from each other at α = 0.05 using a Turkey studentzed range test (HSD).

4. 3 Response of *P. operculella,* Ovipositon on Treated Tubers

The testing of ovipositon on the treated potato tuber, there was no significant difference among the treatments. At (Table 4) result shown, there was least eggs recorded on neem oil extracted by ethanol, followed by pyrethrum, and with unknown reason tubers which were treated with pure water also there was least egg recorded on each per cage and per tuber. There was least percentage of eggs hatched on neem oil extracted by ethanol compared to the remaining treatments, at selected rate followed birbera seed powder extracted using ethanol. The percentage of damaged tubers observed more than 40% on each treatment in (Table 4).

TABLE 4. Effects of treatments on response of ovipositon of adult *P. opercuella* (mean of laying eggs, hatched egg and total percentage of hatched eggs per cage (Mean ± SE).

Treatments	Concen tration	Total eggs / cage	Eggs/ tuber	Total % of hatched eggs	Total % of Damage tuber
Birbera	0.33mg/ml	92.33± 20.91a	18.46 ±4.18 a	43.00 ± 6.02 a	100±0.00 a
Pyrethrum	0.33mg/ml	31.58± 45.25b	5.65 ±2.44 b	68.90 ± 9.24 a	93.33 ± 6.66a
Neem	1ml/ml	20.75± 9.18b	4.15 ± 1.83 b	28.90 ± 17.12 a	40.00 ±30.55 a
Birbera and Pyrethrum	0.33mg/ml	28.75± 17.75b	5.75 ± 3.55 b	77.18 ± 10.37 a	93.33 ±6.66 a
Water		90.75± 18.19a	17.98 ± 3.81 a	24.05 ± 16.76 a	66.67 ±33.33 a
F-value		4.94	4.97	3.44	1.48
P-value		< 0.0185	< 0.0182	< 0.051	> 0.27
%CV		24.87	23.59	44.44	45.47

Means ± SE followed by the same letter (s) with in a column (Lower case letter) are not significantly different from each other at α=0.05 using a Tukey studntized range test (HSD).

In the experimental setting to test ovipositon performance, there was no significant difference between the selected botanical insecticide at the rate of 400mg/1200 ml EEB, PFP, a combination of EEB and PFP, and 1 ml of neem oil extracted by ethanol However, neem oil has a potential to create discomfort for adult *P. operculella* for egg laying compared to the other treatments, followed by the combination.

This experimental result, agree with Moawad and Ebadan (2007) neem oil (*Azadirachta indica*) on potato tuber caused ovicidal action against *P. operculella*. Ayalew Tadesse (2010) reported that birbera in ovipositon response, rates greater than 1.0 mg/ml can prevent ovipositon on leave which extracted with acetic acid, acetone, chloroform and water. Hence, in this experiment setting, at the rate of 0.33mg/ml birbera has shown comparable result with neem and pyrethrum, might be if it goes up to 1mg/ml and more, it will have higher potential compared with neem and pyrethrum. The data taken at the end of the date observed all of treated tubers were damaged by the hatched eggs of *P. operculella.* Because the treatment application was done only one times there was no repeated application on each stages of *P. operculella.* This means the treatments were influenced only on laid eggs, but not including the hatched eggs. Which were continuous their feeding system without influencing. The results indicate that might be due the fact that their active degradability of botanical insecticides, which needs continues, treat on each life stage in order to decrease *P. operculella* damaged.

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. The result obtained from this experiment indicates that the pyrethrum flower powder is more effective in the management of *P. operculella* under laboratory condition at the rate of 0.33mg/ml compare to birbera and neem oil.

2. Ethanol extracted birbera either singly or in combination with pyrethrum flower powder gave the best result for management of *P. operculella* under storage condition.

3. Ethanol extracted birbera was a fast acting insecticides as it killed all 25 exposed larvae per cage within 3-5 days as opposed to other botanicals which killed larvae 9th day after treatment application followed by the combination of birbera and pyrethrum.

4. Ethanol dissolved neem oil, combination of birbera seed powder extracted using ethanol and pyrethrum flower powder were found effective to reduce *P. operculella* egg laying and hatching.

