

**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCES, DEPARTEMENT OF ZOOLOGICAL
SCIENCES**



**The Effects of a Low Toxicity Pesticide on potato Late Blight, Tomato Leafminer, Potato
Tuber Moth and Its Major Parasitoid in Potato and Tomato Intercrops**

By:

Tewodros Mulugeta

A PhD dissertation submitted to the School of Graduate Studies of Addis Ababa
University in partial fulfilment of the requirement for the degree of Doctor of
Philosophy in Zoology (Insect Sciences)

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Addis Ababa University

School of Graduate Studies

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Dedication

This dissertation is dedicated to those who had the potential to pursue further in life but did not get the chance to do so.

Declaration

This is to declare that this dissertation is submitted to the School of Graduate Studies of Addis Ababa University for the degree of Doctor of Philosophy (PhD) in Zoology (Insect science). And I would like to corroborate that it is my own work and all other works used in the dissertation are well acknowledged.

Name: Tewodros Mulugeta

Signature:

Date:

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List of Acronyms

ATP	Adenosine triphosphate
AUDPC	Area under disease progress curve
BABA	Beta amino butyric acid
<i>Bt</i>	<i>Bacillus thuringiensis</i>
BTH	Thiadiazole-7-carbothioic acid S-methyl ester
CHT	Chitosan
CIP	International Potato Center
CSA	Central Statistics Agency
EAD	Electroantennodetector
EAG	Electroantennographic
EBDCs	Ethylenebisdithiocarbamates
EFS	Enemy free space
EIAR	Ethiopian Institute of Agricultural Research
EPN	Entomopathogenic nematodes
EPPO	European and Mediterranean Plant Protection Organization
EPVs	Entomopox viruses
ET	Ethylene
FAO	Food and Agricultural Organization
GVs	Granulosis viruses
HARC	Holetta Agricultural Research Center
IJ	Infective juvenile
INA	2,6-dichloroisonicotinic acid

IR	Induced Resistance
JA	Jasmonic acid
JME	Jasmonic methyl ester
LC50	50% Lethal Concentration
MARC	Melkassa Agricultural Research Center
MeJA	Methyl jasmonate
MeSA	Methyl salicylate
MRK	Marketable
mtDNA	Mitochondrial DNA
NAD	Nicotinamide adenine dinucleotide
NPVs	Nuclear polyhedrosis viruses
OB	Occlusion bodies
Phi	Phosphite
PoGV	PTM granulovirus
PR	Pathogenesis related
PRIs	Plant resistance inducers
PTM	Potato tuber moth
rAUDPC	Relative area under disease progress curve
R-gene	Resistance genes
SA	Salicylic acid
UNMRK	Unmarketable
VOC	Volatile organic compounds
WMV	Watermelon mosaic virus

Abstract

Potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is the most important potato disease worldwide. It causes losses of several billion dollars annually and it is a global threat to potato growers. The pathogen is also equally important in tomato. Even though various resistant cultivars are being released, potato late blight is mainly controlled with intensive application of fungicides. In Ethiopia the pathogen is distributed throughout the potato producing areas. The main rainy season is quite favorable for the growth and development of the disease and loss could reach 100%. Tomato is the second most affected crop by late blight in the country.

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most important potato pests worldwide. Tuber damage in storage facilities is the major problem related to PTM infestation. Even though foliar damage is the source of infestation for the damage in stores, it usually causes insignificant yield losses. In Ethiopia field tuber infestation could reach 42%. In addition to potato PTM attacks tomato, eggplant, tobacco and wild solanum species. *Tuta absoluta* (Meyrick), commonly called tomato leafminer, is a devastating pest of tomatoes. Up to 100% losses have been reported in tomato crops. Even in a condition where control measures have been taken, losses could still exceed 5%. In Ethiopia the pest has been causing severe damage in tomato since 2011. Control of both PTM and tomato leafminer has been mainly with the use of synthetic insecticides.

Due to the non-judicious and continuous use of fungicides/pesticides, insects and pathogens are developing resistance, the environment is polluted and non-target organisms are damaged. Furthermore, lack of knowledge in chemical use and low use of personal protective devices during application, mainly in developing countries, is imposing health problem to poor farmers.

Therefore due to the harmful impacts of pesticides/fungicides use to the environment and human health there is a need to search for a safe alternative to manage pests and pathogens. Phosphite has been identified as a potential alternative to pesticides/fungicides in controlling some herbivorous insects, fungal diseases and oomycetes.

The present study investigated the effect of phosphite against PTM, tomato leafminer and potato late blight. Field trials were conducted for three consecutive years to investigate the efficiency of potassium phosphite, a low toxic inorganic salt with direct and indirect toxicity on oomycetes, and its combinations with the Ridomil fungicide against potato and tomato late blight. Two potato, Belete and Jalene, and one tomato, Melka shola, cultivars with different susceptibility to late blight were used. We demonstrated that phosphite combined with reduced dose of fungicides led to an effective suppression of potato foliar blight same as the full recommended dose of the recommended fungicide. In the moderate resistant potato cultivar Belete phosphite alone had adequate foliar protection against foliar late blight. In tomato, phosphite alone was as effective as the recommended dose of fungicide and combination of phosphite and fungicide. Yield was also affected with phosphite and phosphite and fungicide synergism. Treated plants provided far better yield than untreated control plants. Similarly, three years field trials were conducted to investigate the efficacy of phosphite against PTM and tomato leafminer. The study showed that the phosphite treatment did not affect the population density of tomato leaf miner larvae; however, it reduced PTM larvae population density. Furthermore, the direct toxicity of phosphite on mycelial growth, sporangia production and sporangia germination was investigated in a rye-agar and pea broth plate assays, *in vitro*, in different *P. infestans* isolates. The isolates responded differently to phosphite treatment with LC50 value between 1.4-4.3mM and their response was dose dependent.

These findings show that phosphite can be used efficiently against potato and tomato late blight either alone or combined with reduced dose of fungicides. Phosphite has concentration based direct toxic effect on *P. infestans*, but the toxicity varies between isolates. Therefore phosphite could be a possible a component in the IPM of PTM and late blight.

Key words: Fungicide, isolate, pesticide, plant resistance inducers, PTM, synergism, toxicity,

Chapter 1 General Introduction

1.1. Background of the study

Potato, *Solanum tuberosum* L., is one of the most important starchy edible tuber crops with high nutritive value. More than 325 million tons of potato is produced in the world with over an area of 19.3 million ha (Birch et al., 2012). Potato is the third most important food crop in the world next to wheat and rice and it is grown in more than 160 countries (Dias, 2012; King and Slavin, 2013). More than a billion people feed on potato and it is also source of income for millions of farmers in the world (Devaux et al., 2014). The developed nations used to be high global potato producers, 183.13 million tones, but recently developing nations have taken the lead with a total production of 165.41 million tons which accounts 55% of the global production (FAO, 2008). Like many other countries in the world, potato is a very important food and cash crop, especially in the highland and mid altitude areas of Ethiopia (Borgal et al., 1980). The average potato production throughout Ethiopia is 8-10tons⁻¹. The dependence on potato has dramatically increased and it is now the 9th most important crop in Ethiopia and grown on 179,000 ha area (CSA, 2014). The main production constraints are related to narrow genetic bases of the crop and poor seed qualities distributed across the country, disease susceptibility of the cultivars being cultivated and poor crop management capacity of the farmers (Haverkort et al., 2012).

Next to potato, tomato is the second widely grown and consumed vegetable crop in the world (Dias, 2012). World total tomato production in 2014 was 170.8million tones (FAOSTAT, 2014). In the year 2013 the area devoted for tomato production in Ethiopia was 10,882.83 ha. Currently, tomato is one of the regional export crops of the country

(<http://www.tropentag.de/2012/abstracts/full/659.pdf>). In 2014 the tomato production was 6.7 tons ha⁻¹ (<http://www.factfish.com/statistic-country/ethiopia/tomatoes%2C%20yield>).

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most important potato pests worldwide (Gill et al., 2014). Potato can be damaged by PTM both in field and storage facilities (Rondon, 2010). Potato foliage can be completely destroyed, resulting in substantial yield loss. Especially, high infestations early in the season can directly affect tuber yield (Kroschel et al., 2016). PTM is considered the most damaging insect pest of stored potatoes in the tropical and sub-tropical regions (Gill et al., 2014). It can cause significant economic damage. Different level of crop loss in stored potato has been reported, for instance PTM infestations accounted for losses of 50% in Yemen and Peru; 86% in Tunisia, Algeria and Turkey; 90% in Kenya; and 100% in India and the Philippines (Gill et al., 2014). In Ethiopia, field tuber infestation ranged from 6% to 62% (Sileshi and Teriessa, 2001). Reducing the PTM population density during the potato-growing period is a key to reducing tuber infestation at harvest (Kroschel et al., 2016). In Ethiopia PTM is known to attack tomato as well (Mulatu et al., 2004). *Tuta absoluta* (Meyrick), commonly called tomato leafminer, is another devastating pest of tomatoes and can cause complete damage (Korycinska and Moran, 2009). It has been causing severe damage in Ethiopian tomato system ever since its introduction in 2011 (Gashawbeza Ayalew and Abiy Fekadu, 2013).

The oomycete *Phytophthora infestans*, the causal agent of late blight is one of the most destructive pathogen affecting both potato and tomato (Birch and Whisson, 2001). Late blight causes serious economic losses both in open field and non-heated greenhouses especially under favourable conditions, which are wet and cool temperatures. The genetic diversity, rapid

evolution and a broad range of virulence factors as an effect of dynamic and expanded regions in the genome have made this pathogen to adapt and overcome host resistance quickly (Haas et al., 2009).

The disease occurs throughout the major potato producing areas in Ethiopia and it is difficult to produce the crop during the main rainy season without the use of chemical fungicides (Bekele and Geberemedhin, 2000). The main rainy season is quite favorable for the growth and development of the disease. Consequently, due to high late blight infection in the main season there has been a production shift in time from the main to the off season (Haverkort et al., 2012). The estimated potato yield losses reported in Ethiopia due to late blight are 2.7%-70% (Bekele and Yaynu, 1996), 22–46% (Girma et al., 2013) and 29-57% (Tsedaley et al., 2014b), however, complete crop failures are frequently reported (Bekele and Yaynu, 1996). The second most affected crop from late blight is tomato. Due to the high susceptibility of tomato cultivars found in the country farmers produce tomato mainly in the short rain season, February to April, when disease pressure is low compared to the main season (Ethiopia late blight profile, 2004).

1.2. Rationale of the study

In modern agriculture, chemical use and resistance breeding are the major methods implemented to combat crop damage by pests. However, each method has its drawbacks. Breeding for resistance is time consuming and pathogens sometimes evolve quickly and overcome resistance. Similarly pathogens/pests can develop resistance to fungicides/insecticides (Poland et al., 2008). Furthermore, in developing countries there are problems related to fungicide use. Inadequate knowledge about fungicides, lack of spraying device and safety equipment's are the major problems related to the use of pesticides (Mulatu, 2011; Kromann et al., 2012). This could risk

human health and the environment. Therefore there is a need for the use of safe and environmentally friendly control methods.

One alternative is to induce by enhancing the plant's own innate immunity with plant resistance inducers (PRIs). Phosphite, Beta amino butyric acid (BABA), Chitosan (CHT), Salicylic acid (SA), Methyl salicylate (MeSA), Probenazole and Methyl jasmonate (MeJA) are some examples of PRIs. Unlike fungicides, PRIs perform indirectly by inducing plants innate immunity which makes them environmentally sustainable with less impact to human health. Furthermore, using PRIs like phosphite has the possibility of decreasing selection pressure in pathogens due to the nature of mode of action of this particular PRI, which involves both direct toxicity and indirect protection by inducing the plant's own defence (Alexandersson et al., 2016). To the best of our knowledge no resistance development has been reported by *Phytophthora infestans* against phosphite so far. In this dissertation, phosphite *in vitro* toxicity to *P. infestans* isolates and its integration with fungicides against late blight in field grown potato and tomato crops is demonstrated. The study further demonstrated phosphite effect in the major potato and tomato pests, PTM and *Tuta absoluta*, respectively, under field conditions. The findings of this work could supplement integrated pest management programs.

1.3. Objectives

1.3.1. General objective

To investigate management options through the introduction of safer alternatives to the use of synthetic chemicals in an intercrop system to manage *Phytophthora infestans* and potato tuber moth (PTM) and/or *Tuta absoluta* in potato and tomato.

1.3.2. Specific objectives

- To evaluate the effects of potato-tomato intercrop on the pest pressures, crop quality, yield and natural enemies, particularly parasitoids
- To investigate the effects of foliar application of potassium phosphite on late blight, PTM, *Tuta absoluta* and parasitoids in the intercrops as well as in monocultures of potatoes and tomatoes
- To investigate the behavioral response of PTM to potato and tomato plants treated with the PRIs phosphite and BABA
- To investigate the direct toxicity of phosphite to various *P. infestans* isolates from different location

Chapter 2 Literature review

2.1. Origin and importance of potato and tomato

Potato (*Solanum tuberosum* L.) (Solanaceae) was first domesticated in the mountainous regions around Lake Titicaca between Peru and Bolivia 8,000 years ago (FAO, 2008). Next to wheat and rice, potato is the third most important food crop in the world and it is grown in more than 160 countries (Dias, 2012; King and Slavin, 2013). It is cultivated in diverse climatic conditions of tropic, subtropics and temperate. The developed nations used to be high global potato producers, 183.13 million tons, but recently developing nations have taken the lead with a total production of 165.41 million tons which accounts 55% of the global production (FAO, 2008). China, India and the Russian federation are the three world leading potato producing countries. In the world more than a billion people feed on potato and it is also source of income for millions of farmers (Devaux et al., 2014).

The nutritional value of potato is undeniable. Potato mainly contains carbohydrates, antioxidants, fibers and proteins. Although potato provides the highest energy as compared to cereals and cassava, the amount of protein it has is small, less than 6% but the proteins are of best biological value vis-à-vis other vegetable sources (Dias, 2012). Potato is an important mineral nutrient source, which provides humans with potassium, phosphorus and magnesium. It is also a source of the vitamins B1, B3, B6 and vitamin C (Camire et al., 2009). Studies have shown that the daily vitamin C requirement of an adult can be achieved by consuming a single potato. Though, it contains toxic glycoalkaloids such as solanine and chaconine, the level of the glycoalkaloids could be reduced through storing the tubers under cool and dark conditions, repeatedly frozen over several nights and skin removal and soaking or leaching in running water afterward (FAO, 2008a; Camire et al., 2009). Nowadays, the glycoalkaloid content is monitored in breeding

programs to ensure safe levels in cultivars released to the market (McCue, 2009; Khan et al., 2012).

There are about 5000 potato varieties worldwide and they vary in size, shape, color, starch content, and flavor (Zaheer and Akhtar, 2016). Potato varieties can be classified by a number of criteria such as, days between planting and maturity out in the field, tuber quality, storage property, tuber size and skin colour. Based on maturity cultivars could fall under five categories, very early, early, mid-season, late, and very late maturing. Tubers can also be categorized in to culinary and processing qualities (Camire et al., 2009). Some of the large sized popular varieties include Russet Burbank, White Rose, and Katahdin (Zaheer and Akhtar, 2016). In Ethiopia, 29 varieties are available in the markets most of which are released by EIAR-CIP (Haverkort et al., 2012).

Though it varies based on the cultivar, generally potato requires less time to mature than any other major crop in the field and it yields more per unit of cropland. Furthermore, unlike other cereal products potato is not involved in the major international trade, therefore, it is not at a risk of rough trade activity. Hence, potato could be a trustworthy crop to address the future world food demand (Devaux et al., 2014).

Potato is also a very important food and cash crop especially in the highland and mid altitude areas of Ethiopia (Borgal et al., 1980). The Amhara, Oromia, and Southern Nations, Nationalities and Peoples (SNNP) regions are the main potato producing areas of the country (Haverkort et al., 2012). Potato is thought to have been introduced to Ethiopia some 150 years ago. The dependence on potato has dramatically increased and it is now the 9th most important

crop in Ethiopia and grown on 179,000 ha area (CSA, 2014). The main production season of potato at altitudes higher than about 2500 m.a.s.l is from June to December (Meher in Amharic). The off season production at higher elevation is January to May (Belg in Amharic). The average potato production throughout Ethiopia is 8-10 tons ha⁻¹ (Haverkort et al., 2012). This is relatively low as compared to countries such as Holland, Germany, England and the U.S. with production of 50-65 tons ha⁻¹ (Smirnov and Suslov, 2014), especially when considering the potential of Ethiopia, with its favorable climate at higher elevations, soils and irrigation potential. The main production constraints are related to narrow genetic bases of the crop and poor seed qualities distributed across the country, disease pressure/susceptibility of the cultivars being produced and poor crop management capacity of the farmers (Haverkort et al., 2012).

Tomato (*Solanum lycopersicum* Mill.) also originated in the Andean region of South America, including Ecuador, Peru and Chile (Chetelat et al., 2009). But it was first domesticated and cultivated in Central America. Nutritionally tomato is low in calories but high in vitamins A and C, potassium, magnesium, iron, phosphorus, sodium, niacin, riboflavin, thiamine and the valuable antioxidant lycopene and beta-carotene (Dias, 2012). World total tomato production in 2014 was 170.8million tones (FAOSTAT, 2014). China is the biggest producer of tomato with production of 34 million tones. India and U.S.A are 2nd and 3rd with production of 14 and 11 million tones, respectively (<http://www.worldknowing.com>). The introduction of cultivated tomato (*S. lycopersicum*) into Ethiopian agriculture dates back to the period between 1935 and 1940. Between 1994 and 2011, tomato acreage increased to 5,338 ha. In the year 2013 the area devoted for tomato production increased to 10,882.83 ha. Currently, tomato is one of the regional export crops of the country (<http://www.tropentag.de/2012/abstracts/full/659.pdf>). In Ethiopia, the crop is grown in areas between 700 and 2000 m.a.s.l, which receive about 700 to over 1400

mm annual rainfall, in different soils, under different weather conditions, and provide variable yields. In 2014 the tomato production was 6.7 tons ha⁻¹ (<http://www.factfish.com/statistic-country/ethiopia/tomatoes%2C%20yield>).

2.2. Potato and tomato production constraints

2.2.1. Potato tuber moth (PTM)

2.2.1.1. Origin, distribution, host range and damage symptoms of PTM

The potato tuber moth (PTM), *P. operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most important potato pests worldwide. It was described and named as *Gelechia operculella* by Zeller in 1873 before Povolny changed the genus name to *P. operculella* in 1964. There are two other species in different genera with similar name: *Tecia solanivora* (Povolny) and *Symmetrischema tangolias* (Gyen) (Gill et al., 2014). PTM is believed to be originated together with its favored host, potato, in South America (Rondon, 2010). The pest is mainly distributed in warm temperate and tropical regions where the host plants are widely grown (Golizadeh and Esmaeili, 2012). PTM was introduced to different parts of the world in different periods via various ways including trade: in 1906 to India, mid-70's to Iraq and early 80's to Russia (Rondon, 2010). It is now known to be distributed in 103 countries in the world, including Ethiopia (Cabi, 2015) (Fig 2.1).

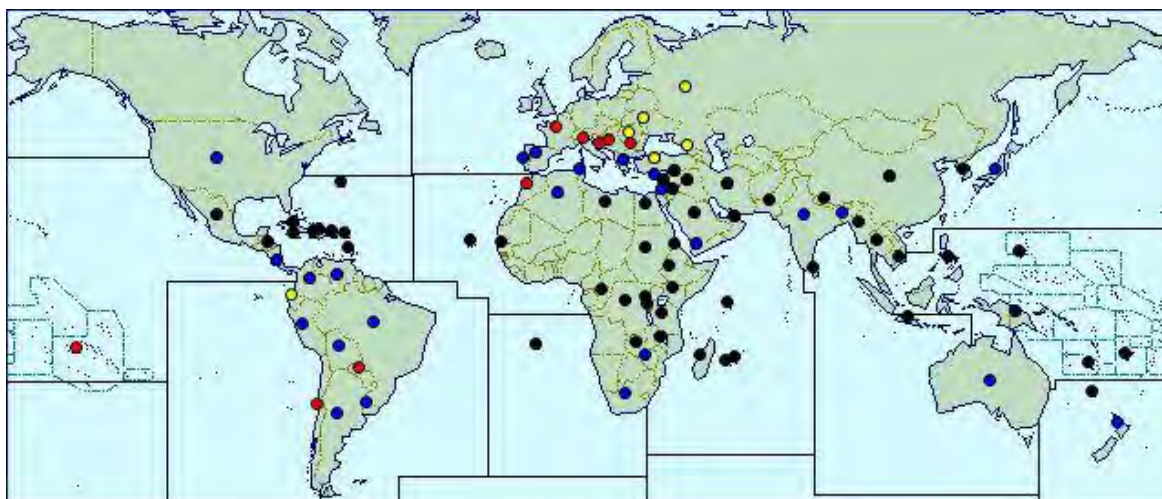


Figure 2.1 PTM distribution, Black= present, no further details, Blue= Widespread, Red= Localized Yellow= Occasional or few reports. Source: Cabi, 2015

Though potato is the primary host, PTM feeds on other solanaceous species such as eggplant (*Solanum melongena* L.), tomato (*Solanum lycopersicum* L.), black nightshade (*S. nigrum* L.), silver leaf nightshade (*S. elaeagnifolium* Cav.), chili pepper (*Capsicum frutescens* L.), tobacco (*Nicotiana tabacum* L.), cape gooseberry (*Physalis peruviana* L.), field ground cherry (*Physalis mollis* D.), prickly nightshade (*S. torvum* Sw.), jimson weed (*Datura stramonium* L.), *P. angulata* L. and *Brugmansia suaveollens* (Bersch) (Rondon, 2010). Insects may not always reproduce in their host plant. For example in U.S. Pacific Northwest, PTM reproduce to perpetuate its generation only in potato plants (Rondon et al., 2007). In Ethiopia it is known to attack potato and tomato (Mulatu et al., 2004). Other hosts like eggplant, tobacco and *wild solanum* could also be attacked by PTM (Tewodros, 2013).

Potato can be damaged by PTM both on field and in storage facilities mainly in non-refrigerated stores while foliar damage usually caused insignificant yield losses. The damage is quite high in store than field (Rondon, 2010). The typical damages from the pest are caused by the larvae and these include; mining of leaves, stems, petioles, and tuber tunneling. When foliage is available,

larvae reaches the stem via the leaves. Brown blistering in leaflets of potato are symptoms of larvae mining. Sometimes these larvae web and mine adjacent leaves. New larvae often mine at growing points of terminal shoots or in the axils of leaves, and, as they develop, tunnel into the stem for up to 5 centimeter (Hamilton, 2003).

The biology of PTM has been studied since 1932, a lot has been known about the nature of the insect, interaction with its hosts and the life cycle ever since. Like other holometabolous insects PTM has four life stages egg, larva, pupa and adult. The egg of PTM is sphere-shaped with a size less than 0.1cm. It is semitransparent and yellowish to light brown in colour (Fig 2.2A). Even though, leaves are the most preferred for oviposition eggs can be laid on soil, plant debris or exposed tubers often singly in fields (Rondon, 2010). The underside of the leaves closer to the midrib is the typical site of oviposition under field condition. Often only a single egg is laid but at times in batch, 3 to 5. Under laboratory conditions eggs are laid on peeled potato skins, fissures, cracks (Rondon, 2010), in vials, gauze, petri dish, plastic bag, and plastic tray (Personal observation), singly or in a batch. While in potato stores at 7.2⁰C female lays egg on tubers mainly near the eye buds. Egg hatching depends on the temperature of the location where the pest is found. Different authors have reported different incubation period ranging 2.3 to 34 days. Attia and Mattar (1939) reported 36⁰C to be the critical temperature for the female to completely stop ovipositing (Rondon, 2010).

PTM has four larval instars. The larvae are light brown in colour with brown head and prothoracic shield. Maturation changes the colour to pink or greenish along with size, 0.94 cm (Fig 2.2B). The stadia of instars are highly influenced by the ambient temperature. The whole larval period could take 12 to 33 days and different authors report different larval period. The

last larval instar closer to pupation drops down to the soil and pupate in the tubers or soil (Rondon, 2010).

The pupa which is protected with fine sediment is around 0.84 cm in size with brown coloration (Fig 2.2C). In the field pupa can be found around tuber eyes (Rondon et al. 2007) and in the soil but sometimes one can find it on leaves. Age of an individual could be determined by eye pigmentation of the pupa. One to two days old pupa will have yellow eyes whereas three days old pupa will have early red colour. Four to six days old pupa will possess a colour ranging from mid red to black (Rondon, 2010). Similar to the egg and larva, pupal incubation period also depends on temperature. Different authors have identified different pupal period based on the temperature. Pupal period of six to nine, 13 to 33, 14 to 17 days were reported by Moregan and Crumb (1914), Graft (1917), and Van der Goot (1926), respectively (Rondon, 2010).

PTM adults are small in size, 0.94 cm, having a distance of 1.27 cm between the wingtips (Fig 2.2D). Forewings are used to identify between the sexes. The female has dark spots on the forewings which forms 'x' mark. In contrast, the male has 2 to 3 spots again in the forewings (Rondon and Xue, 2010) (Fig 2E). A very simple way to identify adults between the sexes is to see the tip of the abdomen; the female has pointed tip while the male has a blunt end with thick set of hairs (Rondon and Xue, 2010). Interestingly, the female can pass through soil cracks and slack soil looking for the tubers to oviposit (Rondon, 2010). Like other moths PTM are active at night, therefore oviposition is nocturnal. The adults do fly well, up to 10 km without stopping, at low wind speed (Rondon, 2010). Mating takes places a couple of hours after emergence (Rondon, 2010). The activity of adult moth is dependent on temperature and they do not usually survive in temperatures below 5°C. However, survival below freezing point has been reported

(Rondon, 2010). The number of generations of PTM per year is dependent on the availability of the host and location. Varying number of generation per year has been reported with the highest in India and in Iraq with 12-13 generations (Rondon, 2010).

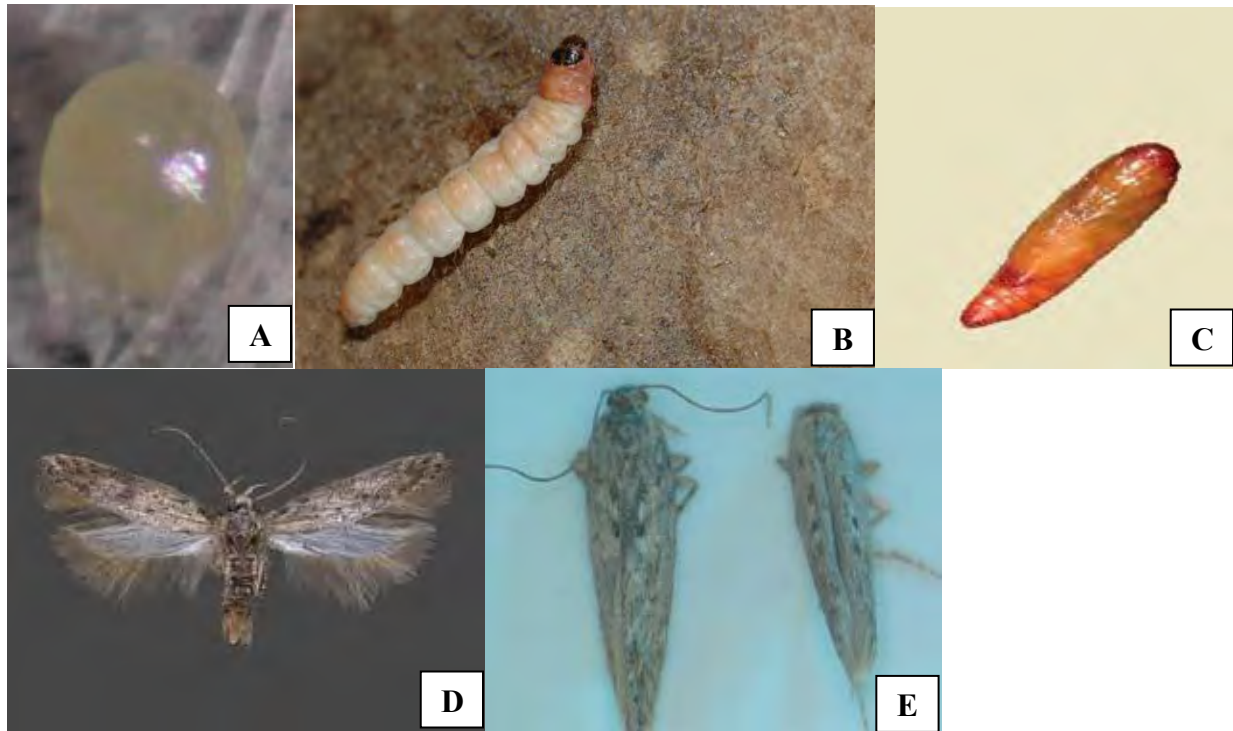


Figure 2.2 PTM (A= Egg, B= Larva, C= Pupa, D= Adult E= Adult, Female (left) and male (right) (Images from Rondon et al., 2007 and Rondon, 2010).