5.2 Recommendations

1. Awareness creation activities should be done with stakeholders (farmers, Developmental agents, NGO, etc.) on the comparative advantage of using botanicals.
2. Non target effects of the botanicals at different rates and frequencies should be studied.
3. All parts of the effective botanicals should be tested for efficacy.
4. In this study the combinations of ethanol extracted birbera and pyrethrum flower powder has potential for management of *P. operculella*, moreover it needs further study its exact rate of the efficacy .
5. Imbalance of the emerging adult sex ratio, which needs clear biological studies about *P. operculella* under Ethiopian agro ecological conditions and to give more attention to develop a base line integrated pest management.
6. At this time most farmers used pesticide for insect pest management without recommended dosage, which leads to dramatic environmental change, hence botanical insecticide is good for environmental sound.

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7 ANNEX

ANNEX 1. Summery table for analysis of variance (ANOVA) for percentage of leaf and petiole damage, larval mortality on the leaf on the treatments

Percentage of leaf damage

Source	**DF**	**Sum of square**	**Mean square**	**F value**	**Pr>f**	**% CV**
Model	4	199.0512822	49.7628206	0.67	0.625	27.88
Error	10	739.3178181	73.9317818			
Corrected Total	14	938.3691003				

Percentage of petiole damage

Source	**DF**	**Sum of square**	**Mean square**	**F value**	**Pr >f**	**% CV**
Model	4	1110.115347	277.528837	2.35	0.1251	28.19
Error	10	1183.426252	118.342625			
Corrected Total	14	2293.541599				

Dead larvae per cage

Source	**DF**	**Sum of Squares**	**Mean square**	**F valu**	**Pr >f**	**% CV**
Model	4	61.24	15.31	45.68	< .0001	13.15
Error	10	3.35	0.33			
Corrected Total	14	64.59				

Total percentage of larval mortality

Source	**DF**	**Sum of square**	**Mean square**	**F value**	**Pr>f**	**% CV**
Model	4	12226.66667	3056.66667	41.68	<.0001	12.97
Error	10	733.33333	73.33333			
Corrected Total	14	12960.00000				

Total emergence of adult

Source	DF	Sum of square	Mean square	F value	Pr>f	% CV
Model	4	122.2666667	30.5666667	41.68	<.0001	25.18
Error	10	7.3333333	0.7333333			
Corrected Total	14	129.6000000				

ANNEX 2. Summery tables for analysis of variance (ANOVA) on response of *P. operculella* larvae on treated tubers.

Number of fecals materials on potato tubers

Source	DF	Sum of square	Mean square	F value	Pr>f	% CV
Model	4	24.30531767	6.07632942	12.73	0.0006	19.91
Error	10	4.77267651	0.47726765			
Corrected total	14	29.07799418				

Number of terminated galleries o tubers

Source	DF	Sum of square	Mean square	F value	Pr>f	% CV
Model	4	0.01165436	0.00291359	0.25	0.9040	13.5
Error	10	0.11720206	0.01172021			
Corrected Total	14	0.12885642				

Number of non-terminated galleries on tubers

Source	DF	Sum of square	Mean squar	F value	Pr>f	% CV
Model	4	5.82037881	1.45509470	11.07	0.0011	26.72
Error	10	1.31489708	0.13148971			
Corrected Total	14	7.13527589				

Total percentage of dead larvae per cage

Source	DF	Sum of square	Mean squar	F value	Pr>f	%CV
Model	4	11033.60	2758.40	18.47	<0.0001	16.30
Error	10	1493.33	149.33			
Corrected Total	14	12526.93				

Dead larvae per cage at count one

Source	DF	Sum of Square	Mean Square	F value	Pr >F	%CV
Model	4	8.93	2.23	1.6	0.24	20.5
Error	10	14.00	1.40			
Corrected Total	14	22.93				

Dead larvae per cage at count two

Source	DF	Sum of Square	Mean Square	F value	Pr>F	%CV
Model	4	98.93	24.73	1.69	0.22	25.55
Error	10	146.00	14.60			
Corrected Total	14	244.93				