2.2.1.2. Parasitism on PTM and other control methods

Entomophagous arthropods may be predatory or parasitic. Predators may feed on several or all stages (egg to adult) of their prey and each predator usually consumes several individual prey organisms during its life (Gullan and Craston, 2005).

The solitary larval–pupal parasitoid, *Diadegma mollipla* (Holmgren), develops on three instars (second–fourth) of one of its hosts, the diamondback moth, *Plutella xylostella* (L.) (Nofemela and Kfir, 2008). *Copidosoma koehleri* (Blanchard) and *Apanteles subandinus* (Blanchard) are believed to be excellent parasitoids of PTM worldwide along with *Trichogramma* (Whiteside, 1985). Earlier study has shown parasitism to be highly efficient on potato leaves, 65%, than tubers, 32%. Larvae on exposed leaves are more accessible than those in buried tubers (Briese, 1981). In Ethiopia there are two identified parasitoids, which are known so far to be effective against PTM: *Diadegma mollipla* and *Chelonus spp.* (Negasi et al., 1985).

PTM control has to involve both field and storage control. Even though, the most important economic damage is attributed to infested tubers in stores, control should also consider for field infestation since these can be the sources of infestation for storage damage. Various control strategies are available to control PTM and avoid economic damage. The desired management can be achieved with integrating the best possible control methods.

2.2.1.2.1. Cultural methods

The cultural methods reported to reduce PTM damage include use of infestation free seed tubers, elimination of cull piles and volunteers, tubers left in the field, and covering hills. Keeping the soil moist after vine kill could also prevent oviposition by eliminating soil cracks which allows the adult to reach the tubers and oviposites easily. The time between vine kill and harvesting must be short (Rondon et al. 2007). Population monitoring using pheromone traps could also be used to decide on insecticide applications. Furthermore, they are used for mass trapping and mating disruption of PTM (Herman et al., 2005).

2.2.1.2.2. Microbial methods

Virus: PTM granulovirus (PoGV)

Many viruses infect and kill insects, however, those with potential for insect control are very few, nuclear polyhedrosis viruses (NPVs), granulosis viruses (GVs) and entomopox viruses (EPVs) (Bravo and Soberon, 2010). PTM granulovirus (PoGV) moved together with PTM from its center of origin, South America and has been identified in different parts of the world: Africa, Asia, Australia, Middle East, North America and South America (Lacey and Kroschel, 2009 and Jukes et al., 2014). The alkali nature of the midgut of PTM larva dissolves the proteinaceous coat or granulin of the ingested PoGV which causes the release of the nucleocapsids (enveloped virion). The released nucleocapsids attaches to microvilli of the midgut epithelium and then they colonize various host cells and produce huge number of occlusion bodies (OBs) or granule. The most important parts for OBs production are fat cells. Eventually, the deceased larva becomes an inoculum source for further infection (Lacey and Kroschel, 2009).

PoGV can be produced *in vitro* by a couple of methods and use to infect neonates or eggs through contact with tubers or dipping in PoGV suspension, respectively. Mostly infected larva died within 2-3 weeks, however, duration could be reduced with increasing dose. Thus, mortality is dependent on the concentration of PoGV used. PoGV has been tried for both field and non-refrigerated stores use (Lacey and Kroschel, 2009 and Lacey et al., 2011a). Kroschel et al., (1996) showed that field application of PoGV at a rate of 5×10^{13} OBs ha⁻¹ in 500 L of water had a marked (70%) PTM larval mortality. However, its use is limited due to its vulnerability to UV-radiation. A laboratory study conducted using lignin has shown the inactivation of PoGV

reduced by mixing PoGV with lignin. They have further reported that lignin alone has no effect on the target pest survival (Bhattarai et al., 2015). PoGV has also been shown to be used in combination with neem-oil based products. The combination caused high larval mortality (86.7%) applied at a rate of 4 mg of azadirachtin L⁻¹ and 10⁴ OBs mL⁻¹ (Mascarin and Delalibera, 2012).

Bacteria and *Bt* transgenics

Bacteria rarely cause disease in insects, although saprophytic bacteria, which mask the real cause of death, frequently invade dead insects. Relatively few bacteria are used for pest control, but several have been proven to be useful entomopathogens against particular pests (Lacey et al., 2001). The only bacterium evaluated to control PTM has been *Bacillus thuringiensis* (*Bt*) (Lacey et al., 2001). *Bt* toxins based biopesticides are widely used against a vast range of pests and they are commercially available. The parasporal crystalline inclusions of *Bt*, which are produced during sporulation, contain toxins made of protein. These proteinaceous toxins bring about death to the insect through lysis of midgut epithelial cells. The efficacy of *Bt* toxins depends on the target insect spp, the stage of the insects and the quantity of *Bt* gulped. *Bt* is used both under field and storage conditions. However, repeated application is required in fields since *Bt* is degradable by sun light and can be simply washed away by rain (Bravo et al., 2007; Lacey and Kroschel, 2009; Sanahuja et al., 2011).

A laboratory bioassay of PTM neonates on leaves and micro-tubers of *Bt* transgenic potato line has shown 18-56% larval mortality in the *Bt* transgenic potato line. In the non-transformed control leaves only 12% neonates' mortality was recorded. No tuber damage has occurred in the

Bt transformed potato line, whereas, non-transformed tubers showed extensive damage (Kumar et al., 2010). Similarly, a laboratory leaf and tuber bioassay conducted in two *Bt* transformed potato lines, Spunta and ND5873-15, revealed a larval mortality of 22-58% on the transgenic Spunta line, whereas in the non-transgenic Spunta line only a 3% mortality was observed. Also in the transformed ND5873-15 larval mortality was higher than the non-transformed potato, 99% and 22%, respectively (Estrada et al., 2007).

Nematodes

Mermithidae, *Heterorhabditidae*, and *Steinernematidae* are the families of nematodes which could potentially be useful for insect pest control (Lacey and Kroschel, 2009). Entomopathogenic nematodes (EPNs) are usually applied inundatively at their infective stages (Lacey and Kroschel, 2009). The symbiotic bacterium which lives in association with the nematode is the agent which kills and digests tissues of the target insect. Infective juvenile (IJ) nematode releases the bacteria after entering the pest and continues its reproductive cycle. After the depletion of host nutrients IJs will be produced to infect another host. PTM has been shown to be highly susceptible to *Heterorhabditis* sp. isolates (Lacey and Kroschel, 2009). A number of factors determine the susceptibility of PTM to EPNs infection such as the developmental stage of insect, the age of the host insect within a given stage (pre-pupa was the most susceptible stage), soil type, EPN species/strain and concentration as well as foraging strategy of the EPN (Hassani-Kakhki et al., 2013). Furthermore environmental factors are also critical in determining efficacy of EPNs application. For instance, the nematodes are highly sensitive to desiccation and ultraviolet light thus application must take these factors into consideration (Lacey et al., 2015).

Hassani-Kakhki et al., (2013) studied the efficacy of different strains, *H. bacteriophora* FUM 7, *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* FUM 2 strains of EPN against PTM under different soil types (loamy, sandy loamy and sandy soil) and stage L2 larvae (5 days old), L4 larvae (12 days old) and prepupa (1 day old), under laboratory condition. Their result has shown that *H. bacteriophora* and *S. carpocapsae* had the lowest LC50 of 61-99 and 71-95 IJs per early larvae. Furthermore, *S. carpocapsae* and *H. bacteriophora* were more lethal on late larvae with LC50 of 48-78 IJs per late larvae. The pathogenicity of EPN was even better in sandy soil.

Fungi

Fungi are the most common disease causing organisms in insects (Lacey et al., 2015). Even though, there are approximately 750 species known to infect arthropods only a few infect agriculturally important insects (Bravo and Soberon, 2010). In the infection process fungal spores that contact and adhere to an insect cuticle germinate and send out hyphae. The hyphae penetrate the cuticle, invade the hemocoel and cause death. Fungal death of insects is in either of the two ways: by the rapid release of toxins or more slowly due to massive hyphal proliferation that disrupts the insect body functions. Fungal disease may spread through the insect population by releasing spores (Bravo and Soberon, 2010). For entomopathogenic fungi infect their hosts through the cuticle they are known to infect both soft and hard bodied insect pests. They are also favored for their minimal effects to not targeted organisms (Lacey et al., 2015). The fungi *Muscodor albus*, *Beauveria bassiana* and *Metarhizium anisopliae* were shown to be effective against PTM (Lacey et al., 2011b and Zeleke et al., 2015). They affect the pest in different ways like reduction in percentage moth emergence, malformation of emerged adult after treatment of the prepupae, low life span of emerged adults and less egg production by the female (Hafez et

al., 1994). Hafez et al., (1994) showed that PTM larvae are susceptible to the pathogen *Beauveria bassiana*. However their efficacy is dependent on the concentration of the pathogen used and the developmental stage of the larva. Their results revealed that the first instar larva was more susceptible than the other instars with and LC50 value of 1.98×10^8 conidia mL⁻¹. In contrast, the 3rd and 4th instars had LC50 values of 4.08×10^8 and 4.7×10^8 , respectively.

2.2.2. Tomato leafminer

2.2.2.1. Distribution and bio-ecology and morphology of tomato leafminer

Tuta absoluta (Meyrick), commonly called tomato leafminer, is a devastating pest of tomatoes. Up to 100% losses have been reported in tomato crops. Even in a condition where control measures have been taken, losses could still exceed 5% (Korycinska and Moran, 2009). Although the primary host is tomato, other cultivated Solanaceous spp such as eggplant, potato, pepper and tobacco could be attacked (Desneux et al., 2010). The pest is native to South America, but now it has invaded and spread through the Mediterranean region, the Middle East, North Africa, and some parts of East Africa since 2006 (Fig 2.3) (Hayden and Brambila, 2013). It has been causing severe damage in Ethiopian tomato system since 2011 (Gashawbeza Ayalew and Abiy Fekadu, 2013).

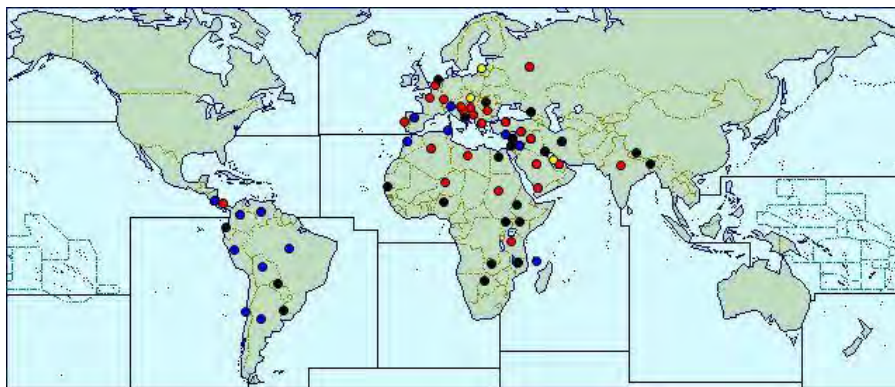


Figure 2.3 *Tuta absoluta* distributions in the world (Cabi, 2017), Black= present no further detail, Blue=wide spread, Red= localized, Yellow= few reports

Tuta absoluta has a high reproductive potential with a lifecycle that could be completed within 29-38 days depending on temperature. The number of generations per year is also reliant on temperature. Like PTM, *T. absoluta* has four larval instars. Female lays up to 260 eggs in its lifetime (Korycinska and Moran, 2009). The egg of *T. absoluta* is very small and cylindrical in shape with a colouration of creamy white or yellow (Molet and Jackson. 2011) (Fig 2.4A). The underside of the host plant is favored for oviposition, buds and calyxes of green fruits are similarly suitable for oviposition (Hayden and Brambila, 2013). Egg incubation period ranges 4-5 days.

After hatching, young larva penetrates into tomato fruit or leaves on which it feeds and develops. On leaves, larva feeds only on mesophyll leaving the epidermis intact. Instars are indeed different in colours and size. The last instar larva for instance is approximately 7.5 mm in size and greenish in colour (Fig 2.4B). The pupa is brown in colour, < 6 mm long (Fig. 4C), and the pupal period is 9-11 days. Adult moths are about 10 mm long with filiform antennae and brown or silver colour. It has black spots on the forewings (Fig 2.4D).

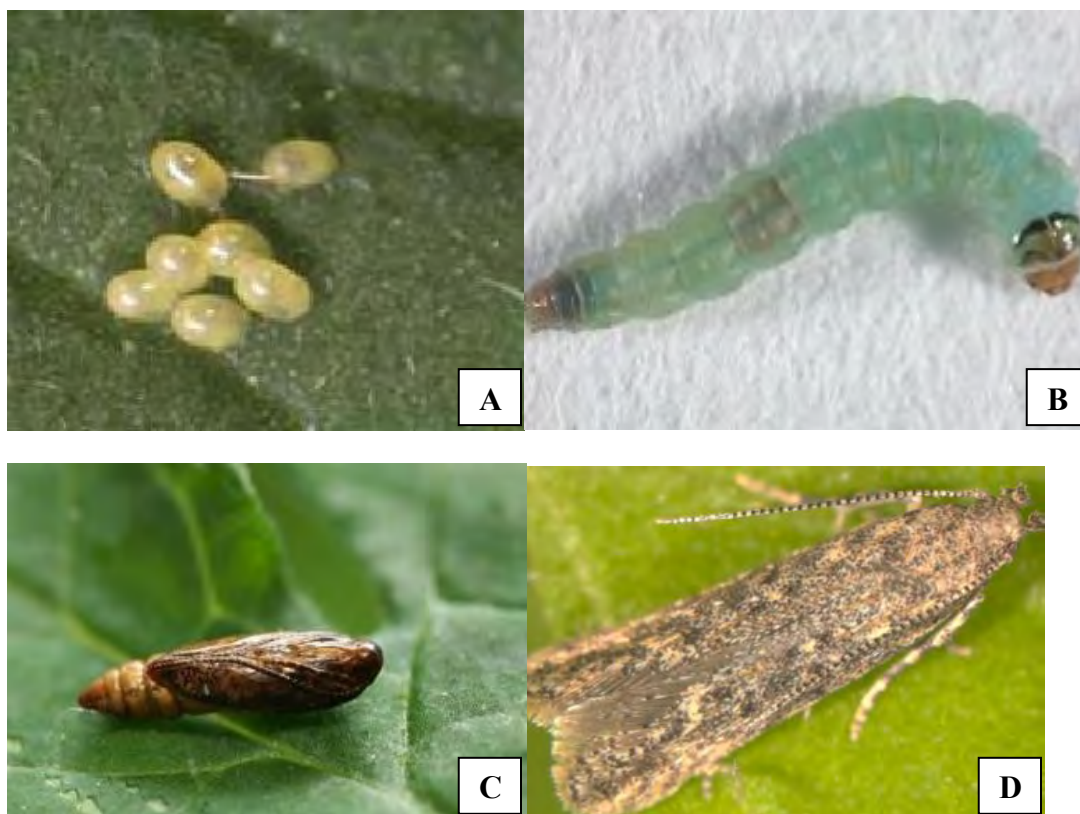


Figure 2.4 *Tuta absoluta* (A= egg, B= larva, C= pupa and D= adult) Source: www.coleacp.org/pip ,Korycinska and Moran, 2009; <http://www.tutaabsoluta.com> ; <http://www.uniprot.org/taxonomy/702717>,

2.2.2.2. *Tuta absoluta* control methods

Whenever *Tuta absoluta* is a threat for production of tomatoes, various control methods could be applied including cultural, microbial, biological and chemical to manage the pest.

Cultural practices

There are a number of cultural control practices which have been suggested for effective control of the pest in a greenhouse or field. Sanitation in nurseries, greenhouses, fields, and packaging and transportation facilities is one of the cultural control methods used to control the pest. In addition, the tools, machineries and equipment used in a greenhouse and field and packaging

materials and transporting vehicles should be well scrutinized and cleaned before they are taken to another place (USDA APHIS, 2011). These could help to limit pest dissemination to other places and become inoculum source. Furthermore, plants must be continuously examined for presence of eggs, larvae, frass and mines on leaves, stems and/or fruits and infested plants or plant parts should be removed (realIPM, undated). Elimination of nearby wild and other cultivated host plants other than tomato such as potato, pepper, and bean should be removed. This could reduce build-up of a potential population reservoir by interrupting the pest life cycle and spread (USDA APHIS, 2011 and EPPO, 2005). Other practices include crop rotation with non-solanaceous crops and sufficient irrigation and fertilization. Soil irrigation could disturb eggs, larvae and pupae, and increase mortality in field populations. Proper disposal of infested plant residues and post-harvest debris are also critical components of the cultural practices which would be inoculum source otherwise (Retta and Berhe, 2015 and Korycinska and Moran, 2009).

Pheromone

Pheromones, chemical signals released by an organism to attract a mate, traps are used extensively throughout Europe, South America, North Africa and the Middle East for the monitoring and mass-trapping of male adult *T. absoluta* (Kaoud, 2014). The pheromone traps are the best method to monitor the presence of *T. absoluta* and the data are indicators of levels of future infestations. Pheromone lures can be used in pan traps, Delta traps, McPhail traps, and bucket traps (USDA APHIS, 2011). Unlike monitoring, in mass-trapping the target is reducing the pest population by trapping as many adult males as possible to imbalance male vs female ratio. For mass trapping a total of 20-25 and 40-50 traps/ha should be installed in greenhouses and fields, respectively, in planned positions within a crop. Reliance on insecticide use and

damage due to the pest could be reduced by combining mass trapping with other measures. (<https://agriculture.gov.mt>). Trap height and position with respect to the vegetation also influence capture (Megido et al., 2013). Ferrara et al. (2001) showed that installing the trap at 60 cm as the best height for effective catch regardless of height of the plants.

Biological

There are many naturally occurring and commercially available biological control agents which act against *T. absoluta*. They are egg, larval, pupal, egg-larval and larval-pupal parasitoids. The egg parasitoids *Trichogramma pretiosum*, *T. achaeae*, *T. fasciatum*, *T. rojasi*, and *T. nerudai* in the order Hymenoptera have been recorded parasitizing *T. absoluta* in different parts of the world. *T. pretiosum* is a widely used generalist parasitoid (Ghoneim, 2014; Consoli et al., 1998). *T. achaeae* has worldwide distribution. A study has shown that *T. achaeae* caused 100% parasitism under laboratory conditions. When 30 *T. achaeae* per plant were released under greenhouse conditions in 3 to 4 days interval, 91% damage reduction was detected on tomatoes (USDA APHIS, 2011). The larval parasitoid *Pseudapanteles dignus* was found in South America parasitizing larvae irrespective of their developmental stages or instars. In late tomato crops up to 46% parasitism can be achieved (USDA APHIS, 2011). *Agathis* sp., *Apanteles gelechiidivoris*, *Apanteles* sp., *Bracon lucileae*, *Bracon* sp., *Earinus* sp., *Origilus* sp., *Dineulophus phtorimaeae*, *Neochrysocharis formosa*, *Cirrospilus* sp., *Horismenus* sp., *Temelucha* sp., and *Diadegma* sp. are other potential parasitoids from different families of the order hymenoptera (USDA APHIS, 2011; Urbaneja et al., 2012; Ghoneim, 2014). *Chelones* sp. and *Copidosoma* sp. are egg-larval parasitoids and *Campoplex haywardi* and *Conura* sp. are larval-pupal and pupal parasitoids, respectively. Likewise, the predators *Nabis pseudoferus*, *Macrolophus pygmaeus* and

Nesidiocoris tenuis feeds on *T. absoluta* eggs and larvae (USDA APHIS, 2011; www.tutaabsoluta.com, 2009; Luna et al., 2012; Ghoneim, 2014).

Microbial

Studies have found that a couple of microorganism like Nematodes, bacteria and fungi can be used effectively against *T. absoluta*. Some of them are also manufactured artificially in laboratories and sold as bio-pesticides (realIPM). *B. thuringiensis*, gram-positive endospore-forming entomopathogenic bacterium, has been found effective in controlling *T. absoluta*. The mode of action of *Bt* is through contact and ingestion of a lethal dose by the caterpillar. A number of studies have shown the efficacy of *Bt* to the different instars of *T. absoluta* (Youssef and Hassan, 2013; Hernandez-Fernandez et al., 2011; Gonzalez-Cabrera et al., 2011 and Urbaneja et al., 2012). Gonzalez-Cabrera et al., (2011) showed *Bt* to be effective against all the larval instars, however, first instars were found to be the most susceptible. Furthermore, a laboratory assay has shown *Bt* efficacy to be isolate and developmental stage dependent. They showed that out of the 12 tested isolates five had a significantly higher larval mortality ranging between 80 and 97%. *Bt* can also be used in synergism with other methods, for instance mass release of *T. pretiosum* combined with *Bt* brought 98% reduction in *T. absoluta* damage (Sabbour, 2013).

Different strains of *Beauveria bassiana* and *Metarhizium anisopliae* have been shown to control *Tuta absoluta* successfully (El Kichaoui et al., 2016 and Contreras et al., 2014). A study showed that *Beauveria bassiana* and *Metarhizium anisopliae* can cause 42-67 and 92-100% larval mortality of *Tuta*, respectively (realIPM). However, egg mortality due to *B. bassiana* was as low

as 12%, compared to *Metarhizium anisopliae*, 92% (realIPM). Contreras et al., (2014) found *M. anisopliae* to control *Tuta absoluta* pupa in addition to eggs and the different larval stages. *M. anisopliae* has also been reported to cause 37% adult female mortality (www.tutabsoluta.com). *B. bassiana* has been shown to be more effective when used in combination with *Bt* (Urbaneja et al., 2012).

Entomopathogenic nematodes in the family Steinernematidae and Heterorhabditidae are important control agents of a number of insect pests with the help of mutualistic bacterial which live within them in obligate parasitism (Lacey and Georgis, 2012). *Steinernema carpocapsae*, *S. affine*, *S. feltiae* and *Heterorhabditis bacteriophora* have been shown to be effective in *Tuta absoluta* management. However, the susceptibility of the insects varies according to the species of EPNs used (Gozel and Kasap, 2015). In comparison efficacy of nematodes is very high in larvae than pupae (Urbaneja et al., 2012). Sabino et al. (2014) showed that EPNs such as *S. carpocapsae* and *H. amazonensis* can be used in synergism with insecticides which are frequently used on tomato crops.

Chemical

Chemical control has been the main control measure used for *Tuta absoluta*. Currently at least 12 classes of insecticides are in use against *Tuta absoluta* in different parts of the world depending on the efficacy of the chemicals and pest resistance. Due to failure of the insecticides in use new alternatives has been released since 1970's (IRAC, 2009). Flubendiamide, thiamethoxam, chlorantraniliprole, chlorfenapyr, abamectin, spinosad and indoxacarb are some of the insecticides in use (USDA APHIS, 2011). Resistance to abamectin and deltamethrin has been

reported in Argentina, while deltamethrin has been effective in Spain (www.tutaabsoluta.com). The reason for insecticide resistance is their non-judicious application. Resistance could be managed by rotation of compounds with varying mode of action (USDA APHIS, 2011).

2.2.3. Origin, geographic distribution and host range of late blight (*Phytophthora infestans*)

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of late blight, is one of the most destructive pathogen affecting both potato and tomato. The horrors of the incident it caused, the Irish potato famine, are still remembered for it caused more than a million people to starve to death or migrate. This was the starting point for the current advancement in research related to late blight control. Worldwide losses caused by late blight have been estimated at billions of U.S. dollars each year (Sedláková et al., 2011). The genus *phytophthora* is not a true fungus and belongs to the oomycetes (water molds) in the kingdom Chromista, phylum oomycota and order *Peronosporales* (Birch and Whisson, 2001). The oomycetes are characterized by producing sporangia. Zoospores within the sporangia have two unequal flagella and often have a single nucleus. Late blight causes serious economic losses both in the open field and non-heated greenhouses especially under favorable conditions, such as wet and cool temperatures. The responsible pathogen is heterothallic and forms oospores with A1 and A2 mating types, which have been found in different parts of the world, in Ethiopia the mating type is believed to be A1 (Gotoh et al., 2005 and Haverkort, et al., 2012). Schiessendoppler and Molnar (2002) found no US-1 isolates in Ethiopia, but rather a population characterized by the mitochondrial DNA Ia haplotype. Genotypes similar to some variants of KE-1 have been found in Ethiopia (Njoroge et al., 2015), who showed that US-1 clonal lineage has been virtually

replaced by KE-1 in Kenya and Uganda. For sexual reproduction to take place both A1 and A2 mating types should be found together in same place at the same time. The genetic diversity, rapid evolution and a broad range of virulence factors as an effect of dynamic and expanded regions in the genome make this pathogen rapidly adaptable and to quickly overcome host resistance (Haas et al., 2009).

Regarding the origin of *P. infestans* there are two views, most researchers in the area supports the idea that late blight was originated in central Mexico highlands (Grünwald et al., 2001; Yoshida et al., 2013) because of the presence of both mating types (A1 and A2) and high genetic diversity (Goodwin and Drenth, 1997; Grünwald et al., 2001; Andrivon, 1996). Since the Andes is centre of origin for the cultivated potato, it is argued that *P. infestans* could also be originated in Andes (Abad and Abad, 1997 and Gomez-Alpizar et al., 2007). The first reported global migration of *P. infestans*, A1, occurred in 1843 to Philadelphia and New York (Ristaino and Hu, 2009, Fry et al., 1993). In two years' time it was identified throughout the maritime provinces of Canada and north eastern United States and then it migrated to Belgium, Ireland, and Germany and the rest of Europe. There is also another hypothesis that it could have been transported directly from Mexico to Europe. The migration to the rest of the world could have been through trade. Growers in South America, Africa, Asia and Middle East could have planted *P. infestans* infected tubers.

Prior to 1970s, global *P. infestans* population outside of South America and Mexico were dominated by a single clonal lineage that had mitochondrial (mtDNA) haplotype Ib and was called US-1 (Goodwin et al., 1994). The second potential migration including both mating types, A1 and A2, is believed to occur in 1970s through infected potato tubers (Goodwin et al., 1994 and

Fry et al., 1993). These days, the population of sexually reproducing *P. infestans* is highly diversified in Europe (Drenth, 1994 and Andersson et al., 1998). Late blight is now distributed in 131 countries worldwide (Fig 2.5).

In Ethiopia potato is attacked by pathogens causing a significant yield loss such as early blight (*Alternaria solani*), bacterial wilt (*Ralstonia solanacearum*), virus, and late blight (*P. infestans*). Late blight is one of the major diseases causing agents. The disease occurs throughout the major potato producing areas and it is difficult to produce the crop during the main rainy season without chemical fungicides (Bekele and Geberemedhin, 2000). The main rainy season is quite favorable for the growth and development of the disease. Consequently, due to high late blight infection in the main season there has been a production shift in time from the main to the off season (Haverkort et al., 2012). The estimated potato yield losses reported due to late blight are 3-70% (Bekele and Yaynu, 1996), 22–46% (Girma et al., 2013) and 29-57% (Tsedaley et al., 2014b) however complete crop failures are frequently reported (Bekele and Yaynu, 1996). The second most affected crop by late blight is tomato. Due to the high susceptibility of tomato cultivars found in the country farmers produce tomato mainly in the short rain season, February to April, when disease pressure is low compared to the main season (Ethiopia late blight profile, 2004).

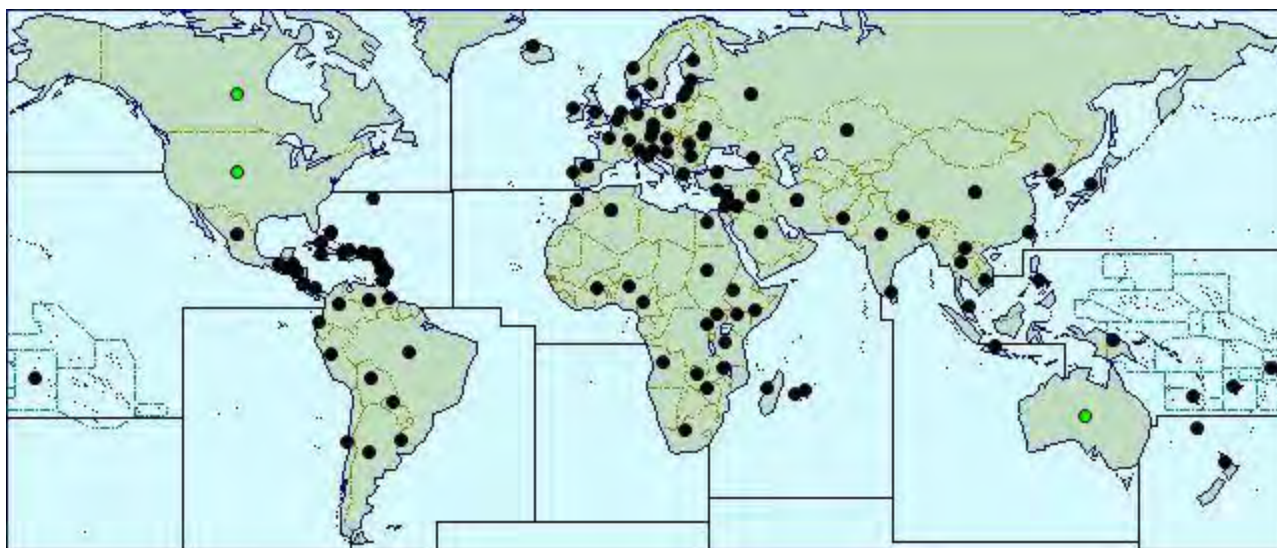


Figure 2.5 Distribution of late blight in the world (Cabi, 2016), Black= present, no further detail, Green= see regional map for distribution within the country

P. infestans are known to infect the economically important tuberous crop potato and of the non-tuber crop tomato is economically important. Other domesticated solanum species such as pear melon (*Solanum muricatum*) and tree tomato (*Solanum betaceum*) are also hosts of *P. infestans* (Oliva et al., 2002). Even though, little evidence is available, eggplant has been reported to be an occasional host (Hooker, 1981). Wild species related to potato and tomato is also part of the host range of *P. infestans*. Plants like nightshades, *S. dulcamara*, *S. sarrachoides* (Vartanian and Endo, 1985), *S. nigrum*, and *S. sarrachodes* have been reported to be infected (Flier et al., 2003b). In Africa a number of species has been identified as hosts of *P. infestans* including *S. indicum*, *S. incanum*, *S. aculosturm* and garden huckleberry (*S. scabrum*) (Nattrass and Ryan 1951). Infection out of the solanum group like *Nolana* species has also been reported (Abad et al., 1995). Weeds like *Datura* species are also other alternative hosts (Sunita and Sen, 1997).

2.2.4. Infection process of *P. infestans* and late blight damage symptoms on potato and tomato crops

The symptoms of late blight first appear as pale-green lesions often at the tip and margin of leaves. The lesion starts to develop rapidly into bigger brown lesion (Fig 2.6A). The lower part of the leaf produces a white mildew particularly around the edge of the lesion, mainly in the morning and damp weather (Fig 2.6B) (Tsedaley, 2014). The white mildew consists of sporangia and spore which assists in spreading the disease to the neighbouring plants. The symptoms of late blight observed on stems are characterized by girdling. Part of the plant above the root mainly the leaves and the infected stem wilt. The symptoms which appear on the stem are brown in colour (Fig 2.6C) (Bohl et al., 2003).

Late blight could attack potato tubers both in storage facilities and fields. Infected tubers show different symptoms based on the stage of infection. The infection starts from the top of the tuber and extends deep into the internal tissue. Infected tubers produce tissue browning (Fig 2.6D). Late blight infected tubers are also vulnerable for secondary infection mainly bacterial. Infected tubers have pungent smell (Douglas, 2012; Secor et al., 2011, and Bohl et al., 2003).

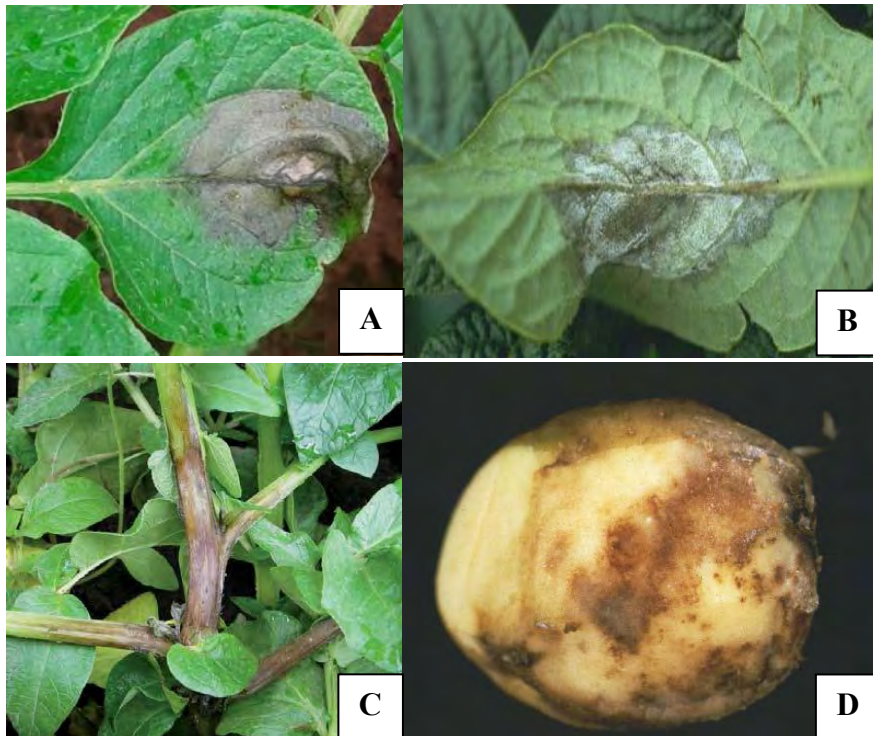


Figure 2.6 A= Late blight on potato leaves B= Sporulation on potato leaves C= *P. infestans* on potato stem D= *P. infestans* on potato tuber Source: Secor et al., 2011

The symptoms in tomato are almost the same with those in potato. The onset of the disease is characterized by the presence of pale green lesions on the leaves which shortly enlarge and turn in to brown-black under favourable conditions (Fig 2.7A). A lesion under sporulation produces a white-grey appearance (Fig 2.7B). The black or brown spore producing lesions are also seen in stems (Fig 2.7C). The symptoms on fruits are golden to chocolate brown in colour and sporulation is possible on the fruits as well (Fig 2.7D) (Nelson, 2008 and Secor et al., 2011).



Figure 2.7 A= Late blight on tomato leaves B= Sporulation on tomato leaves C= *P. infestans* on tomato stem D= *P. infestans* on tomato fruit Source: Douglas, 2012

P. infestans has various source of infection in different situations, time and space. In temperate zone for example the main sources are refuse piles and infected seed (Zwankhuizen et al., 1998; Marshall and Stevenson, 1996). Long distance dispersion of late blight is caused mainly by infected seed tubers. Potato production in subtropical and highland tropical areas is all around the year, thus the disease is always there. Differently, in tropics the disease occurs with rain (Forbes et al., undated). In Ethiopia for example, the disease is apparent in the main rainy season (Haverkort et al., 2012).

P. infestans reproduces largely through asexual reproduction. They can also reproduce sexually but that is rare in nature. *P. infestans* grows and sporulates well under very humid condition. Zoospore movement and sporangia germination need wet leaves or highly humid air (Harrison, 1992).

The main steps in the infection process of *P. infestans* include sporangial germination, zoospore movement, encystment, cyst germination, germ tube development, appresorium formation and penetration. Each step is characterized by a particular process. For example, penetration involves physical pressure and enzymatic activity to facilitate the process of penetration (Harrison, 1992). On the other hand, germination occurs directly through the germ tube or indirectly by freely moving zoospores. Leaf or tissue penetration by germ tubes occurs indirectly through appresorium and subsequently an infection peg. After a successful penetration of a tissue by the pathogen infection vessel is formed, specialized hyphal structure, and hyphal extension and colonization will continue within the cell. The hyphae inside the cell will form haustoria which involves in cell penetration for nutrient absorption (Hohl and Suter, 1976). In course of time via stomatal openings sporangiophores grow out (Agrios, 2005). The lesions become visible within a couple of days.

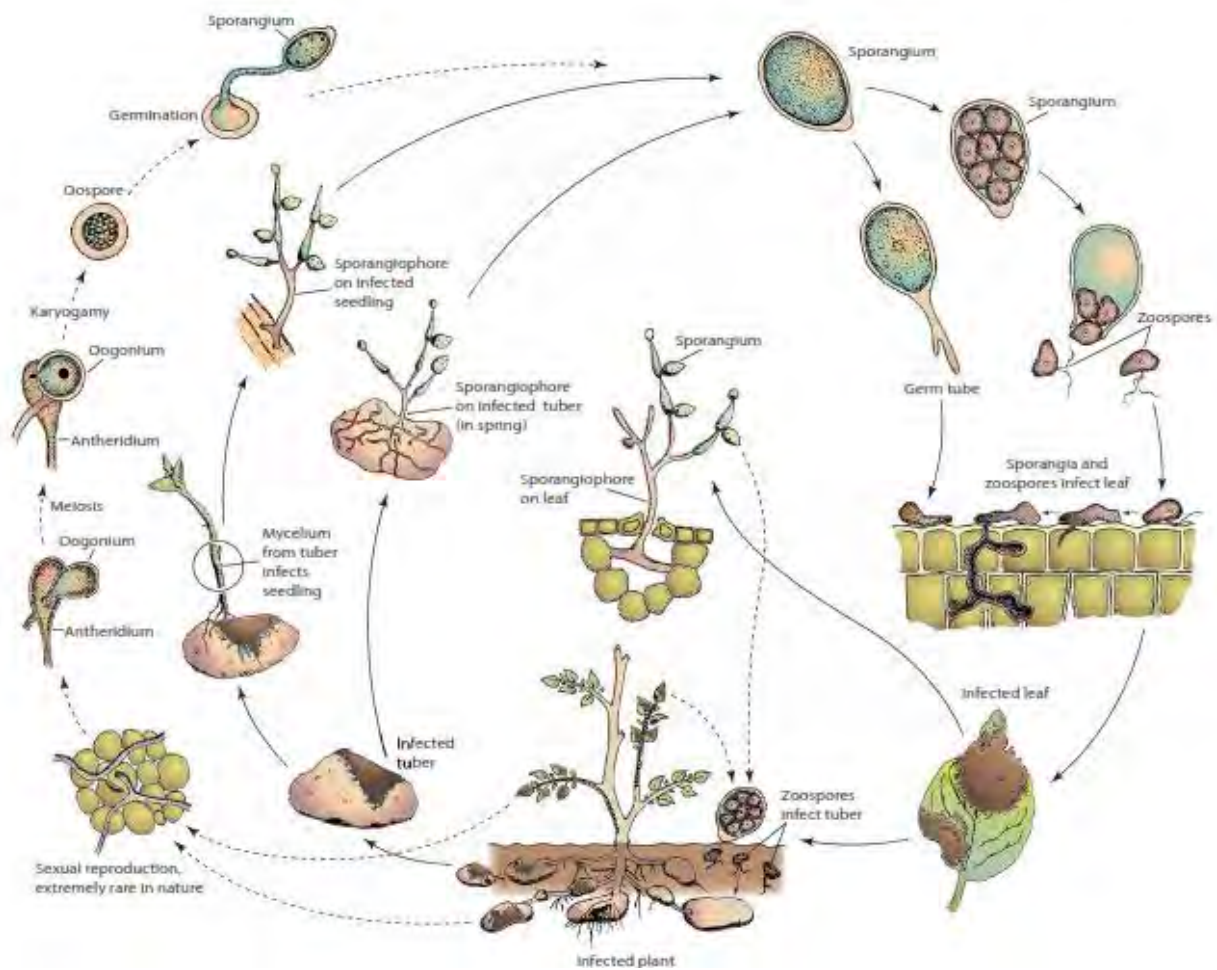


Figure 2.8 Life cycle of *P. infestans* causing late blight Source: Agrios, 2005

The temperature is also an important factor for infection, even though the exact effects are dependent on the genotype (Mizubuti et al., 2000) 15-25°C is known to be the optimal for infection. Studies have also shown its potential to reproduce at a temperature of 30°C high. The temperature above 30°C is not appropriate for reproduction but survival might be possible but not in all phases (Agrios, 2005). Interestingly sporangia survival inside a plant tissue at a temperature of 40°C has been witnessed (Kable and Mackenzie, 1980). Drying causes death of sporangia (Warren and Colhoun, 1975), however viability could be gained via rehydrating

(Minogue and Fry, 1981). Cloudy days are most suitable for late blight since high light intensity, UV radiation, can reduce sporangia viability by 95% within an hour (Mizubuti et al., 2000).

As mentioned above the infection is dependent on temperature, which also affects the time for lesion development. The genotype of the host and pathogenicity of the pathogen also determine the time before lesion development. In a susceptible host, symptoms can be seen in less than three days under favourable condition. Similarly sporulation can occur in a day or two after lesion development. Sporulation also needs appropriate conditions, 10-25°C temperature and 90-100% humidity. Again under favourable conditions sporangia are borne on sporangiophores. Sporangia can then be wind-dispersed in water drops; a single lesion can produce as much 100,000 sporangia (Agrios, 2005).

2.2.5. Management options of late blight on potato and tomato crops

2.2.5.1. Cultural practices and weather forecasting

Cultural late blight control is implemented throughout the growing season up to storage including the major before planting precautions (Secor et al., 2011). Controlling late blight disease starts by avoiding the sources of initial inoculum (Agrios, 2005). Therefore subsequent incidence could be reduced by using certified healthy potato seeds and tomato transplants (Secor et al., 2011). Getting rid of volunteer plants is another way to reduce disease occurrence in the next season. The presence of volunteers, contributed a lot for very early onset of infection and pathogen build-up which causes disease epidemic in the main potato crop. Alternate hosts are also other sources of infection, so removing them is necessary (Flier et al., 2003b). Areas with excessive soil moisture are favourable flashpoints for the disease. Planting in shady and wet areas is not recommendable but rather well drained and sunny areas are more preferred (Kirk et

al. 2013). Another important thing to consider while planting is the timing to avoid the major disease period. For example, in highland tropics, farmers plant before or after rain in order the plants to escape infection. Tuber blight incidence can be reduced by high hilling, which reduce the contact between tuber and washed foliage spores, and early removal of foliage. Storage facilities are the other important factors that contribute a lot for the future tuber blight after harvest. Tubers should be stored under dry conditions which guarantee tuber health (Bohl et al., 2003).

By forecasting the weather necessary fungicide applications can be predicted before the occurrence of disease and avoid excessive application of fungicide (Arora et al., 2014). After the development of the first model in 1940s a number of models have been developed until today. The models are used to make decision. Not all but some of the methods also take into account the cultivars, date of emergence and agro meteorological conditions. This system has been tried in developing nations even though efficient use has not been reported (Singh et al., 2013).

2.2.5.2. Chemical control

Effective chemicals use in late blight control dates back to the 1880s. The copper sulphate and lime based chemical, Bordeaux mixture, discovered in 1882 by the French Professor Pierre Millardet was used effectively against late blight (Fishel, 2013). After successive use for five decades it was replaced by copper oxychloride in 1930s, which was shown to be more effective. Then in 1940s the so called EBDCs (ethylenebisdithiocarbamates) were introduced to the commerce. Which include zineb, maneb, metiran, mancozeb, and propineb. Tin compounds like fentin acetate and fentin hydroxide are effective and durable fungicides but they are also toxic to

young plants. In contrast the fungicide chlorothalonil had low phytotoxicity. Even though, resistance development has reduced its use, metalaxyl has been the most effective systemic fungicide from the phenylamides group, which also include ofurace, oxadixil, and benalaxyl (Schwinn and Margot, 1991). These fungicides are characterized by possessing a curative effect; the pathogen can be killed inside the plant. Regarding tuber activity of chemicals, it is mostly by reducing sporulation, reducing viability of spores on leaves and when repeated applications cause residues to form in the soil that can inhibit formation or motility of zoospores. It is assumed that foliage protection results in tuber safety by reducing sporulation and spore viability in the leaves. In addition chemical residues in the soil inhibit formation and mobility of zoospores (Schepers and van Soesbergen, 1995). Global production cost including fungicides and losses due to late blight is estimated to be € 5.2 billion per annum (Haverkort et al., 2009).

In Ethiopia there are a number of fungicides available for late blight control. The most common are Mancozeb, Mancozeb + Metalaxyl, Ridomil Gold, Agro Laxyl, Mancolaxyl, Unizeb with active ingredients mancozeb and metalaxyl-M (Haverkort, et al., 2012).

2.2.5.3. Host-plant resistance

Resistance is considered to be one of the basic control strategies for many pathogens because of health related and other side-effects of fungicide residual. After the devastating potato famine in Ireland in the 1840s breeding for resistant cultivars was an important interest (Dowley, 1995). The use of resistant varieties is among the most effective and environmentally safe tools of late blight management (FAO, 2008). In the late 1800s, numerous cultivars with resistance to late blight were available (Umaerus et al., 1983). Host plant resistance has a long-term economic benefit for farmers and it also reduces the possibility of fungicides resistance (Tsedaley, 2014).

and Mukalazi et al. 2001). Host plant resistance reduces late blight incidence, delay the onset of disease, reduces rate of disease development and the number of sprays required (Agrios, 2005 and Tsedaley, 2014). It is important to consider the side effect of the discovery of efficient chemical fungicides in the growth of breeding for resistance against late blight in the early days. For instance the discovery of the fungicide, Bordeaux mixture, allowed viable cultivation of highly susceptible cultivars like Bintje and Russet Burbank in use (Robinson, 1996). Host plant resistance against late blight started with introduction of resistance gene (R-gene) which has been broken rapidly by virulent strains. The breeding efforts continued identifying R-genes even though resistance durability is an issue with resistance not lasting more than five to seven years. More than 11 late blight R-genes has been identified (denoted R1 to R11) from potato wild species (Nowicki et al., 2012).

Even though its achievement is not easy, durability of resistance is indispensable (Halterman, 2012; Malcolmson and Black, 1966; Flier et al., 2003a; Vleeshouwers et al., 2011; Nowicki et al., 2012). The problem with breeding against late blight is related to the ability of the pathogen to evolve quickly and overcome resistance. Indeed, different strains of *P. infestans* have been shown overcome all 11 R-genes in potato (Halterman, 2012; Nowicki et al., 2012; Thakur et al., 2016). Thus, researchers are looking for alternative resistance strategies more durable than R-genes which incorporate horizontal or polygenic resistance. Pyramiding resistance genes, allowing several resistance genes to be accumulated in the same genotype or cultivar, often results in stronger and more durable resistance, as has been observed in many plant species, including potato and tomato (Tan et al., 2010).

Potato cultivars resistance against foliage late blight is quite different with their resistance against tuber blight. Cultivars which are resistant to foliage blight might be susceptible for tuber blight (Flier *et al.*, 2003a; Nowicki *et al.*, 2012). For instance Lacey (1967) reported incidence of tuber blight at harvest in the absence of foliar blight. In other cases, low incidence of tuber blight has been reported after high levels of foliar blight (Nyankanga *et al.*, 2007). In breeding, this calls for multiple resistance which works against both tuber and foliar blight (<http://gilb.cip.cgiar.org/what-is-late-blight/economic-impact/social-and-economic-importance-of-late-blight>). In Ethiopia there are more than 29 cultivars released with the most commonly used one are Jalene, Gudane, and Belete. Belete is less susceptible to *P. infestans* (Haverkort *et al.*, 2012).

2.2.5.4. Induced resistance (IR) and inducers

Induced and constitutive resistances are mechanisms by which plants actively defend themselves from pathogens or chewing insects (Karban and Baldwin, 1997). Constitutive resistance is constantly on and already at work in the plant before the pathogen attacks (Sticher *et al.*, 1997). Induced resistance (IR) is a state of enhanced defensive capacity elicited by specific environmental stimuli, whereby the plant's innate defences are potentiated against subsequent challenges (van Loon *et al.*, 1998). IR is effective against various pathogens including viruses, bacteria, and fungi that attack solanaceous plants. However IR does not usually lead to full pathogen control (Vallad and Goodman, 2004 and Deliopoulos *et al.*, 2010). The scope of induced protection obtained using inducers ranges between 20-85% (Walters and Fountaine, 2009). In plants, IR can be triggered by subjecting the plants to virulent, avirulent, and nonpathogenic microbes or artificially by inducers like benzo (1,2,3) thiadiazole-7-carbothioic

acid S-methyl ester (BTH) commercially marketed as Bion®, Actigard® and Boost®, 2,6-dichloroisonicotinic acid (INA), chitosan (CHT), salicylic acid (SA), methyl salicylate (MeSA), ethylene, phosphite, probenazole, methyl jasmonate (MeJA) (Mandal, 2010, Ramamoorthy et al. 2001, Walters et al. 2005 and Alexandersson et al., 2016). Heller and Gessler (1986) were the first to discover the existence of induced resistance against late blight in tomato and Doke et al (1987) in potato.

Different authors have shown various chemicals to be effective in inducing resistance against late blight. Arachidonic acid, eicosapentaenoic acid, linoleic acid, linolenic acid and oleic acid were evident to induce resistance against late blight on potato leaves and 94%, 97%, 82%, 39% and 42% protection were obtained, respectively (Cohen et al., 1991). Foliar application of jasmonic acid (JA) and jasmonic methyl ester (JME) to potato plants (cv. Bintje or Alpha) and tomato (cv. Baby) protect against late blight (Cohen et al., 1993). Leaf infection was reduced by 86% with the application of salicylic acid, a plant hormone which involves systemic resistance and induction of pathogenesis-related (PR) proteins, at a rate of 2.5 mM through petiole of the leaf prior to late blight inoculation (Kombrink and Somssich, 1997; Ke-qiang and Forrer, 2001;). Field foliar application of BABA protected tomato and potato foliage from *P. infestans* without direct toxic effect on the pathogen (Cohen, 2002).

2.2.5.4.1. Phosphites, their importance in agriculture and synergism with fungicides

A number of studies have shown the efficiency of phosphite in inducing resistance against *P. infestans* (Andreu et al., 2006, Cooke and Little, 2002, Lobato et al., 2008, Burra et al., 2014 and Liljeroth et al., 2016). Phosphites are salt derivatives of phosphorous acid (H_3PO_3) (McDonald *et*

al., 2001). Alkali metal cations K^+ or Na^+ and the non-metallic anions such as phosphite (PO_3^{-3}), hydrogen phosphite (HPO_3^{-2}) or dihydrogen phosphite (H_2PO_3) are the two active substances in several phosphites fungicides and “fertilizers”. One can find these inorganic salts in different names viz, phosphonate, hydrogonophosphonates, orthophosphites, phosphonic acid, or phosphorous acid (Landschoot and Cook, 2005 and Deliopoulos et al., 2010). Phosphite moves systemically in basipetal and acropetal direction both in xylem and phloem, thus it is used for the control of both foliar and root fungal pathogens (Cohen and Coffey, 1986, Landschoot and Cook, 2005 and Borza et al., 2017). Phosphite is directly toxic to the target oomycetes, however, it is phytotoxic at high dose, 24 and 96 kg/ha, but the phytotoxic effect was not higher than 10% (Hardy et al., 2001; Barrett et al., 2003). In a recent work by Scott et al., (2016) foliar application of phosphite has been shown to cause minor phytotoxicity in conifers and woody angiosperms under all the concentrations tested.

Phosphite has been shown to effectively ward off various plant diseases and insect pests. For instance Phosphite reduces plant susceptibility to diseases caused by the oomycetes, *Phytophthora* spp. and *Plasmopara* (Gomez-Merino et al, 2015). However, phosphite efficiency depends on the application timing (Johnson et al., 2004); according to Landschoot and Cook (2005) phosphite is efficient if applied preventatively than curatively. Phosphite could be applied to the target plants in different ways, the application method depends on the crop-pathogen combination, however foliar application is the most common (Hardy et al., 2001 and Dorn et al., 2007). Other possible techniques includes fertigation, trunk spray, trunk injection, trunk paint, in-furrow, dip treatments, or root or soil drenches (Deliopoulos et al., 2010, Cooke and Little, 2002; Johnson et al., 2004 and Dorn et al., 2007).

Various field trials indicated the field efficacy of phosphite, for example Cooke and Little, (2002) and Liljeroth et al., (2016) showed the efficiency of phosphite in reducing tuber susceptibility to late blight in repeated field trials. In addition, foliar application suppressed downy mildew of maize, *Peronosclerospora sorghi* (Panicker and Gangadharan, 1999) and grape, *Plasmopara viticola*, however residues of phosphite have been detected in the wine produced (Speiser et al., 2000). Furthermore, phosphite was effective against dieback disease, *Phytophthora cinnamomi*, in feather leaved banksia and on brown rot, *P. citrophthora*, in citrus plants (Oren and Yogev, 2002). Results of field experiments undertaken over 3-years period at various locations in the USA showed that foliar sprays of phosphite reduced tuber rot caused by *P. infestans* in different potato cultivars, which was dependent on the frequency of application. Twice and trice application reduced tuber rot incidence and severity by 67% and 84% and 88% and 91%, respectively (Johnson et al., 2004).

In potato, in comparison, a single phosphite application, 4 kg ha⁻¹ reduced tuber brown rot by around 15% than 2 or 4 times application of 2 kg ha⁻¹ or 1 kg ha⁻¹, respectively (Cooke and Little, 2002). Mostly early treatment is preferred, for instance, in potato where treatment was started 4 weeks after initial tuber bulking provided less protection, 34% (severity) and 38% (incidence), than when it was started two weeks after initial tuber bulking, 41% (severity) and 67% (incidence) (Johnson et al., 2004). In tomatoes, 74 percent control of *P. infestans* was obtained by phosphite application in young tomato plants (Dorn et al., 2007). Pre-plant amendment or post-plant drench of soil substrates with phosphite effectively suppressed damping-off of cucumber seedlings in controlled environment, greenhouse and field. Higher

rates provide almost 100% damping off control whereas lower rates have on average 34–75% healthy plants compared with less than 5% in the control (Abbasi and Lazarovits, 2005). In addition to the foliar protection, phosphite applied to potato seed tubers by spraying at a dose of 3L ha⁻¹ effectively reduced late blight development when the inoculation was made at 0, 1, 2, 4 and 6 hours after phosphite treatment (Miller et al., 2006). Phosphite also effectively managed post-harvest brown and pink rot on potato tubers caused by *P. infestans* (Miller et al., 2006 and Lobato et al., 2008). It is also used against Pythium, downy mildew, fire blight (*Erwinia amylovora*) and potato scab (*Streptomyces scabies*) (Percival and Banks, 2015).

Synergism is defined as the simultaneous action of two or more compounds in which the total response of an organism to the pesticide combination is greater than the sum of the individual components (Evenhuis et al., 1996). Studies on the joint action of pesticides in agriculture started in 1930's for the control of insect pests by combining a less toxic compound with a toxic one. Various contact fungicides have been combined for disease control. Fungicides are combined for a couple of reasons: Firstly to increase the spectrum of antifungal activity in controlling various diseases occurring in a crop at a given time. Secondly, to reduce the amount of fungicides use without losing efficacy. The last is to delay the selection process of resistant pathogen population to one of the component in the mixture (Gisi, 1996). Companies have been selling a prepackaged mixture of fungicides, for instance the systemic fungicide metalaxyl has been sold mixed with contact fungicide for foliar late blight management (Samoucha and Cohen, 1986). A number studies have also shown the potential of combining fungicides in controlling various oomycetes caused plant disease (Baider and Cohen 2003; Liljeroth, Bengtsson et al. 2010).

In addition to sole application, phosphite can be used with other conventional chemicals in synergism. The review by Deliopoulos et al., 2010 reported that synergism between phosphite and other conventional pesticide like Mancozeb in different ratio can provide effective control. Mixed application of 1.275 kg ha⁻¹ of Mancozeb with 2 kg ha⁻¹ of phosphite provided 95% efficacy over Mancozeb alone (Deliopoulos et al., 2010). Combining spray programs between phosphite and fungicide reduced the dose of systemic or non-systemic fungicide needed to suppress foliar and tuber late blight infection and overwinter survival of *P. infestans* in potato tubers (Cooke and Little, 2002 and Liljeroth et al., 2016).

2.2.5.4.2. Phosphite's mode of action

Phosphite has a complex mode of action, at least against oomycetes, and has also been a topic of controversy among researchers for a long time. It includes direct (inhibition of fungal sporulation or causes slow development rate) and indirect (rapid and strong stimulation of plant defense mechanisms) (Smillie et al., 1989; Grant et al., 1990; Guest and Bompeix, 1990; Guest and Grant, 1991; Jackson et al., 2000; Hardy et al., 2001; Brunings et al., 2005; Burra et al., 2014). A molecular study has shown that the indirect responses of phosphite has been observed by the change in the plant transcriptome and secretome proteins, where this change has caused change in the defense response which includes changes in metabolism, oxidative stress and cell wall processes (Burra et al., 2014). Lobato et al., (2008) has shown phosphite under different concentration to inhibit sporangial germination and mycelial growth of *P. infestans in vitro*. The inhibition of sporangial germination and mycelial growth is attributed to interference of Phosphite with key reactions by competing with phosphate for the enzyme catalytic sites, leading

to altered abundances of metabolites such as ATP, nicotinamide adenine dinucleotide (NAD), polyphosphates, and pyrophosphate (Griffith et al., 1990 and Niere et al., 1994).