Dead larvae per cage at count three

Source	DF	Sum of Square	Mean Square	F value	Pr >F	%CV
Model	4	192.93	48.23	6.89	0.0062	22.21
Error	10	70.00	7.00			
Corrected Total	14	262.93				

Dead larvae per cage at count four

Source	DF	Sum of Square	Mean Square	F value	Pr >F	%CV
Model	4	368.93	92.23	10.25	0.0015	37.81
Error	10	90.00	9.00			
Corrected Total	14	458.93				

Dead larvae per cage at count five

Source	DF	Sum of Square	Mean Square	F value	Pr >F	%CV
Model	4	396.26	99.06	6.72	0.0068	38.88
Error	10	147.33	14.73			
Corrected Total	14	543.60				

Dead larvae per cage at count six

Source	DF	Sum of Square	Mean Squar	F value	Pr >F	%CV
Model	4	388.40	97.10	5.58	0.016	40.89
Error	10	174.00	17.40			
Corrected total	14	562.40				

Dead larvae per cage at count seven

Source	DF	Sum of Square	Mean Square	F value	Pr >F	%CV
Model	4	372.40	93.10	5.19	0.0159	30.90
Error	10	179.33	17.93			
Corrected total	14	551.73				

Dead larvae per cage at count eight

Source	DF	Sum of Square	Mean Squar	F value	Pr >F	%CV
Model	4	689.60	172.40	18.47	0.0001	32.37
Error	10	93.33	9.33			
Corrected total	14	782.93				

Dead larvae per cage at count nine

Source	DF	Sum of Square	Mean Squar	F value	Pr >F	%CV
Model	4	689.60	172.40	18.47	0.0001	32.37
Error	10	93.33	9.33			
Corrected total	14	782.93				

ANNEX 3. Summery table for analysis of variance (ANOVA) , response of *P. operculella* ovipostion on treated potato tubers.

Number of eggs per tuber

Source	DF	Sum of square	Mean square	F value	Pr>f	%CV
Model	4	617.47	154.36	4.97	0.0182	23.59
Error	10	310.68	31.06			
Corrected Total	14	928.16				

Number of total eggs per cage

Source	DF	Sum of square	Mean square	F value	Pr>f	%CV
Model	4	15176.500	3907.04	4.94	0.0185	24.87
Error	10	5405.83	790.41			
Corrected Total	14	23582.33				

Percentage of hatched eggs per cage

Source	DF	Some of Squares	Mean Square	F value	Pr>	%CV
Model	4	6641.81	1660.45	3.44	0.051	44.44
Error	10	4820.86	482.08			
Corrected Total	14	11462.67				

Percentage of damage of tuber per cage

Source	DF	Sum of Squares	Mean square	F value	Pr >f	%CV
Model	4	7573.33	1893.33	1.48	0.27	45.47
Error	10	12800.00	1280.00			
Corrected Total	14	20373.33				

ANNEX 4. Experimental setup for rearing

ANNEX 5. Experimental setup of experiment one: larval feeding response on treated potato leaf and petiole in the pots at HARC lab.

ANNEX 6. Experimental setup of experiment two: larval feeding response on treated potato tubers in mica chamber at HARC lab.

ANNEX 7. Honey solution, feeding of adult *P. operculella* during the experiment

ANNEX 8 data collection sheet

To test larval feeding activity and mortality on selected Botanicals on potato leaf and petiole

Treatment	Number of leaf per pot	% damage of leaf per pot	% damage of petiole per pot	% larval mortality

To test larval feeding activity and mortality on tread tuber by selecting Botanicals

Treatmen	Number of fecal material	Terminated Galleries	Active galleries	Dead larvae per counting date	% of larval mo

To test adult *P. operculella* oviopstional response on treated potato tubers

Rep	No	Ca	No of eggs laid per tuber					Total eggs Laid Per Cage	Hatched eggs per cage	Total percentage of hatched eggs	Total damage Percentage Of tubers per cage
			T1	T	T	T4	T5				

This thesis paper work is my own original work which was design my heart of my knowledge. All sources of the materials that I were used to prepared this thesis acknowledged.

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This thesis has been submitted to examination with my approval

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