Phosphite treated plants have shown a quick increase in the production of phytoalexin, an antimicrobial plant substance (Smillie et al., 1989). Leaf lignification, increased cell wall thickness, synthesis of secondary plant metabolites (e.g. beta-1,3-glucanases) and increased enzyme activity of peroxidases are other phosphite related indirect plant responses (Percival et al., 2009). Phosphite application can also alter the metabolic activity of the plant and induces the production of pathogenesis-related proteins (Van Loon, 1997). Many pathogenesis-related proteins have direct antimicrobial activity or are closely related to classes of antimicrobial proteins such as β -1,3-glucanases (Anfoka and Buchenauer, 1997). In addition Eshraghi et al., (2011) showed that phosphite stimulates Arabidopsis for a rapid and intense response to infection by *Phytophthora cinnamomi*, involving heightened activation of a range of pathways that generate the defense related plant hormones SA, JA and ET.

2.3. Genetic diversity and its importance in an agroecosystems

Improved crop varieties, use of chemicals both fertilizers and pesticides, mechanization, urbanization and monoculture have led to agricultural simplification and biodiversity loss (Boudreau, 2013). Biodiversity reduction could cause ecosystem disservice in the agricultural ecosystem (von Döhren and Haase, 2015). Therefore, to achieve sustainable agriculture reestablishing on-farm biodiversity could be a vital strategy (Ekram et al., 2010). Proper spatiotemporal implementation of on-farm biodiversity could create an agroecosystems which per se prevent/reduce pest attack, maintain soil fertility and guarantee sustainable productivity (Daud et al., 2014 and Mishra, 2014). Methods like rotation, intercropping and agroforestry can

be used to improve the biodiversity in agroecosystems (Cook et al., 2007 and Mousavi and Eskandari, 2011).

Intercropping, also called polyculture or mixed cropping, is a practice of cultivating two or more crops at same space and time (Andrews and Kassam, 1976; Ouma and Jeruto, 2010). However, the different components of intercropping do not have to be compatible throughout the growing season, but they should be grown simultaneously for a great part of their growth periods (Mousavi and Eskandari, 2011; Markovi, 2013). In intercropping there is one main crop and one or more companion crop with less importance than the main crop. Usually in an intercrop system, plants from different groups, family or species, are used. However, sometimes two different cultivars of the same species can be used (Lithourgidis et al., 2011). Intercropping could also be between annuals, perennials, or a mixture of the two (Ouma, 2009 and Lithourgidis et al., 2011).

The concept of intercropping is mentioned for the first time in ancient Greece, 300 B.C., where Theophrastus points out the possibility of planting of wheat, barley or pulses together with vines and olives (Hamburda et al., 2015). These days, small-scale holders in tropical countries use intercropping commonly (Raei et al., 2015 and Sangakkara et al., 2012). Surprisingly over 15% of the world's food supply is from these traditional cropping systems. For example in Latin America 60% of the maize is accompanied with another crop and 70-90% of beans are grown together with maize, potato (Lithourgidis et al., 2011). In African 89% of cowpeas are intercropped, furthermore specifically in Malawi the area devoted for intercropping is as high as 94%.

Intercropping is used for different purpose in different parts of the world, for example, in the tropics it is primarily used for food grain production whereas in temperate areas for efficient forage production. In Africa intercropping, as part of traditional farming, is common practice by smallholder farmers in many areas to compensate for small land size and to ensure food security. The condition is far different in mechanized agriculture where Europe, North American and some Asian countries focus on market related production which widely uses monoculture (Lithourgidis et al., 2011).

Intercropping can be advantageous compared to monocropping for a number of reasons. Firstly it is efficient in resource utilization and yield advantage (Boudreau, 2013, Ekram et al., 2010, Mishra, 2014 and Mushagalusa et al., 2008). For example water evaporation can be reduced by maize/cowpea intercropping which increase light interception than sole maize. The yield advantage obtained in intercrop is due to complete utilization of the resources available such as light, water and nutrients (Egbe, 2010 and Mishra, 2014). Complete utilization of resources occurs as a result of differences in competition ability between companion crops, interspecific competition for a given resource is weaker than in intraspecific competition. Free resource utilization, same resource, could also be achieved by component crops through using resources at different places or time. This reduces the overlap for resource utilization. Slow maturing plants require quick maturing companion. Thereby, opting crops with different competitive ability in time or space is an integral part to decide on time of planting, thickness, and composition/arrangement (Hamburda et al., 2015, Jamshidi et al., 2008, Mishra, 2014, Mousavi and Eskandari, 2011 and Rezig et al., 2013). Intercropping is much less risky than monoculture in areas with extreme weather conditions like frost, drought and flood. If a single crop fails due

to bad air conditions farmers reduce risk of total failure. Furthermore through intercropping farmers can compensate for less marketable value crops (Ekram et al., 2010, Gebru et al., 2015, Mishra, 2014, Mousavi and Eskandari, 2011).

Intercropping is also a mean to improve soil fertility and reducing the risk of soil erosion (Ouma and Jeruto, 2010 and Ramert et al., 2002). A study by El-Swaify et al., 1988, showed the importance of intercropping legumes with cassava in reducing runoff and soil loss. Legumes are preferred because of their shallow roots, which allow soil to bind together at the surface and thereby reduce soil erosion. In addition, legumes enrich soil by fixing atmospheric nitrogen (Ouma and Jeruto, 2010). Particularly in areas with low soil nitrogen the advantage of intercropping with legumes is immense (Dusa and Stan, 2013; Ekram et al., 2010; Hamburda et al., 2015; Egbe, 2010).

The problem of weed in agriculture is huge. It causes yield loss and a very high cost of control with hand weeding and/or herbicide use. A number of studies have shown the efficiency of intercropping is weed suppression (Poodineh et al., 2014, Liebman and Dyck, 1993 and Mbah and Aniekwe, 2013). Intercrops that are effective at suppressing weeds capture a greater share of available resources than monocultures and could be more effective in pre-empting resources before the weeds and suppressing their growth (Lithourgidis et al., 2011). Intercrops that grow fast at early stage can cover the soil with their canopy and reduce weed growth by limiting sunlight reaching to the ground which is required by the weed seed for germination (Rajagopal et al., 1998, Jayakumar et al., 2008, Chauhan and Johnson, 2010 and Mallikarjuna et al., 2011). Choudhary et al., (2014) reported that intercropping of maize with different legumes in various

proportions decreased weed density. He explained further that the plot with 1:5 ratio of maize and cowpea intercrop had the lowest grass and sedge weed density. Sole maize plots showed the highest grass, sedge, and broadleaved weeds density. Rice intercropped with cassava or legume reduces weed population and weed dry matter (Gbanguba et al., 2011). In another study pearl millet intercropped with cluster bean and moth bean significantly reduce the density and dry matter of individual and total weeds as compared to sole pearl millet (Kiroriwal and Yadav, 2013). In a coffee orchard intercropping forage peanut and perennial soybean provides good soil cover and reduces weed infestation (Santos et al., 2014). Intercropping maize with French bean, mung-beans, or sunflower can reduce weed coverage by 35-56% (Hussain et al., 2013). Cow pea and corn intercropped plots reduces weed intensity by 20-23% (Silva et al., 2009).

The other important aspect of intercropping is the ability to reduce pest and disease incidence. However, this is a very complex aspect and both beneficial and detrimental effects have been observed (Lithourgidis et al., 2011). In South China intercropping banana (*Musa* spp.) with Chinese chive (*Allium tuberosum* Rottler) has been shown to reduce Panama disease (*Fusarium wilt*) incidence on banana (Zhang et al., 2013). Intercropping castor (*Ricinus communis* L.) with cluster bean, cowpea, black gram or groundnut in 1:2 ratios reduced pest incidence of semilooper, (*Achaea janata* L.), leaf hooper, (*Empoasca flavescens* Fabricius), and shoot and capsule borer, (*Canogethes punctiferalis* Guenee) (Rao et al., 2012). Narrow-leafed lupin intercropped with spring triticale reduces population of Weevils (Coleoptera: Curculionidae) (Hurej et al., 2013). A 1:1 and 1:2 ratios of Pearl Millet (*Pennisetum glaucum* L.) intercropped with groundnut reduces stem borer population in pearl millet (Degri et al., 2014). Björkman et al., (2007) reported that egg-laying behaviour of the turnip root fly (*Delia floralis* Fall.) (Diptera:

Anthomyiidae) on cabbage was reduced in 42-55% through intercropping cabbage with red clover.

Intercropping pumpkin with sorghum results in 43 to 96% disease incidence reduction in aphid-transmitted, non-persistent virus diseases on pumpkin, caused mostly by the potyviruses Watermelon mosaic virus (WMV) and Papaya ring spot virus (Damicone et al., 2007). Similarly, intercropping wheat with rye in China delayed onset of stripe rust (caused by *Puccinia striiformis*) by three to five days and reduced incidence (Peng et al., 2006). A field study conducted in two localities in Ethiopia, Holetta and Galessa, has shown that potato vs garlic, *Allium sativum* (L), intercropping at 3:1 garlic vs potato proportion caused low potato late blight disease development (Kassa and Sommartya, 2006).

The observed decrease in insect population in the intercrop could be explained by “resource-concentration” hypotheses. This states that spatial configuration of diverse vegetation reduces crop susceptibility to herbivore attack by directly interfering with visual and olfactory components of the host plant location and movement of herbivores (Root, 1973).

Intercropping is also known to have a major impact on trophic interactions and biological control (Andow, 1991). Intercropping can reduce phytophagous insect populations and increase beneficial insects’ performance. For instance Push-pull strategy involves driving Stemborers away from the main crop with Napier grass planted on the border of the field as a trap plant (pull) and using a repellent intercrop (push) such as desmodium which also attracts their natural enemies (Khan et al. 2011). Thus, increasing diversity in the field through intercropping, cover cropping, or even tolerating weeds could enhance biological control and reduce the damage by

insect pest. Russell (1989) reviewed studies on the importance of intercropping in improving level of parasitism and he found that in 70% of the cases intercropping increased pest death attributed to natural enemies (Russell, 1989).

Pimentel (1961) and Root (1973) developed the “Enemy Hypothesis”. The hypothesis stated that number of herbivores in intercropped system is less abundant than in monocropped because of the abundance of predators and parasitoids. The abundance of the beneficials in the intercrop might be due to the availability of more resources and habitats than in the monoculture (Linker et al., 2009). Crop systems that are dominated by a single plant species only provide resources to those selected organisms that can exploit that single plant species. Hence, monocultures are an example of agroecosystems with low diversity and may be more susceptible to pest or disease outbreaks (Theunissen, 1994 and Altieri and Nicholls, 2004). Yet, intercropping is not without disadvantages and higher within-field diversity does not always result in better pest control. Increasing diversity may also aggravate pest problems (Andow, 1991) or hinder beneficial insect activity (Andow and Risch, 1985). Other than the allopathic effects (Mousavi and Eskandari, 2011) the major disadvantage of intercropping is management difficulties in mechanized agriculture where plants have different chemical requirements and different methods of chemical application, planting, weeding, and harvesting.

Chapter 3 The Effects of Phosphite and Intercropping on Potato Tuber Moth and Tomato Leafminer in Ethiopia

3.1. Introduction

Phosphites are salt derivatives of phosphorous acid (H_3PO_3) (McDonald et al., 2001). They are marketed as pesticide, fungicide, supplemental fertilizer, plant strengthener and bio-stimulant (Gómez-Merino and Trejo-Téllez, 2015). Phosphite moves systemically from leaves to roots and vice versa and hence it is used for the control of both foliar and root pathogens (Cohen and Coffey, 1986). Phosphite reduces plant susceptibility to diseases caused by the oomycetes, such as *Phytophthora* spp. and *Plasmopara* (Gómez-Merino and Trejo-Téllez, 2015). Cooke and Little, (2002) showed the efficiency of phosphite in reducing potato tuber susceptibility to late blight in field trials. In addition, foliar application suppresses downy mildew of maize, *Peronosclerospora sorghi* (Panicker and Gangadharan, 1999) and grapevine, *Plasmopara viticola* (Speiser et al., 2000).

Several field trials have shown population reduction of insect pests including thrips, *Frankliniella* spp. (Thysanoptera: Thripidae), cotton aphids, *Aphis gossypii* (Glover), (Hemiptera: Aphididae), sweet potato whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and cotton leaf perforators, *Bucculatrix thurberiella* (Busck) (Lepidoptera: Bucculatricidae) with phosphite applications (Collins, 1993). In a recent field and laboratory work phosphite has also been shown to reduce larval population of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Patterson and Alyokhin, 2014).

Phosphite has been shown to stimulate root growth and improve yield and nutritional values of horticultural crops. It also improves nutrient uptake and assimilation, abiotic stress tolerance and

product quality. Foliar or drip application of phosphite improved yield of vegetables and fruits. Celery, potato and sweet pepper yields increase due to phosphite treatment (Gómez-Merino and Trejo-Téllez, 2015). Avocado and citrus fruits yield was also elevated after phosphite foliar treatment (Gómez-Merino and Trejo-Téllez, 2015). In contrast, phosphite could also cause plant toxicity such as leaf chlorosis and stunted growth (McDonald et al., 2001; Thao and Yamakawa, 2009). Jost et al, (2015) showed that treatment of *Arabidopsis thaliana* with phosphite reduced plant biomass production and root elongation.

Potato tuber moth (PTM; *P. operculella* Zeller) (Lepidoptera: Gelechiidae), is a widespread and important oligophagous pest of a range of solanaceous species including potato and tomato (Rondon, 2010). Larva feeds mainly on potato tubers in the field and in storages. In developing countries, the most important economic damage occurs in storage conditions due to larval feeding. Damage in storages could reach 100% (Ahmed et al., 2013), and 42% tuber damage has been reported in Ethiopia (Sileshi and Teriessa, 2001). A study conducted in Ethiopia has also reported 8.7% tuber damage under field conditions (Zelege et al., 2015). In Egypt, PTM has caused up to 100% losses to potato plants in fields as well as in storage (Ahmed et al., 2013). In Ethiopia tomato has associated between PTM's and tomato is recent with damage to fruits (Mulatu et al., 2004, 2006).

T. absoluta (Meyrick), commonly called tomato leafminer, is a devastating pest of tomatoes. Up to 100% losses have been reported in tomato crops (Korycinska and Moran, 2009). Even in conditions where control measures have been taken, losses could still exceed 5% (Korycinska and Moran, 2009). The pest is native to South America, but now it has invaded and spread through the Mediterranean region, the Middle East, North Africa, Asia and some parts of East

Africa since 2006 (Hayden and Brambila, 2013; Cabi, 2017). It has been causing severe damage in Ethiopian tomato system since 2011 (Gashawbeza Ayalew and Abiy Fekadu, 2013).

Various control options are available for the management of PTM and tomato leafminer management, such as intercropping and biological control. Intercropping, also called polyculture or mixed cropping, is a practice of cultivating two or more crops at same space and time (Andrews and Kassam, 1976; Ouma and Jeruto, 2010). In general intercropping is used in developing countries to improve land use and reduce risk (Lal, 1991). It is also advantageous in reduction of pests, diseases and weed damage, and increase yield and soil fertility (Mousavi and Eskandari, 2011). Lal, (1991) showed reduction of PTM infestation on potato by intercropping potato with other plants such as chili, onion and pea.

Copidosoma koehleri (Blanchard) and *Apanteles subandinus* (Blanchard) are believed to be excellent larval parasitoids of PTM along with *Trichogramma* (Whiteside, 1985). *Diadegma mollipla* (Holmgren) and *Chelonus* spp are the only identified larval parasitoids in Ethiopia against PTM feeding on potato plants (Negasi et al., 1985) whereas tomato plants provide an enemy free space (EFS), providing a higher degree of protection against natural enemies than does an alternative host plant (Jeffries and Lawton, 1984), to PTM feeding on tomato leaves (Mulatu et al., 2004). Because of this, there is a need to associate tomato with another host of PTM more attractive than the tomato, such as the potato, which at the same time may allow parasitism to take place.

The most common method of PTM and tomato leafminer control is with use of pesticides (Alvarez et al., 2005; Braham and Hajji, 2012). However, the continuous use of pesticides result

in the development of pesticide resistance, resurgence of pest population and affects human health, the environment and non-target organisms (Douches et al., 2002). In addition, lack of knowledge in pesticide use and low use of protective device during application has been reported in developing countries (Kromann et al., 2012) including Ethiopia (Mengistie et al., 2017). Therefore there is an urge for safe management practices against PTM and tomato leafminer with low risk to human health and non-target organisms.

Phosphite has been identified as a potential alternative for the synthetic pesticides in controlling some herbivorous insects besides some economic fungal diseases, because it has low risk to human health and the environment (Kromann et al., 2012). Such studies on PTM and tomato leaf miner are scanty. In the present study, we therefore investigated the effects of phosphite treatment and intercropping of tomato and potato on field level performance tomato leafminer and PTM and monitored the presence and efficiency of parasitoids at two agro-ecologically locations. We also recorded the impact of intercropping and phosphite treatment on potato and tomato yields.

3.2. Materials and methods

3.2.1. Description of study sites

Field trials were conducted in the years 2014, 2015 and 2016 from December to April under irrigation at Melkassa Agricultural Research Center (MARC), Central Rift Valley of Ethiopia. Melkassa is 115 km from Addis Ababa at an elevation of 1550 m.a.s.l (8024' N 39021'E). The average annual rainfall in the area is 768 mm, which is erratic and unevenly distributed. The site has a mean maximum temperate of 28.5⁰C and mean minimum temperature of 12.6⁰C. Loam and clay loam soil textures are the dominant soils of the area (www.eiar.gov.et). Following the same

experimental set up and sampling procedure as in Melkassa the field trials were conducted in the years 2015 and 2016 from January to May under irrigation at Holetta Agricultural Research Center (HARC). Holetta is located 28 km west of Addis Ababa and positioned at 38° 32'N 90° 3'E at an altitude of about 2,400 m.a.s.l. The average annual rainfall in the area is 1,100 mm and max and min temperature of 22.2 and 6.1°C, respectively (www.eiar.gov.et).

3.2.2. Experimental setup and insect monitoring

The experiments were conducted on separate plots of 3 m*5.25 m planted with potato cv. Belete and tomato cv. Fetan grown in three seasons on onion and haricot bean grown fields. The distance between plants in a row was 30 cm and between rows 75 cm, while the distance between plots and blocks was one and two meters, respectively. Potato was intercropped with tomato at a ratio of 1:1. In addition potato and tomato monocultures were included. The treatments were arranged in a randomized complete block design with four replications. All the plots were fertilized with di-ammonium phosphate (DAP) and urea was applied during planting at the rate of 195 kg ha⁻¹ and 165 kg ha⁻¹, respectively. Half of the tomato, potato and intercropped plots were treated with potassium phosphite (Proalexin, LMI AB, Helsingborg, Sweden) applied at 5 L ha⁻¹ using a 15 L capacity manually-pumped knapsack sprayer. The rest of the plots were left untreated and used as controls. Foliar application of phosphite was made for 8 consecutive weeks. The application was started three weeks after potato emergence or tomato transplant establishment. Data were collected six days after each phosphite treatment. The normal agronomic routines were carried out for all plots.

3.2.3. Sampling procedures and data collection

A total of 24 tomato and potato plants, from the monoculture, and 12 from potato-tomato intercrop plots were selected at random within each plot at weekly interval from inner rows and visually examined for presence of PTM and tomato leafminer larva. Sampling was started as leaf blotch/chlorosis were observed in the control plots and continued for consecutive 8 weeks. PTM and tomato leafminer larva were identified based on the pattern of the prothoracic shield. PTM has uniformly brown coloured prothoracic shield and first abdominal segment, whereas tomato leafminer has distinctly patterned prothoracic shield (Roditakis et al., 2010). The larvae were recovered from the infested leaves and recorded weekly on a whole-plant basis. The recovered larvae were reared to adult stage on their respective host plant leaves and the level of parasitism was determined where applicable. Percentage parasitism was calculated as the ratio of the total number of parasitized larvae to the total larvae collected per plant. The cumulative number of larvae over the whole growth season for each plot was used to study the effect of the treatments for each year, separately. During harvest, tubers from the five inner rows were dugout by hand and sorted into marketable and unmarketable, based on their size, and infestation and pooled together. Similarly, tomato fruits were hand-picked as they matured and sorted to marketable and unmarketable based on insect damage and weighed in the field. In the analysis, insect count and yield data were calculated per plant basis.

3.2.4. Data analysis

JMP 10 (SAS Institute, Inc. 2012) was used to analyze all the data. The data collected on larvae count, percentage parasitism and yield were checked for normality using Shapiro-Wilk W normality test. Since the data was found to be non-normally distributed it was log transformed

before analysis. All the data were analyzed with factorial two-way ANOVA. In the model, block was treated as random effect. Phosphite, intercropping and their interactions were considered as sources of variation. Year effect on larval count was assessed using ANOVA and t-test for Melkassa and Holetta data, respectively. Location effect of the two sites, Holetta and Melkassa, on PTM count was also assessed by t-test. Attributing to their preferences tomato plants were excluded from the analysis when dealing with PTM and potato was excluded when dealing with tomato leafminer. Level of parasitism was determined only from the recovered larvae. The significant level was adjusted to $\alpha = 0.05$.

3.3. Results

3.3.1. Effects of phosphite and intercropping on larval population of tomato leafminer and PTM at Melkassa

There was a statistically significant difference on tomato leafminer larval population density between the years ($F_{2,381} = 23.52$ and $p < 0.0001$) with the overall mean larval density per plant highest in 2015. No statistically significant main effects, phosphite treatment and intercropping, differences were detected on tomato leafminer larval population density ($F_{1,121} = 2.17$, $p = 0.14$ and $F_{1,121} = 1.51$ $p = 0.22$), respectively in 2014 (Table 3.1). Similarly, there was no statistically significant interaction between the effects of phosphite treatment and intercropping on the tomato leafminer larval population density ($F_{1,121} = 0.61$ and $p = 0.44$) (Table 3.1).

Separate analyses of weekly counts in 2014 revealed that there were significantly fewer tomato leafminer larvae on phosphite-treated plots during weeks seven ($F_{1,9} = 19.82$; $P = 0.002$) and eight ($F_{1,9} = 5.63$; $P = 0.041$) of the eight weeks observations. Similarly, intercropped plots had higher number of larvae than the monocrop during week seven ($F_{1,9} = 19.82$; $P = 0.002$) and eight ($F_{1,9} =$

5.63; $P= 0.041$). The interaction between phosphite treatment and intercropping was also significantly different in both these sampling weeks ($F_{1,9}= 19.82$; $P= 0.002$ and $F_{1,9}= 5.63$; $P= 0.041$) with highest number of larvae in the untreated intercropped plots. The trend of average larval density of the leafminer in the different sampling days showed a similar pattern across the treatments where the peak was attained in the 4th sampling week in all the treatments (Fig 3.1).

Table 3.1 Factorial Two-way ANOVA in the effect of phosphite treatment and intercropping at Melkassa, in 2014, 2015 and 2016 (transformed data)

Pest	Year	Sov	Nparm	DF	DFDen	F ratio	Prob> F
Tomato leafminer	2014	Trt	1	1	121	2.17	0.14 ^{ns}
		Cropping system	1	1	121	1.51	0.22 ^{ns}
		Trt*Cropping system	1	1	121	0.61	0.44 ^{ns}
	2015	Trt	1	1	121	5.34	0.02*
		Cropping system	1	1	121	0.28	0.60 ^{ns}
		Trt*Cropping system	1	1	121	0.01	0.94 ^{ns}
	2016	Trt	1	1	121	0.01	0.91 ^{ns}
		Cropping system	1	1	121	2.07	0.15 ^{ns}
		Trt*Cropping system	1	1	121	0.003	0.95 ^{ns}
PTM	2015	Trt	1	1	121	32.17	<.0001*
		Cropping system	1	1	121	1.41	0.24 ^{ns}
		Trt*Cropping system	1	1	121	0.01	0.91 ^{ns}
	2016	Trt	1	1	121	3.93	0.04*
		Cropping system	1	1	121	5.08	0.026*
		trt*Cropping system	1	1	121	0.17	0.68 ^{ns}

* Significant and ^{ns} not significant at $p<0.05$ Trt= phosphite treatment, Cropping system (monocrop and intercrop)

In the year 2015 like the previous year there was no significant interaction between the two factors, phosphite treatment and intercropping ($F_{1,121}= 0.01$ and $p= 0.94$). The result also revealed that there was no effect of cropping system on leafminer larva population density ($F_{1,121}= 0.28$ and $p=0.60$) (Table 3.1). There was, however, a significant main effect of phosphite treatment on tomato leafminer larval population density ($F_{1,121}= 5.34$, $p=0.02$), suggesting that

plants treated with phosphite had less number of tomato leafminer larva than the untreated ones (Table 3.2).

Table 3.2 Mean $\pm$ SE tomato leafminer larvae per tomato plant at Melkassa and under different treatments in 2014, 2015 and 2016

Mean $\ln(x+1)$ +SE tomato leafminer larval per plant						
Phosphite	2014		2015		2016	
	Intercrop	Monocrop	Intercrop	Monocrop	Intercrop	Monocrop
without	0.36 $\pm$ 0.06 ^a	0.22 $\pm$ 0.06 ^a	0.55 $\pm$ 0.07 ^a	0.51 $\pm$ 0.07 ^{ab}	0.11 $\pm$ 0.04 ^a	0.17 $\pm$ 0.04 ^a
with	0.20 $\pm$ 0.06 ^a	0.17 $\pm$ 0.06 ^a	0.38 $\pm$ 0.07 ^{ab}	0.33 $\pm$ 0.07 ^b	0.11 $\pm$ 0.04 ^a	0.17 $\pm$ 0.04 ^a

Means followed by the same superscript (s) are not significantly different from each other according to Tukey's HSD at 5%

Mean $\ln(x+1)$ =Least square mean for $\ln(x+1)$ transformed data

Table 3.3 Mean $\pm$ SE PTM larvae per potato plant at Melkassa and under different treatments in 2015 and 2016

Mean $\ln(x+1)$ +SE PTM larval per plant				
Phosphite	2015		2016	
	Intercrop	Monocrop	Intercrop	Monocrop
without	0.97 $\pm$ 0.06 ^a	0.88 $\pm$ 0.06 ^a	0.45 $\pm$ 0.08 ^{ab}	0.62 $\pm$ 0.08 ^a
with	0.58 $\pm$ 0.06 ^b	0.50 $\pm$ 0.06 ^b	0.22 $\pm$ 0.08 ^b	0.47 $\pm$ 0.08 ^{ab}

Means followed by the same superscript (s) are not significantly different from each other according to Tukey's HSD at 5%

Mean $\ln(x+1)$ =Least square mean for $\ln(x+1)$ transformed data

At early sampling dates the mean leafminer larval density was very low in all the treatments (Fig 3.1). The highest peak of larval density was recorded at the fourth and sixth sampling week in all the treatments. In the last sampling no larvae were recorded whatsoever.

In the year 2016 the larval population pressure was very low compared to the remaining years and no statistically significant differences were detected in the two main effects, treatment and

cropping system, and their interaction ($F_{1,121} = 0.01$, $p = 0.91$; $F_{1,121} = 2.07$, $p > 0.15$; and $F_{1,121} = 0.003$, $p = 0.95$) (Table 3.1). In 2016, unlike 2014, the separate analysis of weekly tomato leafminer counts did not revealed significant effect of phosphite application, intercropping and their interaction on leaf miner count in all the weeks. The larval density trend on the respective sampling dates had somehow overlapping pattern with those of the previous years, 2014 and 2015 (Fig 3.1).

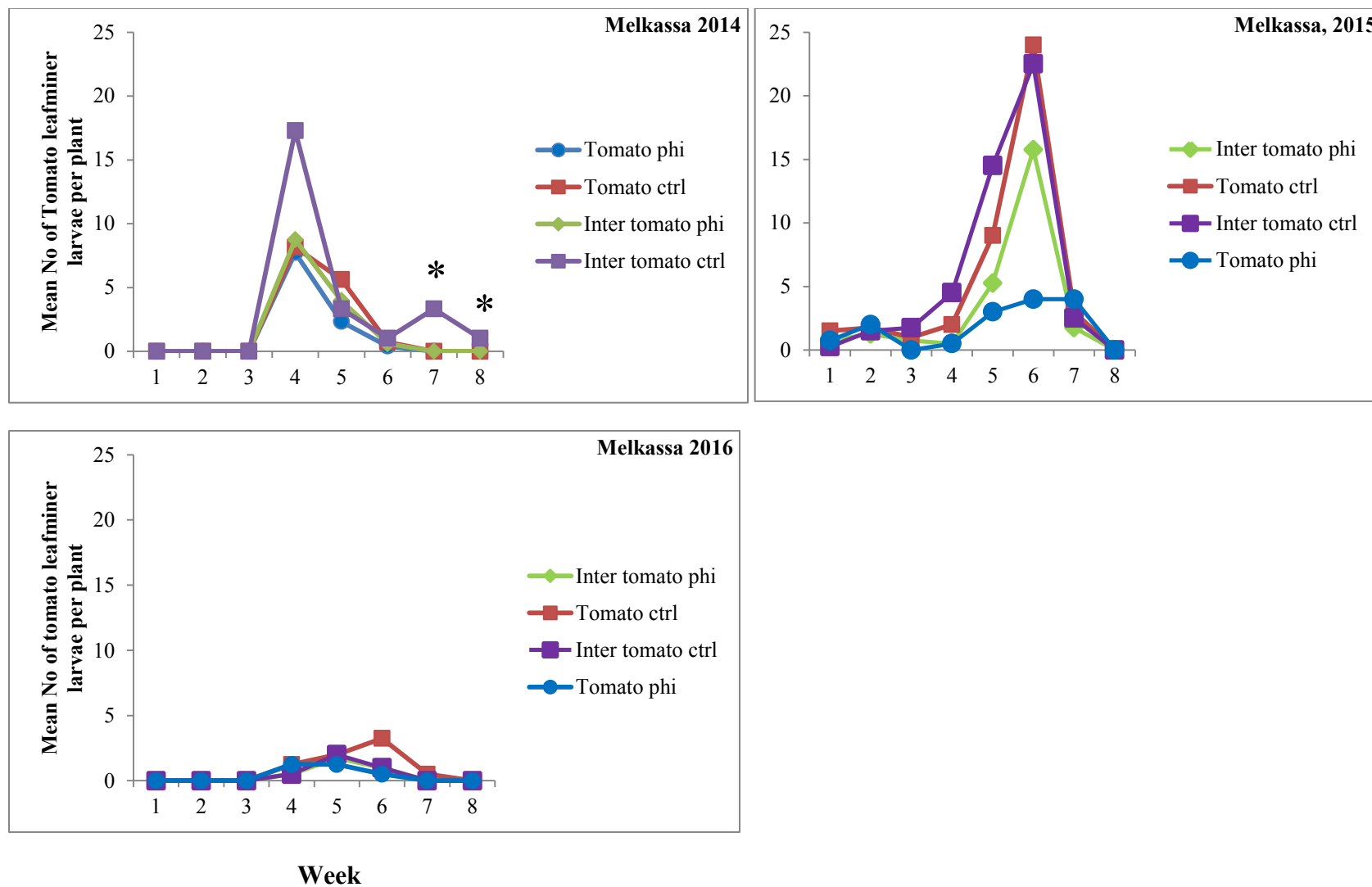


Figure 3.1 Mean number of tomato leafminer larva per plant in the leaves of phosphite treated and untreated monocrop and potato intercropped tomato plants at Melkassa in different experimental years. Phi= Phosphite, Ctrl= Control, Inter= intercrop

At Melkassa, PTM did not occur in the year 2014 and was replaced by tomato leafminer. However, in the next two consecutive years PTM returned, and the effects of phosphite treatment and intercropping were investigated. There was a significant difference between the two locations, Melkassa and Holetta, had different PTM larval population densities ($F_{1,1} = 5.99$ and $p = 0.015$), where in Holetta populations were higher. Significant effect on PTM larval density was found between years, with highest larvae number per plant in the year 2015 ($F_{2,254} = 23.83$ and $p < 0.0001$) at Melkassa. Here, the results showed no statistically significant interaction between phosphite treatment and cropping system on PTM larval population density ($F_{1,121} = 0.01$, $p = 0.91$; $F_{1,121} = 0.17$ $p = 0.68$ in 2015 and 2016, respectively) (Table 3.1). There was, however, a statistically significant difference due to the effect of phosphite treatment in both the years, 2015 and 2016 ($F_{1,121} = 32.17$, $p = 0.0001$ and $F_{1,121} = 3.93$, $p = 0.04$), with the highest number of PTM larvae per plant recovered in the control potato plots (Table 3.1 and 3.3). Furthermore, unlike 2015 more PTM larvae were recorded on monocrop plots than the intercropped in 2016 (Table 3.1 and 3.3). In 2015 when the pressure was high, a few PTM larvae were observed to mine into tomato leaves but it was very low compared with that of the potato leaves, whereas in 2016 PTM was confined to potato plants.

3.3.2. Effect of phosphite treatment and intercropping on potato and tomato yield at

Melkassa

Though phosphite reduced PTM larva numbers, phosphite treatment or intercropping had no significant effect on marketable, unmarketable and total potato yield in 2014, 2015 and 2016, except for that the unmarketable potato yield was significantly higher in intercropped plots in 2014 and the interaction between phosphite treatment and intercropping had a significant effect

on marketable potato yield in 2015. Where untreated intercropped plots had the highest marketable potato yield per plant.

Significant difference was found on the mean marketable tomato yield in 2014, due to intercropping. Indicating that, high marketable yield was obtained in the intercrop than the monocrop. In addition, a significant effect of cropping system was detected on unmarketable and total tomato yield. Intercropped plots had low unmarketable and higher total tomato yield per plant than the monocrops. There was also a significant interaction between treatment and cropping system on unmarketable tomato weight per plant (Table 3.6).

3.3.3. Effects of phosphite and intercropping on PTM larval population at Holetta

A significant year effect was detected between the two years on the number of PTM larvae collected with fewer larvae in 2016 ($F_{1,245} = 6.29$ and $p = 0.013$). Phosphite treatment lowered PTM larval density in both years ($F_{1,121} = 6.65$ $p = 0.01$; $F_{1,121} = 5.83$ $p = 0.02$) (Table 3.4). Untreated plots had higher larval density on average than phosphite treated plots (Table 3.5). On the contrary, intercropping had no significant effect on the larval density ($F_{1,121} = 0.47$ $p = 0.49$ and $F_{1,121} = 0.18$ $p = 0.67$), however, larval population still peaked in the monocropped plots (Table 3.4 and 3.5). Likewise, the interaction between the two main effects, treatment and cropping system, was not different ($F_{1,121} = 0.16$, $p = 0.69$ and $F_{1,121} = 0.16$ $p = 0.69$) (Table 3.4 and 3.5). Furthermore, PTM was confined to potato plants. The mean number of larva per plant for the different sampling dates is presented in Figure 3.3.

Table 3.4 Factorial Two-way ANOVA in the effect of phosphite treatment and intercropping at Holetta, Site II, in 2015 and 2016

Pest	Year	Sov	Nparm	DF	DFDen	F ratio	Prob> F
PTM	2015	Trt	1	1	121	6.65	0.01*
		Cropping system	1	1	121	0.47	0.49 ^{ns}
		Trt*Cropping system	1	1	121	0.17	0.69 ^{ns}
	2016	Trt	1	1	121	5.83	0.02*
		Cropping system	1	1	121	0.18	0.67 ^{ns}
		Trt*Cropping system	1	1	121	0.16	0.69 ^{ns}

* Significant and ^{ns} not significant at $p < 0.05$ Trt= phosphite treatment, Cropping system (monocrop and intercrop)

Table 3.5 Mean+ SE PTM larvae per plant potato at Holetta and under different treatments in 2015 and 2016

Phosphite	Mean $\ln(x+1)$ +SE PTM larval per plant			
	2015		2016	
	Intercrop	Monocrop	Intercrop	Monocrop
without	0.68+0.09 ^a	0.71+0.09 ^a	0.50+0.08 ^{ab}	0.57+0.08 ^a
with	0.43+0.09 ^b	0.52+0.09 ^{ab}	0.33+0.08 ^b	0.34+0.08 ^b

Means followed by the same superscript (s) are not significantly different from each other at 5%, Tukey HSD

Mean $\ln(x+1)$ =Least square mean for $\ln(x+1)$ transformed data

3.3.4. Percentage parasitism in PTM larvae

Significant difference was detected between years on the level of parasitism and 2016 had the highest percentage parasitism ($t = 3.65$ and $p = 0.0004$). The tests of within treatment effects showed that level of parasitism was not affected by treatment, cropping system or interaction between these in 2015 ($F_{1,89} = 1.12$, $p = 0.30$; $F_{1,89} = 0.06$, $p = 0.81$ and $F_{1,89} = 0.79$, $p = 0.38$) and 2016 ($F_{1,65} = 0.001$, $p = 0.97$; $F_{1,65} = 0.47$, $p = 0.46$ and $F_{1,65} = 0.001$, $p = 0.98$). However, there was a trend for parasitism to be higher in the intercropped plots in 2015. The main parasitoids found in

the area were *Diadegma mollipla* and two other unidentified species. The combined parasitism level from three of the parasitoids ranges between 17 and 27% in 2015 and 31 and 42% in the following year (Fig 3.2).

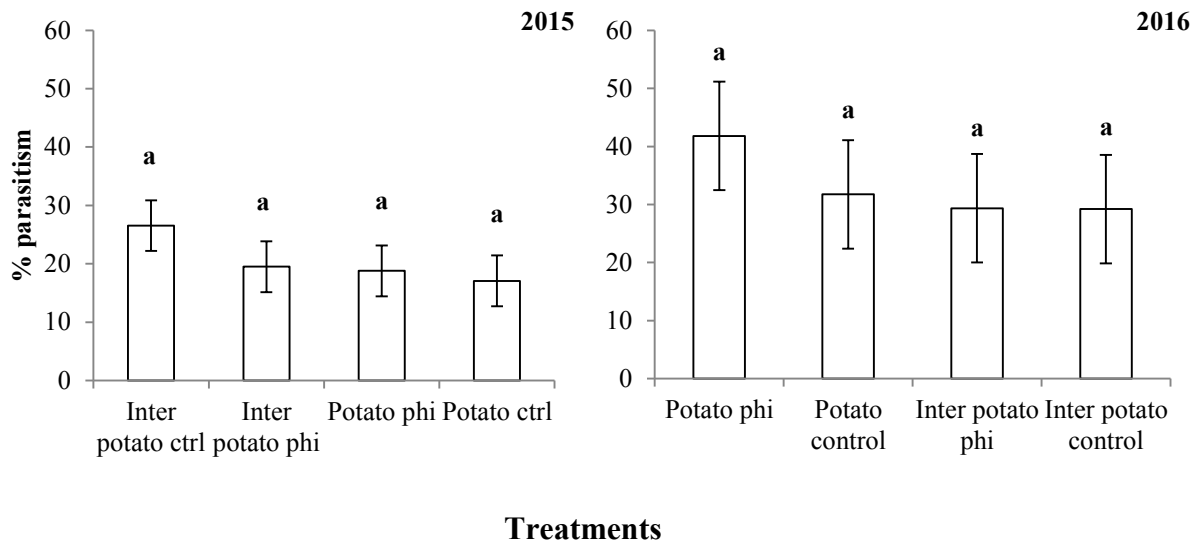


Figure 3.2 Rate of parasitism of PTM infesting potato planted as monocrop and intercropped with tomato in 1:1 ratio at Holetta in different experimental years. Inter= intercropping, Phi= Phosphite, Ctrl= control

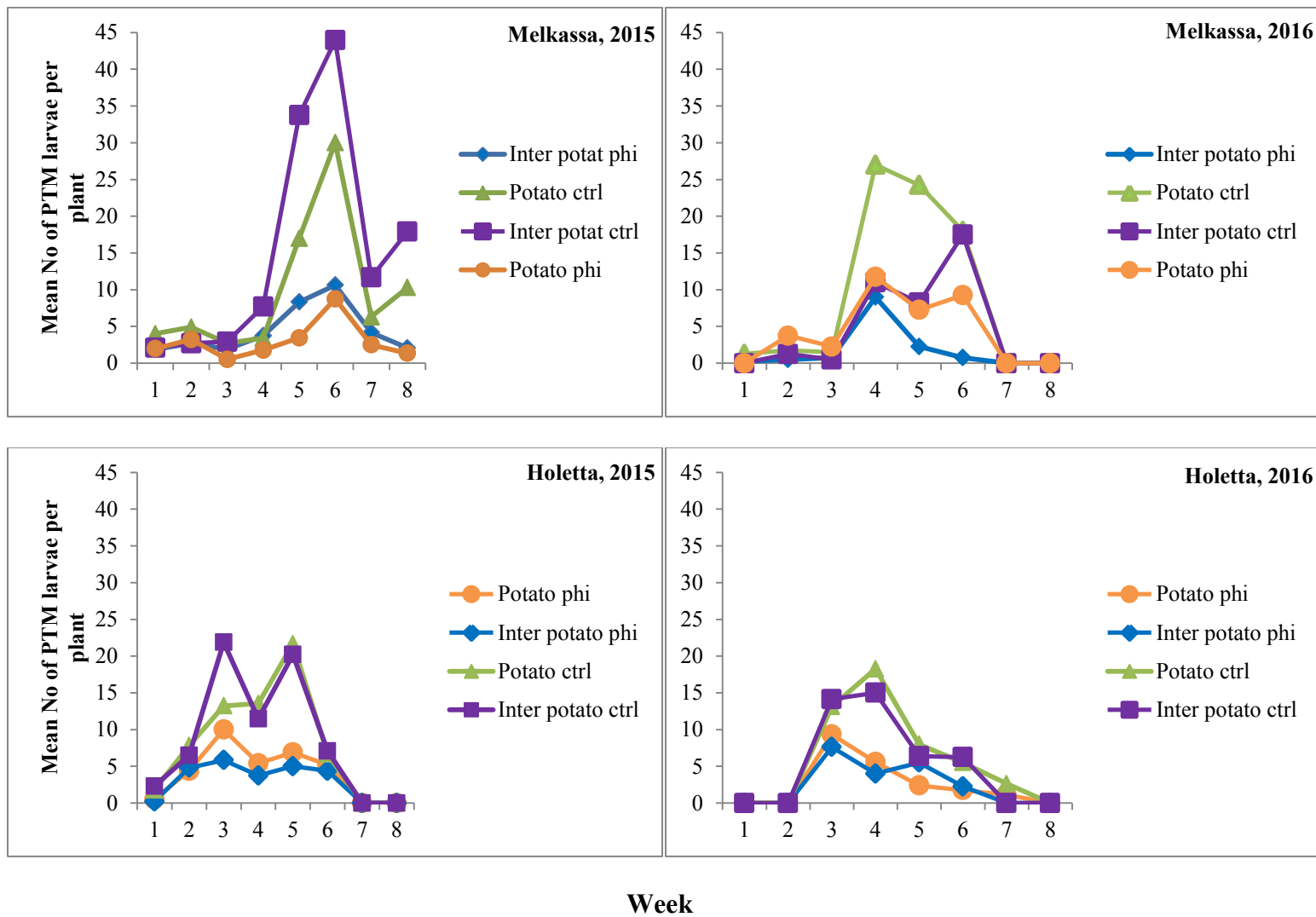


Figure 3.3 Mean number of PTM larva per plant in the leaves of phosphite treated and untreated monocrop and tomato potato intercropped plants at Melkassa and Holetta in different experimental year

3.3.5. Effect of Phosphite and intercropping on potato and tomato yield at Holetta

There was no significant interaction between treatments and intercropping on both tomato and potato yields (Table 3.7). On the contrary, the main effect cropping system caused a significant variation on marketable and total potato yield in the year 2015. Likewise, in 2016 there was a significant effect of cropping system on unmarketable, marketable and total potato yield. Potato yield was high in intercropped plots in both years. In both years tomato yield as a whole was not affected by both the treatments and their interaction (Table 3.7).

Table 3.6 Mean $\pm$ SE potato and tomato marketable, unmarketable and total yield per plant/kg at Melkassa

Location	Year	Treatment(T)	Cropping system (CS)	Mean MRK potato per plant/kg	Mean UNMRK potato per plant/kg	Total potato per plant/kg	Mean MRK Tomato per plant/kg	Mean UNMRK Tomato per plant/kg	Total yield Tomato per plant/kg
Melkassa	2014	Phi	Monocrop	0.15	0.02 ^a	0.17	0.03 ^a	0.08 ^a	0.11 ^a
		Control	Monocrop	0.12	0.03 ^{ab}	0.15	0.04 ^a	0.09 ^a	0.13 ^{ab}
		Phi	Intercrop	0.16	0.06 ^{ab}	0.22	0.1 ^{ab}	0.07 ^{ab}	0.17 ^c
		Control	Intercrop	0.12	0.08 ^b	0.20	0.1 ^b	0.06 ^b	0.16 ^{bc}
		T		ns	ns	ns	ns	ns	ns
		CS		ns	*	ns	*	*	*
		T*CS		ns	ns	ns	ns	*	ns
	2015	Phi	Monocrop	0.09 ^{ab}	0.01	0.10	0.05	0.08	0.13
		Control	Monocrop	0.06 ^a	0.02	0.08	0.03	0.07	0.10
		Phi	Intercrop	0.06 ^a	0.01	0.07	0.03	0.07	0.10
		Control	Intercrop	0.1 ^b	0.01	0.11	0.04	0.08	0.12
		T		ns	ns	ns	ns	ns	ns
		CS		ns	ns	ns	ns	ns	ns
		T*CS		*	ns	ns	ns	ns	ns
	2016	Phi	Monocrop	0.13	0.07	0.20	0.10	0.13	0.23
		Control	Monocrop	0.10	0.06	0.16	0.07	0.10	0.17
		Phi	Intercrop	0.13	0.07	0.20	0.11	0.12	0.23
		Control	Intercrop	0.12	0.05	0.17	0.10	0.13	0.23
		T		ns	ns	ns	ns	ns	ns
		CS		ns	ns	ns	ns	ns	ns
		T*CS		ns	ns	ns	ns	ns	ns

* Significant and ^{ns} not significant at p= 0.05 Phi = Phosphite treated, MRK= marketable, UNMRK= unmarketable

Table 3.7 Mean potato and tomato marketable, unmarketable and total yield per plant/kg at Holetta

Location	Year	Treatment (T)	Cropping system (CS)	Mean MRK potato per plant/kg	Mean UNMRK potato per plant/kg	Total potato per plant/kg	Mean MRK Tomato per plant/kg	Mean UNMRK Tomato per plant/kg	Total yield Tomato per plant/kg
Holetta	2015	Phi	Monocrop	0.28 ^a	0.05	0.33 ^a	0.13	0.02	0.15
		Control	Monocrop	0.29 ^{ab}	0.04	0.33 ^{ab}	0.15	0.02	0.17
		Phi	Intercrop	0.40 ^b	0.05	0.45 ^b	0.16	0.05	0.21
		Control	Intercrop	0.32 ^{ab}	0.03	0.35 ^{ab}	0.10	0.03	0.13
		T		ns	ns	ns	ns	ns	ns
		CS		*	ns	*	ns	ns	ns
		T*CS		ns	ns	ns	ns	ns	ns
	2016	Phi	Monocrop	0.32 ^a	0.04 ^a	0.36 ^a	0.07	0.09	0.16
		Control	Monocrop	0.32 ^a	0.04 ^a	0.36 ^a	0.06	0.07	0.13
		Phi	Intercrop	0.37 ^{ab}	0.06 ^{ab}	0.43 ^{ab}	0.08	0.08	0.16
		Control	Intercrop	0.41 ^b	0.08 ^b	0.49 ^b	0.05	0.04	0.09
		T		ns	ns	ns	ns	ns	ns
		CS		*	*	*	ns	ns	ns
		T*CS		ns	ns	ns	ns	ns	ns

* Significant and ^{ns} not significant at p= 0.05 Phi = Phosphite treated, MRK= marketable, UNMRK= unmarketable

3.4. Discussion

Our results revealed that phosphite was unable to suppress tomato leafminer larva in two of the three years tested. However, the pressure in 2016 was so low that it is difficult to draw conclusions from this year. Even though phosphite is known to have direct and indirect effect in a number of plant diseases (Daniel and Guest, 2006), the effect was ambiguous in the current study. However, in 2015, the year when the density of tomato leafminer was the highest, phosphite application significantly reduced larval density. Therefore phosphite effect in tomato leafminer might be effective in years with a high larval pressure. This is something that will need further study. Tomato leafminer larval density was not different between intercrop and monocrop; this could be due to the variation in the companion crop used in the present experiment, potato. In contrast, another intercropping system showed that tomato leafminer larval density on tomato leaflets decreased significantly by intercropping tomato with aromatic plants such as geranium, spearmint, rosemary and sweet basil which is a deterrent effect (Khafagy, 2015). The larval density was similar over the season in all the experimental years with a slow, initial increase and sharp decline at the end. This shows that the peak larval density in the leaves occurred under similar growth stage of the plant indicating that this could be the right time for control measures such as chemical application.

PTM larval population density was very low on phosphite treated potato leaves in both localities, showing that phosphite has an effect on PTM. However, a significant effect of the potato-tomato intercropping system on larva density was only observed in 2016 at Melkassa. A previous field and laboratory study conducted to assess the effect of phosphite on Colorado potato beetle showed that phosphite treated plants were less defoliated (Patterson and Alyokhin, 2014). In addition, beetle number was reduced due to phosphite treatment both in field and laboratory

conditions. Even though PTM pressure varied between years, the same phosphite effect was observed on the larval density of PTM. The observed PTM preference to potato over tomato during the vegetative stage is in agreement with a previous oviposition preference study under a laboratory condition (Mulatu et al., 2007). According to this study, the female lays significantly more eggs on potato leaves than tomato. In regard to PTM larval density in the tomato-potato intercrop, little effect was seen in contrast to results from intercropping of potato with chilies, onions and peas which reduced both PTM potato leaf infestation and tuber damage (Lal, 1991).

Natural enemies play an important role in the control of wide variety of insect pests. *Diadegma mollipla* and two other unidentified species were the main parasitoids found attacking PTM in our study at Holetta. One of the possible drawbacks of commercially available chemical insecticides is that they are toxic to non-target beneficial insects like parasitoids and predators. However, our study showed that phosphite treatment did not interfere with the performance of the major parasitoids in the area even if it at the same time reduced the PTM population. The level of parasitism between treated and untreated plants was the same, indicating that phosphite did not affect the parasitoids. Intercropping had no clear effect on the level of parasitism but there was an increasing trend in the intercropped potato plants in 2015. There was a correlation between number of larvae and percentage parasitism in 2015 and 2016 ($r = 0.65$; $p < 0.0001$) and $r = 0.40$; $p < 0.0008$, respectively). Although PTM larva existed in the system at Melkassa no parasitism was detected. But the indirect effect of phosphite application on parasitoids and predators is important to note as this affects the availability of host/prey to the natural enemies.

Our findings suggest that even if phosphite reduces PTM larva numbers, this treatment nor intercropping have a significant effect in the total potato yield at Melkassa, in all the

experimental years. The yield obtained in Melkassa was quite low compared to that obtained at Holetta, which could be due to the climatic differences between the sites, potato is not well-adapted to the area. On the other hand, marketable and total tomato yield was higher in the intercropped plots in one of the three experimental years at Melkassa suggesting that intercropping could increase tomato yield and quality. Significant correlation was detected between marketable tomato and PTM larval density ($r = -0.68$, $p = 0.0033$) and unmarketable tomato and tomato leafminer larval density ($r = 0.9546$; $p < 0.0001$) in 2016 and 2014, respectively. Similar results are obtained by Obedoni et al., (2005), described the potential of intercropping in tomato yield. Obedoni et al (2005) showed that intercropping tomato with equal or less proportion of cowpea increases tomato yield significantly (Obedoni et al., 2005). Hussain et al., (2008) also showed high yield in consequence high gross income obtained by intercropping tomato with okra (Hussain et al., 2008). Marouelli et al., (2013) showed that tomato fruit damage by tomato leafminer is reduced by intercropping tomato with Coriander.

Similar to Melkassa potato yield did not increase by phosphite treatment in Holetta. Rickard (2000) reviewed that potato yield increased due to phosphite used as fertilizer (Rickard, 2000). Yet, intercropping significantly increased both marketable and total potato yield. The yield advantage obtained in intercrop could be due to complete utilization of the resources available such as light, water and nutrients. More efficient utilization of resources occurs as a result of differences in competition ability between companion crops, interspecific competition for a given resource is weaker than in intraspecific competition, and in this case maybe more space for potato tubers to develop (Egbe, 2010 and Mishra, 2014). Similar yield trend were reported by different authors. For example Choudhuri and Jana, (2015) found that highest potato equivalent yield per hectare ($23.61 \text{ tons ha}^{-1}$) obtained from potato and mustard intercropping with 2:1 row

ratio (Choudhuri and Jana, 2015). Additional evidence comes from two other studies conducted in China and Iran showing that potato yield increases through intercropping with maize due to the height difference which could resulted in creating favorable microclimate for potato by reducing light intensity and increase photosynthesis efficiency (Li et al., 2009 and Jamshidi et al., 2008).

3.5. Conclusion

In addition to pathogens such as oomycetes phosphite salts has been shown to reduce insect pest population in cotton, sweet potato, and potato (Collins, 1993; Patterson and Alyokhin, 2014). Our study showed that phosphite treatment reduces PTM larval population density in potato plants and it has no observed direct harm on the performance of the existing parasitoids. Therefore it is possible to consider phosphite in developing a suitable control for PTM. However, in our study the decrease in PTM did not seem sufficient to increase the marketable tomato yield.

Chapter 4 Behavioural Response of Potato Tuber Moth Adults to Phosphite and Beta amino butyric acid (BABA) Treated Tomato and Potato Plants

1.1. Introduction

Plant defense mechanism is the means plants protect themselves from being eaten or damaged by birds, mammals, diseases and insects. Defence is either structural, which involves morphological features such as thorns, spines/trichomes, smooth/slippy leaves, tough tissues, wax cover and resin secretion, or chemical, through production of diverse secondary metabolites most of which are volatile compounds (Ryan, 2014). Plant volatiles are secondary metabolites, which are lipophilic with low molecular weights and high vapor pressure, which are produced and emitted from different parts of a plant (Knudsen et al., 2006; Shrivastava et al., 2010 and Irchhaiya et al., 2015). Plant volatile includes isoprenoids, carotenoids, benzenoids, phenylpropanoids, fatty acid derivatives, and amino acid derivatives (Dudareva et al., 2004). Plants make use of them for various physiological processes and to interact with their immediate environment, including interaction with insects such as plant cues that attract herbivores, direct and indirect defense and interplant priming (Shrivastava et al., 2010; Himanen et al., 2010). The production of compounds by plants which are antifeedant, antinutritive, repellent, and/or toxic to herbivores is an example of direct plant defense (Shrivastava et al., 2010). Interplant priming is signaling from herbivore damaged plants to undamaged nearby plants resulting in preparation or sensitization of the undamaged plant's defensive traits, hence facilitating a faster and stronger response upon subsequent attack (Shrivastava *et al.*, 2010 and Girón-Calva et al., 2014).

A range of biotic factors, such as fungi, oomycetes, bacteria, and herbivores, and abiotic factors, such as nutrient stress, drought, UV radiation, and temperature have been found to induce the release of certain plant volatile compounds which change plant interaction with other organism

(Holopainen and Gershenzon, 2010; Dong et al., 2016). In addition to other plant attributes, the interaction between plants and other organism is caused by volatiles that are emitted from the vegetative part of the plant. Their interaction could be beneficial or harmful to the plant (Pichersky and Gershenzon, 2002).

Various studies have shown that phosphite application triggers the production of plant defence compounds (Hardy et al., 2001). In addition, even though the mechanism was not identified, a study has shown that phosphite also reduced Colorado potato beetle larval density and survival (Patterson and Alyokhin, 2014).

BABA applied as a soil drench in a potted winter wheat has shown to induce resistance against the aphid *Sitobion avenae* by weight and growth reduction of the nymph (Cao et al., 2014). BABA soil drench treatment of legumes such as tic bean (*Vicia faba* var. *minor*), pea (*Pisum sativa*), broad bean (*Vicia faba* var. *major*), runner bean (*Phaseolus coccineus*), red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*) in a glasshouse increased mortality of the pea aphid *Acyrtosiphon pisum* through reduction of nymphal growth rate (Hodge et al., 2005). Root drench application of BABA has also shown to reduce the performance of the aphids *Myzus persicae* and *Brevicoryne brassicae* and Lepidoptera *Trichoplusia ni* and *Plutella xylostella* (Hodge et al., 2006).

PTM is one of the most important potato pests worldwide. As the name indicates potato is the primary host of PTM (Rondon, 2010). It also attacks tomato plants (Mulatu et al., 2004). PTM damages potato both under field and storage conditions, however, damage is aggravated in storages. Field infestation is the source of inoculum for storage damage (Rondon, 2010). In our

field studies foliar phosphite treatment of potato plants had reduced the number of PTM larval density; however the reason behind the reduction is not known (See chapter 3). Therefore, in the present study we investigated the behavioural response of PTM to phosphite and BABA treated and untreated potato and tomato plants to determine whether or not the larval population density reduction observed in the field after phosphite treatment could be linked to any change in the plant odour occurring after foliar phosphite treatment. We also assessed the preference of PTM between potato and tomato plants.

1.2. Materials and methods

1.2.1. Insect rearing

Five pairs of adult PTM collected from potato fields and storage in Holetta Research Center were introduced into a plastic tray with perforated and meshed lid and provided with potato tuber for oviposition and larva food source. A drop of water and honey was also provided from top of the perforated and meshed lid. Pupae were then transferred into a Petri dish for hatching. The emerged adults were used for the olfactometer bioassay.

1.2.2. Experimental plants

Seed tuber of the potato cultivar Jalene and tomato seed of the cultivar Melka shola were used in the experiment. Seeds were planted in 70 plastic pots in a greenhouse and urea was supplied during planting at a recommended rate. Twenty five days after emergence the plants were arranged for the experiment. A total of 20 potato and 20 tomato plants were treated with phosphite and BABA (β -amino-butyric acid; Sigma) by spraying the canopy 24 hours before each set of experiment was carried out at the rates of 1.25% concentration of phosphite (Burra et al., 2014) and 1gL^{-1} of BABA (Liljeroth et al., 2010). The remaining 15 potato and 15 tomato

plants were left untreated and used as a control. Phenotypically similar plants were used in the experiment.

1.2.3. Olfactometer bioassay

The behavioral responses of PTM to potato and tomato plants were tested in a Y-tube olfactometer. The olfactometer was a Y-shaped glass tube with an arm of 14 cm and two side arms of 16 cm and 3 cm diameter. Activated charcoal-filtered and humidified air was pumped through the olfactometer arms. The airflow was set to 0.2 L min^{-1} and passed through Teflon tubes connected to each arm. To avoid visual distraction due to light the Y-tube was placed in a box and the positions of the odour sources were reversed between the two arms after each observation.

A total of thirteen different olfactometer experiments were conducted. First the whole untreated potato and tomato plant enclosed in a Teflon bag were tested for their attractiveness against an empty Teflon bag (control). Phosphite treated potato plant was compared against untreated potato and empty Teflon bag (control). BABA treated potato plant was compared against phosphite treated potato and untreated potato and tomato. Untreated and phosphite treated potato was compared with phosphite and BABA treated and untreated tomato. To simulate the natural environment for the insect all the plants were placed in the plastic bag along with the rhizosphere.

Mated female individual was introduced into the Y tube one at a time and their choices for the odours were tallied. A total of 60 adult moths were released per experiment. Individuals that did not decide within 5 min time were regarded as non-choosers. The Y-tube was replaced after

every five test and washed. Each insect was used only once in the experiment. The insects were deprived of food for 24 h before the bioassays. All trials were carried out in a walk in laboratory at room temperature.

1.2.4. Data Analysis

The total number of adult moths that made a choice for a particular odour were analysed using the Chi-square test to compare the attractiveness between the arms in the Y-tube at $\alpha=0.05$ using JMP 10. No choice data were excluded from this analysis.

1.3. Results

4.3.1. Attractiveness of phosphite and BABA treated potato plants to adult PTM

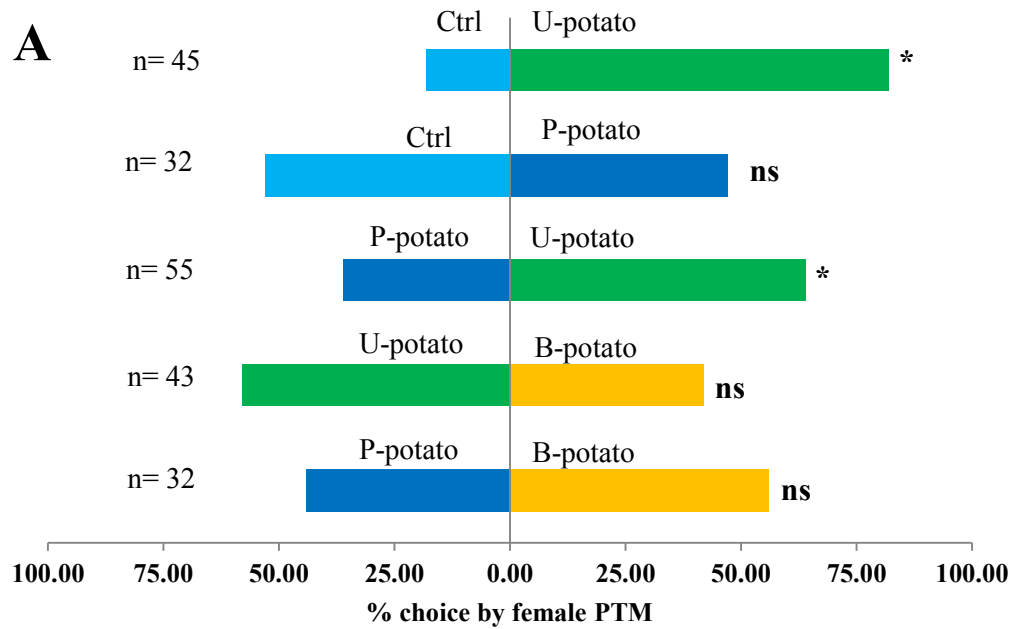
PTM adults were attracted to untreated potato over phosphite treated potato and control ($\chi^2=4.10$, d.f= 1, $p=0.043$ and $\chi^2=18.69$, d.f= 1, $p<0.0001$). However, adults respond equally between BABA and phosphite treated potato plants ($\chi^2=0.5$, d.f= 1, $p=0.48$). Similarly PTM showed no preference for untreated potato and phosphite treated potato over BABA treated potato and control respectively ($\chi^2=1.14$, d.f= 1, $p=0.29$ and $\chi^2=0.13$, d.f= 1, $p=0.72$) (Fig 4.1 panel A).

4.3.2. Attractiveness of phosphite and BABA treated potato and tomato plants to adult

PTM

PTM adults were attracted by phosphite and BABA treated as well as untreated potato compared to BABA and phosphite treated and untreated tomato plants ($\chi^2=19.57$, d.f= 1, $p<0.0001$; $\chi^2=16.2$, d.f= 1, $p<0.0001$; $\chi^2=5.76$, d.f= 1, $p=0.02$; $\chi^2=24.2$, d.f= 1, $p<0.0001$; $\chi^2=19.57$, d.f= 1,

$p < 0.0001$; $\chi^2 = 6.56$, d.f. = 1, $p = 0.01$ and $\chi^2 = 16.2$, d.f. = 1, $p < 0.0001$). The insects did not show any significant preference between untreated tomato and control, $p = 1$ (Fig 4.1 panel B).



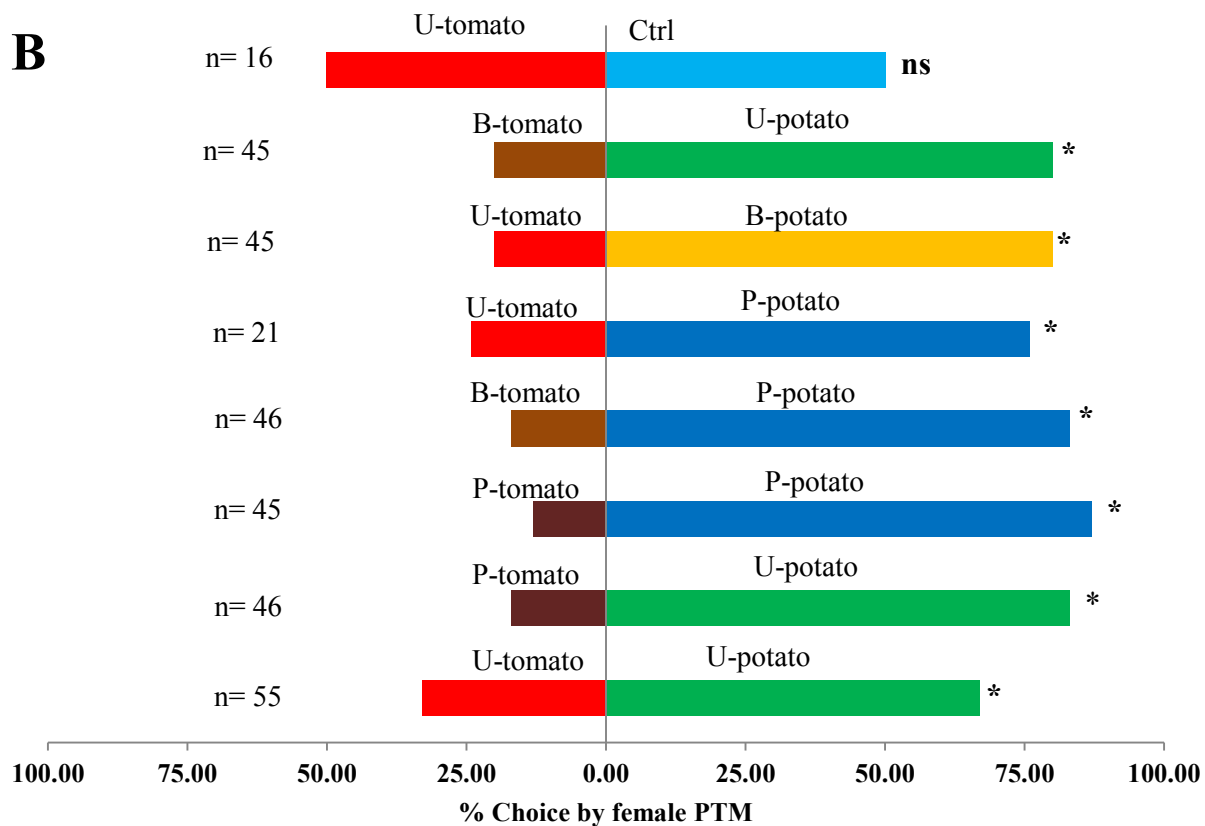


Figure 4.1 Responses of adult female PTM to potato and tomato plants in an olfactometer, U= untreated, P=Phosphite, B= BABA, Ctrl= control

1.4. Discussion

Our result revealed that both phosphite and BABA treated and untreated potato plants were preferred by mated adult female PTM over BABA and phosphite treated and untreated tomato. Adults, however, had no preference to phosphite treated potato over the control (empty bag). These indicated the greater preference of PTM to potato over tomato regardless of phosphite or BABA treatment. In agreement with the present study field and laboratory studies have corroborated that PTM showed preference to potato in the presence of tomato plants (Mulatu et al., 2007). Several studies have shown that in addition to the visual plant attributes such as growth form, leaf shape or color, host location process in insects is also attributed to plant

released volatiles (Bruce et al., 2004; Prokopy and Owens, 1983). Volatile compounds released from different potato cultivars have been shown to attract various potato pests (Bolter et al., 1997; Eigenbrode et al., 2002). Female Guatemalan moth, *T. solanivora*, responded to several Sesquiterpenes and Monoterpenes emitted from potato foliage (Karlsson et al., 2009). Electroantennographic studies (EAG) have shown the capability of the peripheral olfactory system of PTM adult to perceive a broad range of volatiles identified from potato leaves and tubers (De Cristofaro et al, 1999; Das et al, 2007). Therefore the observed preference of PTM to potato might be due to the nature of volatile compounds released from potato plants that attracted the insect more than tomato. The decision to feed or oviposit on a plant might also be depend on the plant defense status and the neighboring plant (Arab et al., 2007).

The presented results also show that female adult PTM had significant preference to phosphite untreated potato plant over the treated plant. As the preference of PTM between phosphite treated and untreated potato plants differ, it is possible that the larval density reduction observed in phosphite treated potato plants under field condition could be due to the change in the volatile compounds of the potato plant emitted after phosphite treatment which could repel ovipositing adults or affected larval performance and survival. Furthermore, the reduction could possibly be due to the indirect effect of phosphite treatment which is the induction of plant defense system. Leaf lignification, increased cell wall thickness, synthesis of secondary plant metabolites (Beta-1,3-glucanases) and increased enzyme activity of peroxidases are possible phosphite related indirect plant responses (Percival et al., 2009). A previous study has shown that phosphite application did not interfere with the oviposition of Colorado potato beetle. However, field treatment indicated that it reduced beetle number and potato defoliation as compared to untreated

plants; furthermore, phosphite dipped potato leaves showed longer development period and low beetle survivorship in a laboratory (Patterson and Alyokhin, 2014).

1.5. Conclusion

Plant volatiles are compounds which are used by plants for various purposes including defense against plant herbivorous. Volatiles are also used by herbivorous as a host plant cues. Plant volatiles emission can be induced by insect herbivores or synthetic compounds. We have shown that volatiles emitted by phosphite treated potato plant caused a change in the preference of PTM. Furthermore PTM preferred potato plant over tomato irrespective of phosphite or BABA treatment.

Chapter 5 Toxicity Evaluation of Phosphite in Different *Phytophthora Infestans* Isolates

5.1. Introduction

Plant disease causing pathogens evolve together with their respective host plants thus they develop a mechanism to cause infection (Ivic, 2010). Pathogens basically vary but they have something in common. Most of them possess spores which can germinate and penetrate into a plant tissue as they come in contact with their hosts under favorable conditions. Fungi and oomycetes cause a number of plant disease and hence brings about yield loss (Wegulo *et al.*, 2012). *P. infestans* the causative agent of the devastating late blight is a disease on solanaceous plants. The pathogen has been classified as high risk due to its evolutionary potential. A number of studies have shown the appearance of many new genotypes through sexual reproduction, which requires both mating types, and migration (Cooke *et al.*, 2003, Deahl *et al.*, 2003 and Hermansen *et al.*, 2000).

Fungicides are continuously applied to control fungal or oomycetes caused disease of various plant crops with the ultimate goal of increasing economic returns by preventing yield loss (Wegulo *et al.*, 2012). Most of the fungicides until the late 1960s and 1970s were protective/contact in nature, killing the fungal spores or interfering with growth on the plant surface (Ivic, 2010). The next groups of fungicides on the market were the systemic fungicides. Unlike the protective fungicides most of the systemic fungicides move through the plant xylem and some of them translocate through the phloem. A number of factors determine the performance of these fungicides such as fungicide's movement within the plant, application, dose and pathogen sensitivity (Ivic, 2010). In 1960-70's other groups of compounds nowadays often referred to as plant resistance inducers (PRIs) were also commercialized. PRIs work by activating plant's own defense mechanism against the encountered pathogen. They are active

against a number of pathogens such as bacteria, virus, fungi and oomycetes in *Solanum* species (Alexandersson et al., 2016).

Different morphological, physiological and biochemical changes have been seen in fungi and oomycetes based on the nature of the pathogen, fungicide applied and host plant. Some of the effects on the pathogen due to fungicides treatment are break down of subcuticular and intracellular mycelia, abnormal growth, change in hyphal morphology, swelling and distortion of hyphae, cytoplasm collapse, hyphal collapse, mycelial density reduction, abnormal cell shrinkage, break down of cell membrane, and haustoria deformation (Ivic, 2010). Suppression of spore production on infected plant tissues can be seen as an indirect effect of fungicide activity on fungi and oomycetes (Ivic, 2010).

Phosphite has a complex mode of action. It has both a direct (inhibition of fungal sporulation or slow development rate) and indirect (rapid and strong stimulation of plant defense) effect (Smillie et al., 1989; Grant et al., 1990; Guest and Bompeix, 1990; Guest and Grant, 1991; Jackson et al., 2000). Leaf lignification, increased cell wall thickness, synthesis of secondary plant metabolites (Beta-1,3-glucanases) and increased enzyme activity of peroxidases are some of the indirect plant responses after the application of phosphite (Percival et al., 2009; Machinandiarena et al., 2012; Lim *et al.*, 2013 and Eshraghi et al., 2011). *In vitro* studies have shown that phosphite, depending on concentration, directly inhibit *P. infestans* and *Streptomyces scabies*, *Rhizoctonia solani* and *Fusarium solani* growth on agar plates (Borza et al., 2014 and Lobato et al., 2010). The objective of the present study was to evaluate the toxicity of phosphite and investigate whether there are differences in sensitivity between *P. infestans* isolates,

Pi1.4.10, 88069, Pink6_H7, Blue13, SE-Halland_2.5 and two more collected in Ethiopia and further investigate its effect on sporangia germination between the different isolates.

5.2. Materials and methods

Phytophthora infestans isolates: SE-Halland_2.5 and Pi1.4.10 were collected in Sweden and kindly provided by Björn Andersson Department of Forest Mycology and Pathology, SLU, Sweden (Table 5.1). Isolate 88069 was collected in the Netherlands and Pink6_H7 and Blue13 were collected in the UK. Two isolates were also collected in Ethiopia from two different locations, Holetta and Addis Ababa. Each isolate was aseptically maintained in rye agar media (Caten and Jinks, 1968) and sporangia from 11-day old plates were used in the toxicity assays.

5.2.1. *P. infestans* isolation and media preparation

To isolate the Ethiopian strains, *P. infestans* directly, a small piece of infected leaf from the sporulating border, including a little bit of green tissue, was cut out and passed through a 5% commercial bleach solution for 30 seconds, then rinsed in distilled water twice, and dried off with filter papers. The leaf piece was then placed on top of sliced potato tubers and a rye agar medium amended with antibiotics, 125mg of doxycycline for 500ml of media. The plate, 9cm petridish, and tuber were incubated 18°C for 5-10 days, or until the fungus starts growing and feeding on the agar or tuber. Hyphal tips were then transferred to other rye agar plates both from the tubers and plates.

Rye agar medium was prepared according to the protocol in Grenville-Briggs et al., (2008). Sixty g of organic Rye seeds were surface sterilized using 1% chlorine bleach for 30-60 s in a beaker. The seeds were then washed with flowing water in a sieve until no bleach smell was left. The

washed seeds were spread in a box and soaked in water and kept for 16-24 h in dark at 25⁰C to germinate. Germinated rye seed were then macerated for 60 s using a blender after adding 200 mL of water until each seed was halved. Then it was incubated in a water bath for 3hrs at 50⁰C. Then it was transferred to a sieve and pressed slightly to get the nutrients out by adding water until it reached 1 L. It was then separated in to two 500 mL Duran bottles. A total of 3.75 and 10 g of bactoagar and sucrose was added to each bottle, respectively. It was autoclaved at 120⁰C for 25min afterward. When it cooled to 50⁰C antibiotics was added and poured onto petri dishes.

5.2.2. Determination of lethal concentration of phosphite against mycelial growth

To assess the effects of Phosphite on the different isolates of *P. infestans*, the pathogens were grown on rye-agar medium. Agar plugs of 7 mm diameter were taken from the edge of 11 days old cultures of *P. infestans* and placed mycelium side facing down at the center of 9 cm in diameter petri dishes containing 20 mL rye-agar medium supplemented or not (control) with different concentrations of potassium phosphite marketed as the product ProalexinTM (0.05, 0.2, 0.8, 1.6, 2.3, 3.2 and 6.4 mM) and then sealed with Parafilm. Each treatment had four biological replicates. The plates were incubated at 20⁰C and the radial mycelial growth, estimated by measuring the diameter of the growth area twice at right angles, was recorded five times: at 3, 5, 7 and 9 days post inoculation (dpi). In this experiment, percentage inhibition due to phosphite amendment was calculate using the formula

$$\text{Percentage inhibition (PI)} = (a-b)/a \times 100$$

Where a= average perpendicular colony diameter of the control plates

b= perpendicular colony diameter of an amended plate

5.2.3. Sporangia production estimation in inoculated plates

Three plates from each treatment of Pi1.4.0, 88069, Pink6_H7, Blue13 and SE-Halland_2.5 isolates containing *P. infestans* in the above experiment at 12 dpi were washed with 5 mL of sterile deionized water and the hyphal growth was gently scraped off with a plastic scraper in order to release the sporangia. The solution was first filtered through a cell strainer with 100 µm nylon mesh (BD Falcon, USA) to remove the hyphal material and then the density of sporangia was counted with a haemocytometer (FuchsRosenthal chamber).

Table 5.1 Isolates of *P. infestans* used in the toxicity

Isolate	Mating type	Origin
Pi 1.4.10	unknown	Sweden
88069	A1	Netherlands
Pink6-H7	A1	UK
Blue13	A2	UK
SE-Halland2.5	A2	Sweden
Unknown	unknown	Ethiopia, Holetta
Unknown	unknown	Ethiopia, Addis Ababa

5.2.4. *In vitro* sporangia germination test

The effect of phosphite on sporangial germination was assessed by inoculating the 88069, SE-Halland 2.5 and Pink6-H7 *P. infestans* isolates in 2 mL of phosphite supplemented pea broth at the concentrations 0.05, 0.2, 0.8, 1.6, 2.3, 3.2 and 6.4 mM in 24 well plates. The broth was prepared by boiling 120 g of Birds eye pea in 1 L of distilled water for one hour. The boiled pea was drained and filtered through muslin and the broth was maintained to a PH of 7.25 after NaOH was added and autoclaved. Petri dishes containing the 3 isolates of *P. infestans* grown for two weeks on a rye agar medium were washed with sterile deionized water and the hyphal growth was gently scraped with a plastic scraper in order to release the sporangia. The solution was filtered through a cell strainer with 100 µm nylon mesh to remove the hyphal material and

the density of sporangia was counted with a haemocytometer (FuchsRosenthal chamber) and the number of sporangia was adjusted to 15,000 sporangia mL⁻¹. One mL of the solution was transferred into each of the plates and incubated for 24 hours at 20°C. Four biological replicates were done. The percentage of sporangial germination was calculated by counting germinated and non-germinated sporangia of 10 µL of the incubated solution using a haemocytometer.

5.2.5. Data analysis

All the analysis were made using JMPIN 10 (SAS Institute, Inc. 2012) with alpha value set at 0.05. LC₅₀, the concentration of phosphite inhibiting growth by 50%, for each of the *P. infestans* isolate tested was calculated by plotting percentage inhibition against phosphite concentration at 3, 5, 7 and 9 dpi independently. Treatment effects on mycelial growth inhibition, sporangia production and sporangia germination were investigated with two-way full factorial ANOVA, where phosphite, isolates and their interaction were sources of variation. The effect of phosphite on mycelial growth inhibition, sporangia production and sporangia germination at different level of phosphite concentrations was evaluated with one-way ANOVA for each isolate independently. A posteriori multiple comparison tests (Tukey HSD test) was performed when significant ($p < 0.05$) differences between means were detected.

5.3. Results

5.3.1. Lethal concentration of phosphite against mycelial growth of different *P. infestans* isolates

Phosphite treatment, isolates and their interaction affected percentage growth inhibition significantly ($F_{7,7} = 881.7$, $p < 0.0001$, $F_{4,4} = 188.3$, $p < 0.0001$ and $F_{28,28} = 11.4$, $p < 0.0001$) at 9dpi. The highest inhibition was detected in Blue13 but it was not significantly different with

Pi.1.4.10. Pink6_H7 had the least percentage growth inhibition by phosphite which was significantly different with the other treatments. Furthermore no significant difference was observed between the isolates 888069 and SE-Halland2.5 (Fig 5.1). There was a direct relationship between mycelial growth inhibition and phosphite concentration in all the *P. infestans* isolates tested (Fig 5.2).

Estimated LC50 values for the five isolates ranged from 4.3 to 1.4 mM during the last sampling time, 9 dpi. Results showed that the highest LC50 value was observed for the isolate Pink6_H7 meaning that this isolate is the most phosphite tolerant. The lowest value was observed in the isolate Blue13, 1.4mM. Furthermore, the data showed that the efficiency of phosphite in inhibiting *P. infestans* increased along with time except in the isolate 88069 and Pink6_H7, where LC50 increased at the last sampling point (Table 5.2). Similarly, the Ethiopian isolates had shown direct relationship between mycelial growth inhibition and phosphite concentration (Fig 5.2). During the last sampling time, 9 dpi, the Holetta isolate had higher LC50 value (3.3 mM) than the Addis Ababa isolate (2.6mM) which showed that Holetta is less sensitive to phosphite treatment than that of Addis Ababa. In addition, growth inhibition efficiency of phosphite increased along with time in both the isolates (Table 5.2).

Table 5.2 Lethal concentrations (LC50) of phosphite for 50% inhibition in the mycelial growth of *P. infestans* isolates at 3, 5, 7 and 9 dpi

<i>P. infestans</i> isolate	LC ₅₀ 3dpi	LC ₅₀ 5dpi	LC ₅₀ 7dpi	LC ₅₀ 9dpi
88069	2.70	2.15	1.60	2.00
Blue13	1.17	1.01	1.41	1.40
SE-Halland2.5	3.05	2.53	2.19	2.10
Pi.1.4.10	2.10	1.83	1.79	1.50
Pink6_H7	4.14	3.52	3.47	4.30
Holetta	5.30	3.10	3.50	3.30
Addis Ababa	3.10	2.80	2.70	2.60

LC50 is in mM

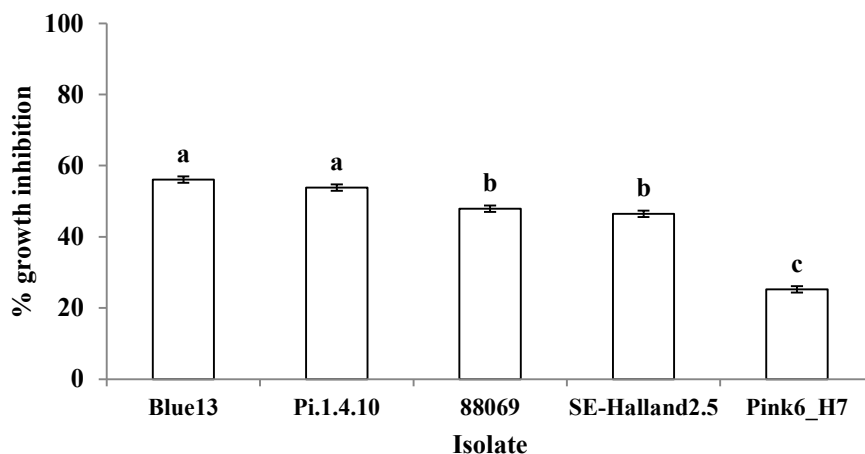
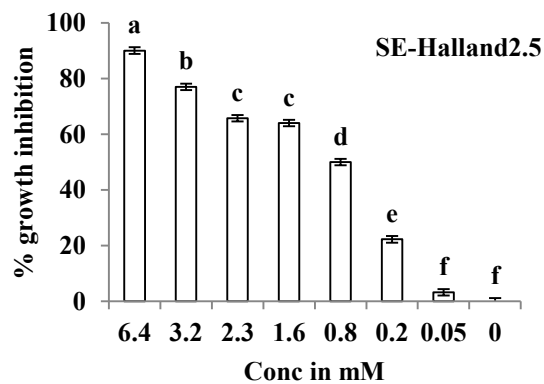
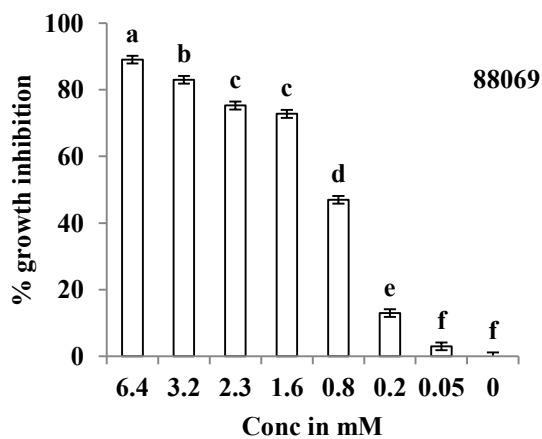
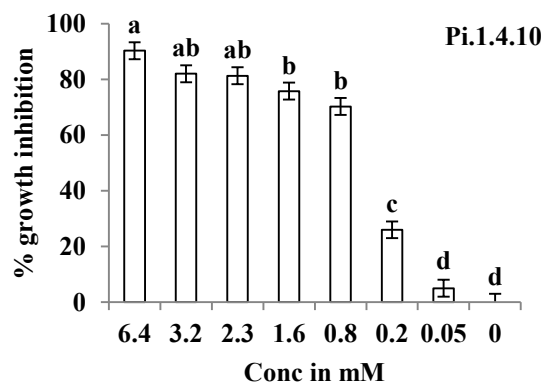
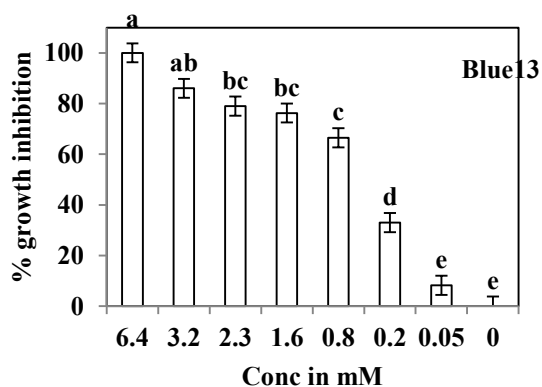


Figure 5.1 Growth inhibition of five *P. infestans* isolates by potassium phosphite in percent at 9 dpi. Bars with the same letters are not significantly different by Tukey-Kramer HSD at a p value of 0.05



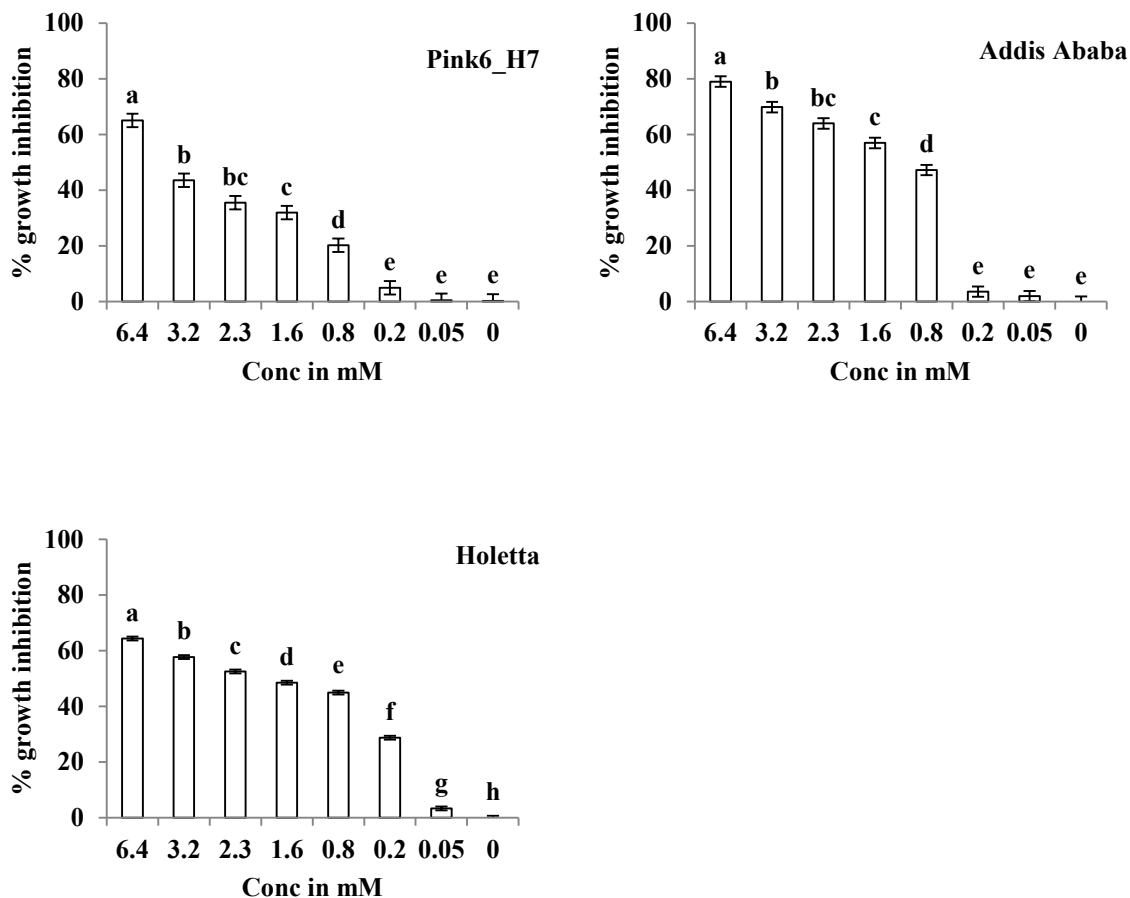


Figure 5.2 Growth inhibition of *P. infestans* isolates by different concentration levels of potassium phosphite in percent at 9 dpi. Bars with the same letters are not significantly different by Tukey-Kramer HSD at a p value of 0.05

5.3.2. Sporangia produced in phosphite amended plates inoculated with different *P. infestans* strains

The results showed that both phosphite concentration, isolate and their interaction had significant effect on sporangia production ($F_{7,7} = 644$, $p < 0.0001$, $F_{4,4} = 655$, $p < 0.0001$ and $F_{28,28} = 51$, $p < 0.0001$). Pink6_H7 had the highest sporangia production and it was also significantly different with the remaining isolates. The lowest sporangia production was in the isolate Blue13. Pi1.4.10 and 88069 had similar sporangia production (Fig 5.3). Increase in phosphite concentration

reduced sporangia production. Except the isolate Pink6_H7, the remaining isolates at the highest concentration, 6.4mM, did not produce sporangia (Figure 5.4). In Pink6_H7 the highest concentration of phosphite was significantly different from the rest of the other concentrations, which also were not significantly different from the no-phosphite control plate. In Blue13 there were no sporangia at all phosphite concentrations above 0.2 mM phosphite. Furthermore, Pi1.4.10 and 88069 isolates there did not produce any sporangia above 0.8 and 1.6 mM, respectively. At the two lowest concentrations of the strains 88069, Halland2.5 and Pi1.4.10, were not significantly different compared to the control treatment (Fig 5.4). There was a significant positive correlation between percentage growth and sporangia production in all the isolates (Table 5.3).

Table 5.3 Correlation between sporangia production (log-scale) and percent growth of five different *P. infestans* isolates

Pairs	<i>Phytophthora infestans</i> isolates	r	P
Sporangia production vs % growth	88069	0.86	<0.0001*
Sporangia production vs % growth	Blue13	0.81	<0.0001*
Sporangia production vs % growth	Halland2.5	0.76	<0.0001*
Sporangia production vs % growth	Pi.1.4.10.	0.84	<0.0001*
Sporangia production vs % growth	Pink6_H7	0.78	<0.0001*

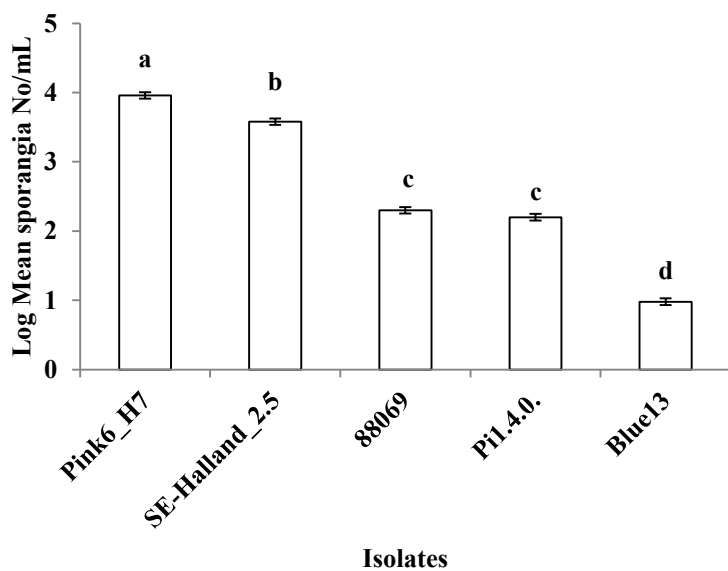
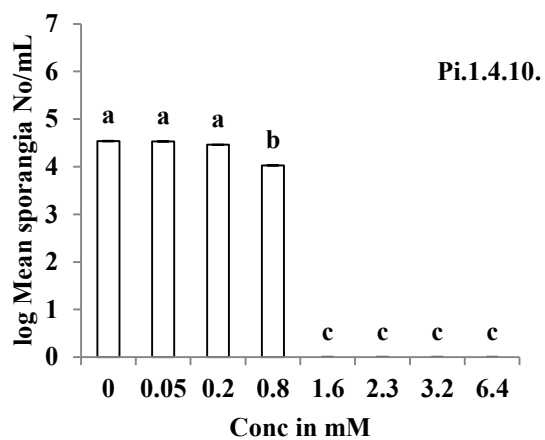
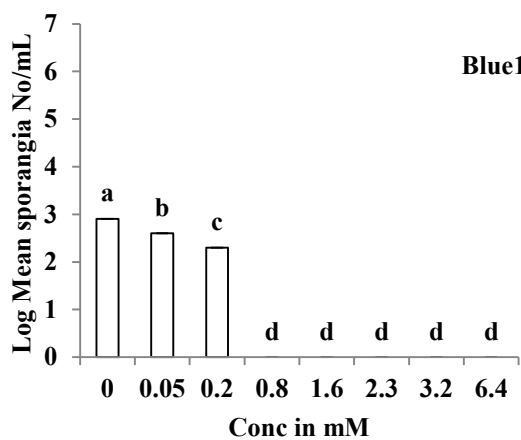


Figure 5.3 Sporangia produced from plates: Sporangia per mL of five isolates of *P. infestans* at 12 dpi. Letters indicate significance differences by Tukey-Kramer HSD at $p < 0.05$.



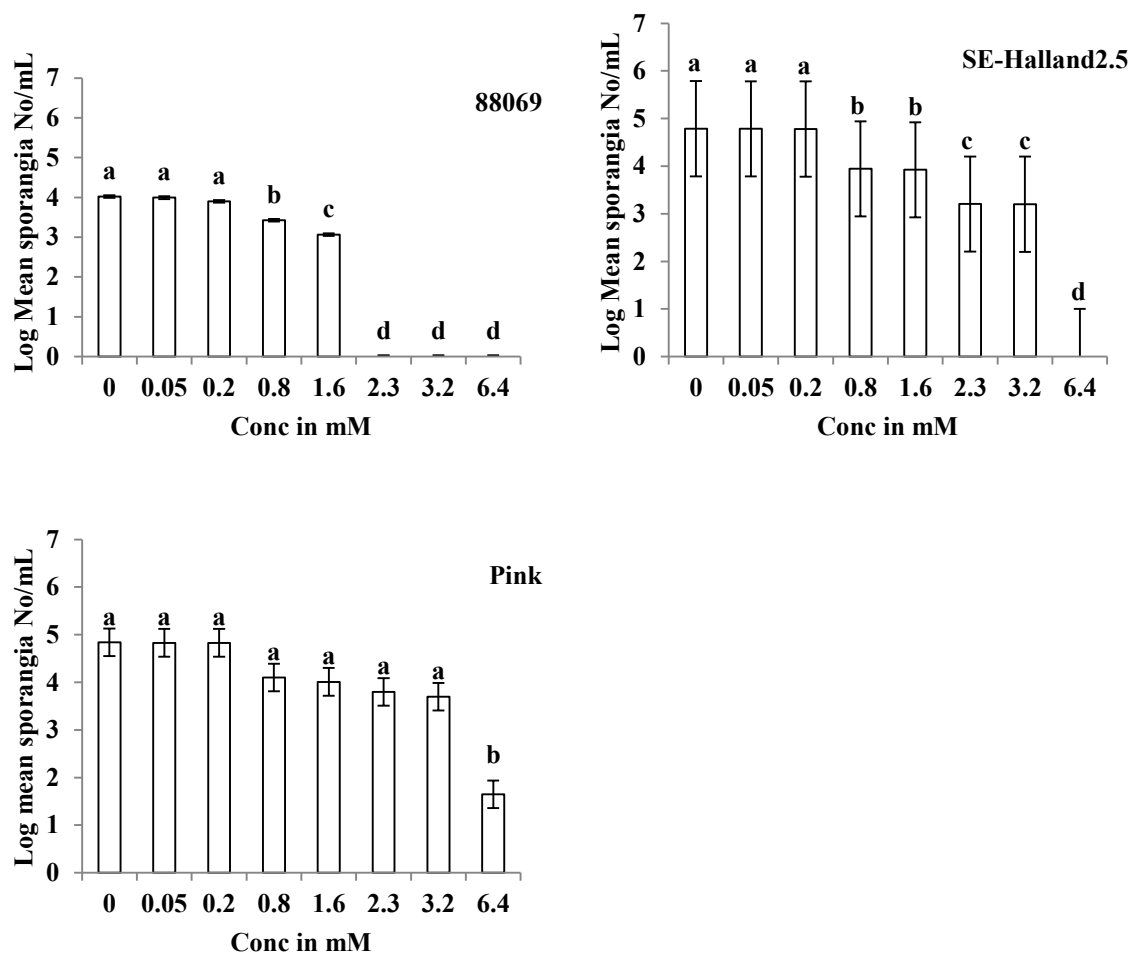


Figure 5.4 Sporangia produced from plates: Sporangia per mL of five strains of *P. infestans* at different concentration levels of phosphite at 12 dpi. Groups that do not share a letter are significantly different by Tukey-Kramer HSD at p value of 0.05

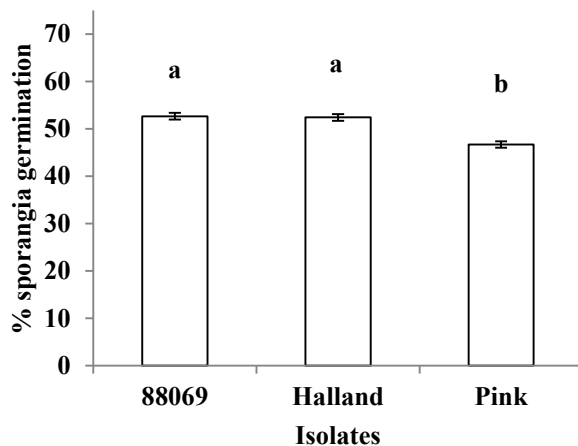


Figure 5.5 Sporangia germination: Number of germinating sporangia after growth in different concentrations of phosphite. Groups that do not share a letter are significantly different Tukey-Kramer HSD at p value of 0.05

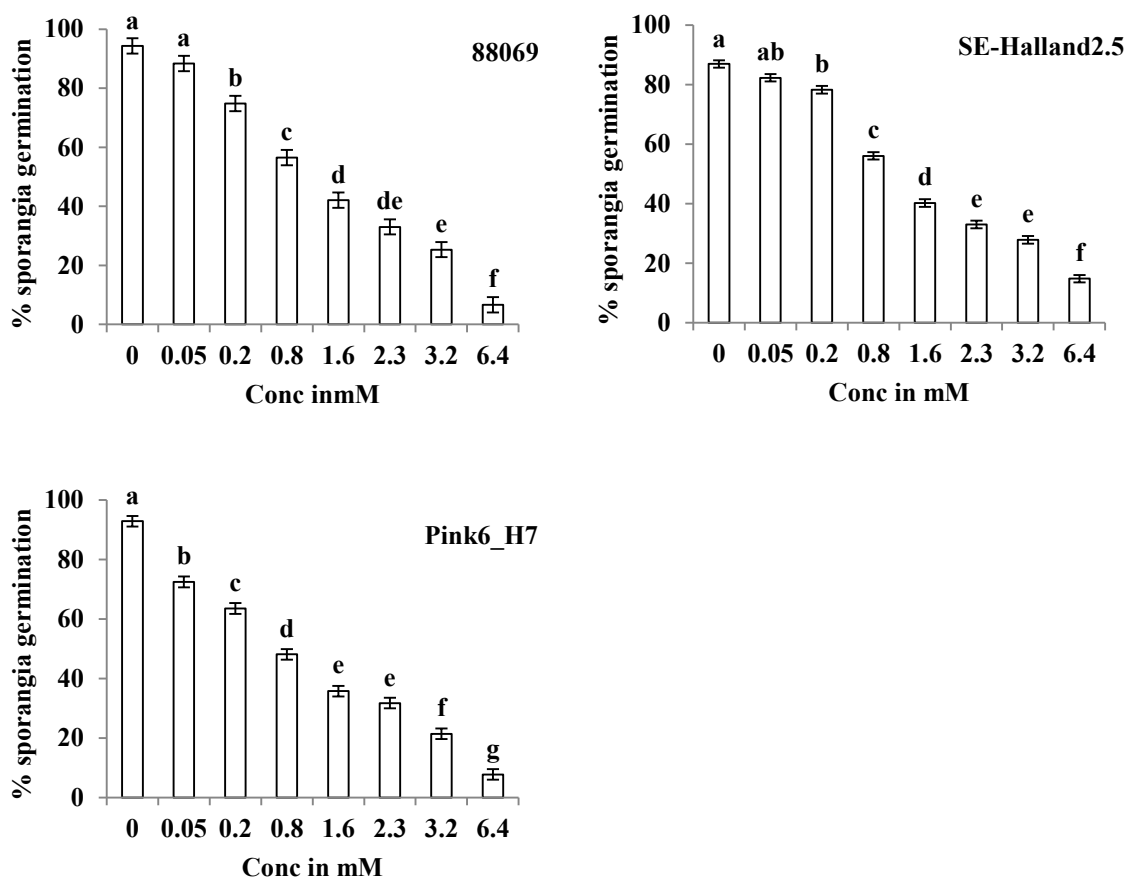


Figure 5.6 Percentage sporangia germination: Number of germinating sporangia after growth in different concentration levels of phosphite. Groups that do not share a letter are significantly different Tukey-Kramer HSD at p value of 0.05

5.3.3. *In vitro* sporangia germination test

The mean percentage sporangia germination was significantly affected by the phosphite treatment, isolates and their interaction ($F_{7,7}= 175.21$, $p<0.0001$, $F_{2,2}= 7.83$, $p= 0.0011$, and $F_{14,14}= 2.73$, $p= 0.0049$). The number of germinating sporangia significantly varied between isolates. Isolate 88069 had significantly higher percentage of sporangia germination compared to Pink6_H7 but no variation was seen with that of SE-Halland2.5 (Fig 5.5). The mean percentage sporangia germination of the three tested isolates varied with concentration and decreased with the increased phosphite concentration. Sporangia germination was very high in the control and at the lowest concentration, 0.05 mM, of 88069 and SE-Halland2.5. The highest concentration, 6.4 mM, had the least mean percentage sporangia germination in all the tested isolates. Therefore the effect of phosphite on sporangia germination was dose dependent (Fig 5.6).

5.4. Discussion

The study showed that the isolates tested had sensitivity differences to phosphite treatment. The isolate Blue13 had the lowest LC50 value and isolate Pink6_H7 the highest. Isolates 88069, Blue13, SE-Halland2.5 and Pi.1.4.10 were sensitive to potassium phosphite treatment and had LC50 values ≤ 2 mM at 9 dpi. Isolate Pink6_H7 was less sensitive than the rest of the isolates and had EC50 value greater than 4mM at 9 dpi. Using plant resistance inducers like phosphite has the possibility of decreasing selection pressure due to the nature of mode of action which involves both direct and indirect activity (Alexandersson et al., 2016). Resistance by *P. infestans* isolates against phosphite has not been reported so far. However, Dobrowolski et al., (2008) have shown that prolonged use of phosphite results in a decreased in sensitivity of *Phytophthora cinnamomi* to phosphite treatment. In the study, different *P. cinnamomi* isolates collected from an avocado orchard that had a long history of phosphite use were shown to highly colonize

phosphite treated *Leucadendron* sp., lupin seedling roots and *Eucalyptus sieberi* cotyledons (Dobrowolski et al., 2008). Therefore, intensified use of phosphite in potato cultivation may lead to increased resistance of *P. infestans* against phosphite salts.

Of the two isolates of Ethiopian origin, the one from Addis Ababa was more sensitive to phosphite than that of from Holetta with about one order magnitude LC50 difference between the two. Holetta is known for intensive production of potato so the *P. infestans* isolate from this area is under high fungicide pressure with a possibility of increased fitness and population structure difference with Addis Ababa. Therefore the observed relative insensitivity of the isolate from Holetta might be due to the change in the vulnerability of the pathogen due to successive exposure to fungicides with similar mode of action such as Ridomil or Mancozeb (Brent and Hollomon, 2007). Even though we used phosphite for the first time in Ethiopia, three years field study at Holetta indicated that phosphite was less effective as compared to sole Ridomil and synergism between phosphite and Ridomil. However, phosphite treatment still provided a clearly better protection than the untreated control treatments (see chapter 6).

Moreover, the effect of potassium phosphite observed on the tested isolates was highly dependent on dose. Even though there was variation among isolates; the direct inhibitory effect of phosphite on the mycelial growth peaked at the highest concentrations, 6.4 mM, in all the isolates tested. These findings are in agreement with a previous study which showed the antimicrobial nature of phosphite (CaPhi, KPhi and CuPhi at concentration of 0 up to 80.95, 72.35 and 65.75mM respectively) in different groups of plant disease causing pathogens: *P. infestans*, *F. solani*, *R. solani* and *S. scabies* (Lobato, et al., 2010). Their results showed that *P. infestans* was the most inhibited of the pathogens tested and had the lowest EC50 values.

Furthermore, they found that the observed inhibition was totally dose dependent; the highest inhibition was obtained at the maximum concentrations. Copper phosphite completely inhibited the growth of *P. infestans* at lower concentration, 6.58 mM. Similar result was obtained by Borza et al., (2014) when they compared different concentrations of phosphite on the average mycelial growth of *P. infestans* at various time points on plate assays. The dose dependent inhibition observed in their experiment might be due to the phosphite anion differences between the concentration, the fact that phosphite accumulation in leaves and tubers depends on the frequency of applications and the concentration of phosphite used. For instance high performance ion chromatography (HPIC) result indicated four times application of 1% phosphite caused 890 mg g⁻¹ of fresh tissue phosphite while twice application of 0.5% caused only 167 mg g⁻¹ of fresh tissue phosphite in potato leaves (Borza et al., 2014). The dose dependent response to phosphite is also seen in other species of phytophthora. For instance *P. palmivora* and *P. cinnamomi* mycelial growth was reduced with increased phosphite concentrations (Griffith et al., 1993 and Wilkinson et al., 2001). Species with mating types have been reported to show variation in their sensitivity to phosphite; in an *in vitro* test of the 72 A1 and A2 isolates the A1 mating types of *P. cinnamomi* were more tolerant (Wilkinson et al., 2001). However, in our study we cannot say that phosphite effect is dependent on the mating type of the tested *P. infestans* isolates because we tested only a few isolates of which only four have known mating types. However, the isolates reacted differently to the dose dependent application of phosphite, particularly isolate Pink6-H7 (A1 mating type) was exceptionally tolerant. From these we can conclude that phosphite efficacy might be affected by the mating type, which requires further investigations.

The significant positive correlation between phosphite concentration and reduction of mycelial growth of *P. infestans* isolates indicated that the direct, dose-dependent effect of phosphite played a major role in the inhibition process. The efficacy of phosphite on plants and under field condition is not only dependent on the direct effect but it also involves indirect effect by stimulation of plant defense response (Cohen and Coffey, 1986; Panicker and Gangadharan, 1999). Massoud et al., 2012 showed the effect of phosphite in Arabidopsis (*Arabidopsis thaliana*) against the oomycete *Hyaloperonospora arabidopsidis* (Hpa) to be dependent on the concentration of phosphite used. At low concentration (<10mM) the plant responded indirectly by triggering the plant immunity and at higher concentration (>50mM) it led to both direct, mycelial growth inhibition, and indirect effect by induced resistance in the plant. Although phosphite is systemic and reaches all parts through acropetal and basipetal movements, a molecular study in potato showed that a direct leaf contact is needed for foliar protection to occur against *P. infestans* (Burra et al., 2014). Therefore, under field conditions the indirect effect of phosphite could possibly be observed at a lower concentration. Phosphite treatment also reduced potato tuber damage by *P. infestans* and residuals up to 35% of the total amount of phosphite applied was detected in tubers after five months storage (Liljeroth et al., 2016). The observed tuber blight protection could be related to the accumulation of phosphite in tubers.

The phosphite treatment not only inhibited the mycelium of *P. infestans* but also sporangia production. Similar to the mycelial growth, the sporangia reduction observed was isolate specific and dependent on the concentration of phosphite. At higher concentration no sporangia was produced in all the isolates tested, except Pink6-H7. This illustrates how the effect of phosphite varies among isolates. Borza et al., 2014 showed that phosphite treatment affects sporangia production in a dose-dependent manner in a pea-agar medium, and no sporangia production was

detected at the highest concentration, 500 $\mu\text{g mL}^{-1}$ (6 mM), of phosphite, which is similar to our observation.

As expected, sporangia germination was significantly different between concentrations in the three isolates tested with the largest number of sporangia present in the untreated control. However, sporangia of some *P. infestans* isolates were capable of germinating even at the highest phosphite concentrations. This effect was similar to the detached leaf assays by Borza *et al.*, 2014. They reported a dose dependent delay in sporangia development by phosphite treatment on potato leaves, with sporangia development observed at 10, 6 and 5 dpi in 1%, 0.5% phosphite and control treatments, respectively. Unlike the *in vitro* plate assays, field application of phosphite often involves several consecutive applications, with an expected accumulation of phosphite ions in the plant organs which increased protection.

Generally, the observed direct mycelial inhibition and suppression of sporangia production and growth together with the indirect phosphite activity in combination seem enough to hinder growth and reproduction of the pathogen, which ultimately leads to sufficient protection to secure yield and good quality. The efficiency under field conditions, however, can be expected to be isolate dependent.

5.5. Conclusion

Phosphite acts against *Phytophthora* pathogens both directly and indirectly. The direct effects could be inhibition of mycelial growth, sporangia production and sporangia germination. In the present study we showed that the mycelial growth, sporangia production and germination of the tested isolates of *P. infestans* were differentially inhibited in response to phosphite treatment in a

dose dependent manner, *in vitro*. Therefore, we conclude that the effect of phosphite to oomycetes is dependent on the isolate present and the concentration of phosphite used.

Chapter 6 Phosphite protects Potatoes and Tomatoes against Late Blight tropical cool highland

6.1. Introduction

Potato, *Solanum tuberosum* L., is the most important starchy edible tuber crop with high nutritive value. It is the third most consumed food crop in the world after rice and wheat. More than a billion people worldwide eat potato (<http://cipotato.org/potato/facts/>). About 325 million tons of potato is produced in the world over an area of 19.3 million ha in 2012 (Birch et al., 2012). Potato is a very important food and cash crop especially in the highland and mid altitude areas of Ethiopia (Borgal et al., 1980 and Haverkort et al., 2012). Potato is thought to have been introduced to Ethiopia some 150 years ago, whereas tomato came much later. The dependence on potato has dramatically increased and it is now the 9th most important crop in Ethiopia and is grown on about 179,000 ha area (CSA, 2014). However, due to various reasons productivity is quite low, 8-10 tons ha⁻¹, compared with the country's potential (Haverkort et al., 2012) and the global average productivity, which is 16.8 tons ha⁻¹ (FAO, 2008).

Tomato (*Solanum lycopersicum* Mill.) was introduced into Ethiopian agriculture in the period between 1935 and 1940. The area devoted for tomato production has been increasing since then. In 2013 the total production area reached 10,882.83 ha. Currently, tomato is one of the regional export crops of the country. Similar to potato, the average tomato productivity in Ethiopia is very low, 7 tons ha⁻¹, compared to production in America, Europe, Asia which have average yields of 51, 41, and 36 tons ha⁻¹, respectively (Balcha et al., 2015). According to Haverkort et al., (2012) the main production constraints affecting potato production and productivity in Ethiopia are poor seed quality, farmers' poor management practice and disease pressure. Shortage of alternative chemical fungicides supply is also another problem affecting potato and tomato production (Ethiopia late blight profile, 2004). Even those who have access to chemicals lack the right skills

on how to use the chemicals. Furthermore, low use of personal protective devices to avoid health hazard using pesticides has been reported (Mulatu, 2011), which could affect human health and the environment.

Late blight caused by the oomycete *P. infestans* (Mont.) de Bary, early blight caused by *Alternaria solani*, potato bacterial wilt (*Ralstonia solanacearum*), and different potato virus diseases such as Potato virus S (PVS), Potato virus Y (PVY), Potato virus M (PVM), Potato virus X (PVX) and Potato leaf roll virus (PLRV) are the major threats of potato production. However, late blight is the number one farmers concern (Haverkort et al., 2012 and Bekele et al., 2011). The second most affected crop by late blight is tomato (Ethiopia late blight profile, 2004).

The oomycete *Phytophthora infestans* is one of the most destructive pathogen affecting both potato and tomato crops. In Ethiopia, the disease is widespread all over the major potato producing areas, which makes it hard to produce during the main rainy season without application of chemicals (Bekele and Geberemedhin, 2000). Therefore, a shift in time of production of potato has been observed from the main season to the off season in order to avoid late blight development (Haverkort et al., 2012). Losses in potatoes due to the disease were estimated to be 65-70% and complete crop failures has also been reported (Bekele and Yaynu, 1996).

Use of fungicides and late blight resistant cultivars are the major control strategies in the country. Integrated disease management of late blight, which combines host resistance, cultural practices like early planting, and reduced fungicide use, has also been tried (Ethiopia late blight profile, 2004). However, the availability of fungicide and the understanding of farmers and shopkeepers on good practice of fungicide use are very limited. For instance Mancozeb and a co-formulation

of Mancozeb and Metalaxyl-M are the only available fungicides which are being used by farmers against late blight (Haverkort et al., 2012). There is fear that over use of these fungicides may induce resistance in *P. infestans*. In Ethiopia there are some reports mentioning that these fungicides have been applied up to 20 times per cropping season to control different fungal diseases. Such level of use is unheard of and is certainly creating a selection pressure for the emergence of pesticide resistant races of late blight causing oomycetes in potatoes and tomatoes (Personal communication, Bayeh Mulatu, 2016). The producer of Ridomil Gold (Mancozeb + Metalaxyl_M) cautions that insensitive fungi strains may be found due to repeated use of this fungicide. Furthermore the susceptibility of potato cultivars to *P. infestans* infection and the poor management of potato crops by farmers are the other challenges constraining potato production in Ethiopia (Haverkort et al., 2012). Therefore there is a need to investigate alternatives to the use of the above synthetic fungicides in the country in order to reduce the risk of promoting the occurrence of resistant strains of oomycetes and also reduce the effects of the fungicides on human health due to low use of protective devices and miss use and for the environment. For instance the use of induced host plant resistance in potatoes against *P. infestans* can be considered as one option to include in the list of options that may be used to manage late blight on potatoes.

Induced resistance, state of enhanced defensive capacity elicited by specific stimuli, whereby the plant's innate defenses are potentiated against subsequent biotic challenges, has been recommended by different authors. And it works against pathogens and parasites, including fungi, oomycetes, bacteria, viruses, nematodes, parasitic plants, and even insect herbivores (Vallad and Goodman, 2004).

In plants, induced resistance can be triggered by subjecting the plants to virulent, avirulent, and nonpathogenic microbes or artificially by inducers like benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), 2,6-dichloroisonicotinic acid (INA), chitosan (CHT), salicylic acid (SA), methyl salicylate (MeSA), ethylene, BABA, phosphite, probenazole, and methyl jasmonate (MeJA), etc. (Ramamoorthy et al. 2001; Vallad and Goodman, 2004; Walters et al. 2005; Mandal, 2010). Different authors have shown the importance of BABA and phosphite in inducing plant resistance against *P. infestans* (Cohen, 2002, Cooke and Little, 2002; Andreu et al., 2006, Lobato et al., 2008, Burra et al., 2014). Phosphite has also been shown to be effective under field conditions in developing countries in suppressing foliar potato late blight (Kromann et al., 2012). A very recent study has shown the efficiency of phosphite combined with conventional fungicides in suppressing potato late blight. According to their results phosphite and its combination with fungicides (Shirlan, Ranman Top, Revus, Epok and Infinito) had improved defense against *P. infestans* equally with use of only conventional fungicides in potato (Liljeroth et al., 2016). Despite the need to control late blight on potato and tomato, to date few alternatives to fungicides are in use to control late blight on potato and tomato. In line with this no phosphite products, either solitary or in combination with synthetic fungicides has ever been tested to try and control late blight on potato and tomato in Ethiopia. Therefore this study was carried out to determine the efficacy of phosphite product in controlling late blight on potato and tomato in a tropical highland environment. In the present study, we evaluated the efficiency of phosphite and combinations of phosphite with Ridomil against late blight on potato and tomato and measured the yields obtained from these treatments.

6.2. Materials and methods

6.2.1. Experimental design and field setup

Field trial was conducted during the main cropping seasons of 2014 at Holeta Agricultural research center (HARC), Holeta, Ethiopia. In the years 2015 and 2016 the experiment was repeated with the number of chemical treatments increased by 2 more treatments than the previous year.

Two potato cultivars with different level of resistance to late blight were used: Jalene, susceptible to late blight but with a wide range of environmental adaptations in Ethiopia and Belete (CIP-393371.58), resistant, released in 2009 (MoARD, 2009). Seed Potato tubers, supplied by Holeta Agricultural Research Center (HARC), were hand-planted at 30cm and 75cm within and between rows spacing, respectively. Distance between plots within a block was 1m and blocks were separated by 1.5 m space. The treatments were arranged in a randomized complete block design within block replicated four times.

The plots were fertilized with di-ammonium phosphate (DAP) and urea applied during planting at the rate of 195 kg/ha and 165 kg/ha, respectively. Starting with the appearance of the first late blight symptom, foliar application of phosphite (Proalexin, LMI AB, Helsingborg), synthetic fungicide (Ridomil gold) and combination of phosphite and the fungicide was made for 10 consecutive weeks using a manually-pumped knapsack sprayer of 15 liter capacity. There were a total of 6 treatments including the recommended doses of phosphite and the fungicide, doubled the recommended dose of phosphite, different combinations of phosphite and fungicide and untreated control (Table 6.1).

Table 6.1 Descriptions of treatments for the field experiment

Treatment	Active ingredient	Dose
Phosphite	K ₂ PO ₃	5L/ha (recommended dose)
Phosphite	K ₂ PO ₃	10L/ha
Phosphite + Ridomil gold	K ₂ PO ₃ , Mancozeb, Metalaxyl	2.5L/ha + 1.25kg/ha
Phosphite + Ridomil gold	K ₂ PO ₃ , Mancozeb, Metalaxyl	3.75L/ha +0.625kg/ha
Ridomil gold	Mancozeb and Metalaxyl	2.5 kg/ha
Untreated control	-	-

With the same field trial procedure and consideration of the six treatments described in Table 6.1 another experiment was set and executed on tomato in two consecutive years, 2015 and 2016. The cv. Melkasholla, with moderate susceptibility to late blight, obtained from Melkassa agricultural research center (MARC) was used (MoARD, 2009).

6.2.1.1. On-farm verification of phosphite on potato

Four farmers' fields with a size of 15m*15m were selected randomly within the vicinity of HARC to which potato cv. Jalene was planted following similar protocols as in the trials described above. Treatments applied against late blight were recommended dose of the fungicide Ridomil and combination of half the recommended dose of phosphite and Ridomil. The experimental plots were monitored daily and application of treatments started following the appearance of the first late blight symptom and made every three week with a total of four applications over the season.

6.2.2. Foliar late blight and potato and tomato yield assessment procedure

With the onset of the first late blight symptom, 50 inner row plants within each plot were evaluated visually for foliar infection occurrence and was continued on weekly interval and

carried out for ten more subsequent weeks. Late blight infection was scored on the basis of the number of infected leaflets out of the whole leaves in a sampled plant following the assessment key developed by Syren and Wiik (1993), which was modified by the EPPO (European and Mediterranean Plant Protection Organization) procedure and scale (OEPP/EPPO, 2004).

At maturity, the vines were removed and after 15 days potato tubers from inner rows on each plot for each treatment were dug out by hand. Based on the presence or absence of blighted tubers and size, tubers were sorted to marketable and unmarketable categories and weighed. Similarly, tomato fruits were hand-picked as they mature and sorted as marketable and unmarketable and weighed.

6.2.3. Data analysis

The area under the disease progress curve (AUDPC) for both potato and tomato was calculated for each plot with the following standard formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(Y_i + Y_{i+1})/2] [X_{i+1} - X_i]$$

Where Y_i is disease severity in percent at the i^{th} observation, X_i is time (days) at the i^{th} observation and n is the total number of observations. The maximal area under the disease progress curve was calculated as:

$$\text{maxAUDPC} = \sum_{i=1}^{n-1} [(100)(X_{i+1} - X_i)]$$

Relative AUDPC (rAUDPC) was calculated as:

$$\text{rAUDPC} = \text{AUDPC} / \text{maxAUDPC}$$

Treatment effects on rAUDPC and potato yield were investigated with a full factorial two-way ANOVA using JMP SAS, version 10. In the model, block was treated as random effect and chemical treatment, variety and their interactions were considered as sources of variation. Both rAUDPC and yield of tomato trials and farmers' field trials were analyzed using one way ANOVA. Means were compared using Tukey's HSD test. In both tomato and potato year effect

on rAUDPC and yield was assessed using T-test between the years 2015 and 2016. The significant level was adjusted to $\alpha = 0.05$.

6.3. Results

6.3.1. Effects of phosphite and synergism with fungicide on foliar *P. infestans* and yield on potato in 2014

The main effects (variety and chemical treatments) had significant effect on the rate of late blight development, measured in terms of rAUDPC ($F_{1,21} = 396.90$, $F_{3,21} = 1200.09$ and $p < 0.0001$). There was also a significant interaction between the two factors, chemical treatment and cultivar, ($F_{3,21} = 247.35$, $p < 0.0001$) (Table 6.2) where the variety Jalene had higher level of late blight infection than Belete. In relation to the chemical treatment, untreated control plants were highly infected by late blight especially in the cultivar Jalene where infection reached almost 100%. In the control treatments untreated Jalene was highly susceptible to *P. infestans* than the untreated Belete. Likewise, the two cultivars responded differently for phosphite treatment, where rAUDPC value was lower in the cultivar Belete due to phosphite treatment than Jalene. Furthermore, treatment with phosphite at the recommended dose resulted in higher rAUDPC in Jalene compared to recommended dose of Ridomil and combination of half dose of phosphite and Ridomil in both cultivars (Table 6.3). The efficacy of combination of half the recommended dose of phosphite and Ridomil was as effective as the full dose of Ridomil, therefore we decided to reduce the dose of Ridomil by 75% in the following year's trials.

Table 6.2 The effect of variety, chemical treatment and their interaction on relative area under disease pressure curve (rAUDPC) in potato at Holetta

Year	Sov	Nparm	DF	DFDen	F Ratio	Prob > F
2014	Variety	1	1	21	396.90	<.0001*
	CT	3	3	21	1200.09	<.0001*
	Variety *CT	3	3	21	247.35	<.0001*
2015	Variety	1	1	33	12.17	0.001*
	CT	5	5	33	133.67	<.0001*
	Variety *CT	5	5	33	1.53	0.21 ^{ns}
2016	Variety	1	1	33	50.02	<.0001*
	CT	5	5	33	2008.70	<.0001*
	Variety *CT	5	5	33	22.64	<.0001*

CT= chemical treatment, ^{ns} not significant and * significantly different at $\alpha=0.05$

Table 6.3 Effects of phosphite, Ridomil, combination of the two and cultivars represented as relative area under disease pressure curve (rAUDPC) of potato late blight and yield at Holeta in 2014

Treatment	rAUDPC	Mean MRK ¹ yield (tons ha ⁻¹)	Mean UNMRK ² yield (tons ha ⁻¹)	Mean total yield (tons ha ⁻¹)
Jalene				
Untreated Control	0.470 ^{a*}	10.00 ^c	0.25 ^a	10 ^c
5L/ha phi	0.070 ^c	23.25 ^b	0.25 ^a	24 ^b
2.5kg/ha Rid	0.004 ^d	48.75 ^a	0.00 ^a	49 ^a
2.5L/ha phi + 1.25kg/ha Rid	0.004 ^d	42.75 ^a	1.25 ^a	44 ^a
Belete				
Untreated Control	0.170 ^b	27.00 ^b	1.00 ^a	28 ^b
5L/ha phi	0.013 ^d	33.50 ^b	0.50 ^a	34 ^b
2.5kg/ha Rid	0.001 ^d	48.25 ^a	1.00 ^a	49 ^a
2.5L/ha phi + 1.25kg/ha Rid	0.003 ^d	47.75 ^a	1.00 ^a	49 ^a

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

There was significant effect of crop variety, chemical treatment and their interaction on marketable and total potato yield ($F_{1,21}= 24.23$, $F_{3,21}= 77.33$, $p< 0.0001$, $F_{3,21}= 5.36$, $p= 0.007$ and $F_{1,21}= 25.31$, $F_{3,21}= 78.77$, $p< 0.0001$, $F_{3,21}= 5.40$, $p= 0.007$). All the treatments resulted in

significantly higher yield compared to the untreated control in the cultivar Jalene. Marketable and total yield in untreated Belete was not significantly different with phosphite treated Jalene and Belete. The recommended dose of phosphite treatment had significantly lower marketable and total potato yield compared to Ridomil and combination of half the recommended dose of phosphite and Ridomil in both cultivars. The average highest marketable potato yield was obtained with the variety Jalene at recommended dose of Ridomil; however, the difference was not statistically significant from combination of half the recommended dose of phosphite and Ridomil treated Belete and Jalene and Ridomil treated Belete (Table 6.3). There was no main effect variety and chemical treatment and their interaction on average unmarketable potato yield (Table 6.3).

6.3.2. Effects of phosphite and synergism with fungicide on foliar late blight and yield on potato in 2015

There was statistically significant difference on rAUDPC between the years 2015 and 2016 ($t=5.0$ and $p=0.0001$). The overall mean rAUDPC was significantly higher in the year 2016 than the year 2015. Generally, there was lower infection in 2015 compared with the remaining two years.

The result showed that interaction of variety and chemical treatment was not statistically significant ($F_{5,33}=1.53$, $p=0.21$). The main effects of variety and chemical treatments on foliar late blight infection level and development were significantly different ($F_{1,33}=12.17$, $p=0.001$ and $F_{5,33}=133.67$, $p<0.0001$) (Table 6.2). There was a significant variation between untreated control and the rest of the treatments in both cultivars in terms of rAUDPC. The rAUDPC values of the treatments recommended and double dose of phosphite, recommended dose of Ridomil and the combination of one fourth the recommended dose of Ridomil + three-fourths the

recommended dose of phosphite and half the recommended dose of phosphite and Ridomil treated plots were not significantly different with each other in both cultivars (Table 6.4). In Belete the lowest infection was observed in the combined treatments of phosphite and Ridomil, whilst in Jalene all the chemical treatments had equal rAUDPC values except combination of one fourth the recommended dose of Ridomil + three-fourths the recommended dose of phosphite. There was also a significant variation between cultivars where Jalene had the higher mean rAUDPC value (Table 6.4).

Table 6.4 Effects of phosphite, Ridomil, the combination of the two and cultivars represented as the relative area under disease pressure curve (rAUDPC) of potato late blight and yield at Holeta in 2015

Treatment	rAUDPC	Mean MRK ¹ yield (tons ha ⁻¹)	Mean UNMRK ² yield (tons ha ⁻¹)	Mean total yield (tons ha ⁻¹)
Jalene				
Untreated Control	0.14 ^{a*}	17.75 ^b	3.00 ^a	20.75 ^b
5L/ha phi	0.01 ^c	34.50 ^a	3.00 ^a	37.50 ^a
3.75L/ha phi+0.625kg/ha Rid	0.02 ^c	41.25 ^a	2.50 ^a	43.50 ^a
10L/ha phi	0.01 ^c	34.75 ^a	2.50 ^a	37.25 ^a
2.5L/ha phi + 1.25kg/ha Rid	0.01 ^c	35.50 ^a	2.75 ^a	38.25 ^a
2.5kg/ha Rid	0.01 ^c	39.50 ^a	3.00 ^a	42.50 ^a
Belete				
Untreated Control	0.11 ^b	37.75 ^a	2.25 ^a	40.00 ^a
5L/ha phi	0.01 ^c	38.50 ^a	1.75 ^a	40.25 ^a
3.75L/ha phi+0.625kg/ha Rid	0.003 ^c	39.75 ^a	2.00 ^a	41.75 ^a
10L/ha phi	0.01 ^c	39.50 ^a	2.25 ^a	41.75 ^a
2.5L/ha phi + 1.25kg/ha Rid	0.003 ^c	35.50 ^a	2.50 ^a	38.00 ^a
2.5kg/ha Rid	0.01 ^c	39.00 ^a	1.25 ^a	40.25 ^a

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

Table 6.5 Effects of phosphite, Ridomil, their combination and cultivars on relative area under disease pressure curve (rAUDPC) of potato late blight and yield at Holeta in 2016

Treatment	rAUDPC	Mean MRK ¹ yield (tons ha ⁻¹)	Mean UNMRK ² yield (tons ha ⁻¹)	Mean total yield (tons ha ⁻¹)
Jalene				
Untreated Control	0.82 ^{a*}	1 ^e	1 ^a	2 ^e
5L/ha phi	0.20 ^c	18 ^d	1 ^a	19 ^d
3.75L/ha phi+0.625kg/ha Rid	0.02 ^e	40 ^{bc}	1 ^a	41 ^{bc}
10L/ha phi	0.16 ^{cd}	22 ^d	1 ^a	23 ^d
2.5L/ha phi + 1.25kg/ha Rid	0.02 ^e	38 ^c	1 ^a	39 ^c
2.5kg/ha Rid	0.02 ^e	44 ^{abc}	1 ^a	45 ^{abc}
Belete				
Untreated Control	0.67 ^b	3 ^e	1 ^a	4 ^e
5L/ha phi	0.15 ^d	25 ^d	1 ^a	26 ^d
3.75L/ha phi+0.625kg/ha Rid	0.03 ^e	43 ^{abc}	1 ^a	44 ^{abc}
10L/ha phi	0.14 ^d	26 ^d	1 ^a	27 ^d
2.5L/ha phi + 1.25kg/ha Rid	0.02 ^e	49 ^{ab}	1 ^a	50 ^{ab}
2.5kg/ha Rid	0.02 ^e	51 ^a	1 ^a	52 ^a

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

Table 6.6 Correlation between rAUDPC* and MRK1 potato yield in two potato cultivars

Pairs	Variety	Year	R	P
rAUDPC vs	Belete	2014	-0.6871	0.0033 [*]
MRK yield	Jalene	2014	-0.847	<0.0001 [*]
	Belete	2015	-0.1268	0.5549 ^{ns}
	Jalene	2015	-0.8301	<0.0001 [*]
	Belete	2016	-0.8816	<0.0001 [*]
	Jalene	2016	-0.8948	<0.0001 [*]

* rAUDPC= relative area under disease pressure curve

¹MRK= Marketable yield

In the year 2015, no tuber blight was found in all the treatments including untreated control, the same was true the previous year. There was a significant difference on marketable, unmarketable

and total potato yield between the years 2015 and 2016 ($t = 2.23$, $p = 0.03$; $t = 9.13$, $p = 0.0001$ and 2.66 , $p = 0.01$). Again there was a significant difference on marketable and total potato yield due to variety, chemical treatment and the interaction of the two ($F_{1,33} = 16.98$, $p = 0.0002$; $F_{5,33} = 8.925$, $p < 0.0001$; $F_{5,33} = 7.23$, $p = 0.001$ and $F_{1,33} = 14.54$, $p = 0.0006$; $F_{5,33} = 9.02$, $p < 0.0001$; $F_{5,33} = 8.07$, $p < 0.0001$). The average marketable and total yield was highest for combination of one-fourth the recommended dose of Ridomil and three-fourth the recommended dose of phosphite in both cultivars. However, it was not significantly different with the rest of the treatments except in the untreated Jalene. Marketable and total yield in untreated Belete was not significantly different when compared with all the chemical treatments except with untreated Jalene. Recommended dose of Ridomil and phosphite, the two phosphite and Ridomil combinations and double dose of phosphite were not significantly different to each other in terms of both marketable and total yield (Table 6.4).

6.3.3. Effects of phosphite and synergism with fungicide on foliar late blight and yield on potato in 2016

There was statistically significant interaction between the effects of chemical treatment and variety on rAUDPC ($F_{5,33} = 22.64$ and $p < 0.0001$). There was statistically significant main effects of variety and chemical treatments on the rate of late blight development in potato, which was measured as rAUDPC ($F_{1,33} = 50.02$, $p < 0.0001$ and $F_{5,33} = 2008.70$, $p < 0.0001$, respectively) (Table 6.2). The two cultivars had significantly different late blight infection level with higher susceptibility shown in the variety Jalene. The rAUDPC value in Jalene at recommended dose of phosphite was significantly higher than the corresponding Belete but it was not different with double dose of phosphite treated Jalene (Table 6.5). The two phosphite treatments were equally effective in the cultivar Belete. No significant difference was detected between the two phosphite

treatments and the two phosphite+Ridomil combinations in both cultivars. Nonetheless they were significantly different with the other treatments. Unlike the previous years, 2014 and 2015, untreated control treatment of the cultivar Belete was fully destroyed before the last sampling date. There was however a delayed infection compared to untreated Jalene.

There was a significant difference on marketable and total potato yield due to variety and chemical treatment, however, no significantly different interaction of the two factors was detected ($F_{1,33}=21.55$, $p= 0.0001$; $F_{5,33}=130.51$, $p< 0.0001$; $F_{5,33}=1.32$, $p= 0.28$ and $F_{1,33}= 19.58$, $p= 0.0001$; $F_{5,33}= 121.02$, $p< 0.0001$; $F_{5,33}= 1.21$, $p= 0.33$). The variety Belete at the recommended dose of Ridomil had the highest marketable and total potato yield. However, yields were not significantly different for the same variety when it was treated with one-fourth dose of Ridomil +three-fourth dose of phosphite and half the recommended dose of Ridomil +phosphite. In addition, the yields were not also significantly different with Jalene at recommended dose of Ridomil. The marketable and total potato yield at double the recommended dose of phosphite and recommended dose phosphite was almost the same in both cultivars whilst, both marketable and total yield was significantly different with the rest of the treatments on both cultivars and untreated control treatments. The yield in the untreated control of both cultivars was very low compared to all the other treatments. Unmarketable potato yield was exactly the same in all the treatments including the untreated control treatments (Table 6.5). There was a significant strong negative correlation between marketable potato yield and foliar late blight severity, rAUDPC, in both cultivars. The higher the mean rAUDPC the lower the MRK yield was (Table 6.6).

6.3.4. Effects of phosphite and synergism with fungicide on foliar late blight and yield on tomato in 2015

There was statistically significant difference on rAUDPC between the years 2015 and 2016 ($t=2.66$ and $p=0.0107$). Calculated rAUDPC value was significantly higher in the year 2016 than the year 2015. The average late blight infection, rAUDPC, was very high on untreated control plots compared to the rest of the treatments and all the plants in the control plots, which were completely destroyed before the last sampling date (Table 6.7). Furthermore, all the treatments except the untreated control were not significantly different with each other. However, the differences were not significantly different from the rest of chemical treatments (Table 6.7). The recommended dose of phosphite treatment and the double dose of phosphite treatment were not significantly different.

Table 6.7 Effects in tomato of phosphite, Ridomil and the combination of the two represented as the relative area under disease pressure curve (rAUDPC) of tomato late blight and yield at Holeta in 2015

Treatment	rAUDPC	Mean MRK ¹ yield (tons ha ⁻¹)	Mean UNMRK ² yield (tons ha ⁻¹)	Mean total yield (tons ha ⁻¹)
	2015			
Untreated control	0.158 ^{a*}	0 ^b	0 ^c	0 ^b
10L/ha phi	0.011 ^b	29.50 ^a	3.50 ^b	33 ^a
5L/ha phi	0.009 ^b	30.25 ^a	2.75 ^{ab}	32.75 ^a
2.5Kg/ha Rid	0.003 ^b	30.00 ^a	4.50 ^b	34.60 ^a
3.75L/ha ph+0.625kg/ha Rid	0.006 ^b	29.75 ^a	3.25 ^{ab}	33 ^a
2.5L/ha of phi+1.25kg/ha Rid	0.005 ^b	29.75 ^a	2.75 ^{ab}	33 ^a

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

Table 6.8 Effects on tomato of phosphite, Ridomil and the combination of the two represented as the relative area under disease pressure curve (rAUDPC) of tomato late blight and yield at Holeta in 2016

Treatment	rAUDPC	Mean MRK ¹ yield (tons ha ⁻¹)	Mean UNMRK ² yield (tons ha ⁻¹)	Mean total yield (tons ha ⁻¹)
	2016			
Untreated control	0.78 ^{a*}	0 ^b	0 ^a	0 ^a
10L/ha phi	0.11 ^b	19 ^a	5 ^a	24 ^b
5L/ha phi	0.07 ^b	19 ^a	6 ^a	25 ^b
2.5Kg/ha Rid	0.05 ^b	19 ^a	6 ^a	25 ^b
3.75L/ha ph+0.625kg/ha Rid	0.05 ^b	19 ^a	6 ^a	25 ^b
2.5L/ha of phi+1.25kg/ha Rid	0.04 ^b	20 ^a	6 ^a	26 ^b

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

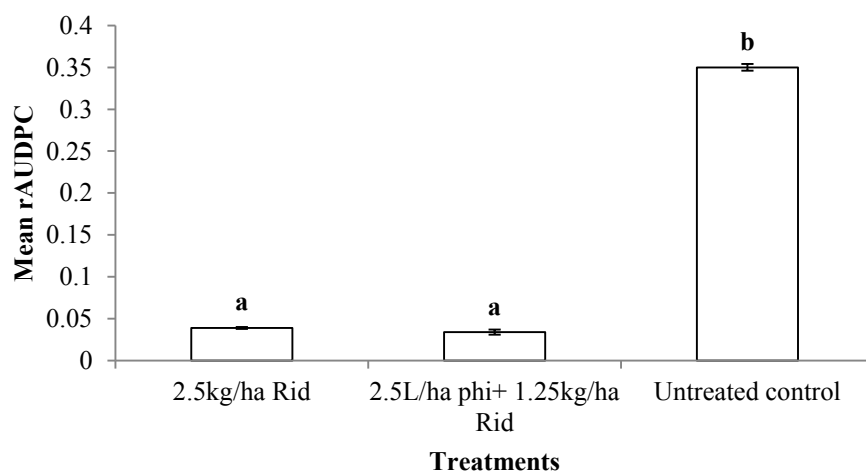


Figure 6.1 Mean rAUDPC of potato plants in 2015 farmers field, phi= phosphite Rid= Ridomil

A significant difference was detected in the marketable and unmarketable tomato yield between the two years, 2015 and 2016 ($t = 2.83$, $p = 0.01$ and $t = 2.54$, $p = 0.02$). In the year 2015 treatments had significant effect on marketable, unmarketable and total tomato yield ($F_{5,18} = 56.52$, $p < 0.0001$; $F_{5,18} = 4.09$, $p = 0.01$; $F_{5,18} = 52.42$, $p < 0.0001$). All the treatments were significantly different from the untreated control. No yield was collected from the untreated plots for all the plants died before setting fruits (Table 6.7). There was significant negative correlation between

6.3.5. Effects of phosphite and synergism with fungicide on foliar late blight and yield on tomato in 2016

The result also revealed that, similar to the previous year, 2015, all the treatments rAUDPC was not significantly different to each other except in the untreated control treatment, which had higher late blight development (Table 6.8). Same as the previous year, 2015, untreated control plants were fully destroyed from damage sustained due to late blight infection before the last sampling date.

There was significant effect of chemical treatment in marketable and total tomato yield in the year 2016, however, unmarketable yield difference was not significant between the treatments ($F_{5,18} = 3.42$, $p = 0.02$; $F_{5,18} = 3.72$, $p = 0.02$ and $F_{5,18} = 2.68$, $p = 0.06$, respectively). All treatments resulted in significantly higher marketable and total tomato yield compared to the untreated control (Table 6.8). However, no significant difference was detected between the other treatments. There was significant negative correlation between rAUDPC and marketable tomato yield in 2016 ($r = -0.67$, $p = 0.0003$).

6.3.6. On farm verification: Effects of phosphite and synergism with fungicide on foliar late blight and yield on potato

There was significant effect of treatments on the average rAUDPC ($F_{2,9}= 3820$, $p< 0.001$). The mean rAUDPC value of untreated plots was almost ten times higher than the treated plots. No difference was observed between the recommended dose of Ridomil and combination between half the recommended doses of Ridomil and phosphite (Fig 6.1).

The effect of chemical treatment on average marketable and total potato yield was significant ($F_{2,9}= 7.79$, $p= 0.01$ and $F_{2,9}= 9.15$, $p= 0.007$, respectively). The overall average marketable and total potato yield of Ridomil and combination of half the recommended dose of Ridomil and phosphite treated potato plots exceeded the untreated control. However no difference was detected between the two chemical treatments. The yield in the treated plots exceeded the untreated control with more than 5 folds (Table 6.9).

Table 6.9 Mean potato marketable, unmarketable and total yield per tons ha⁻¹ on farm verification

Variety	Chemical treatment	Mean MRK ¹ potato yield (tons ha ⁻¹)	Mean UNMRK ² potato yield (tons ha ⁻¹)	Total potato yield (tons ha ⁻¹)
Jalene	2.5L/ha phi + 1.25kg/ha rid	26 ^{a*}	5 ^a	30 ^a
	2.5kg/ha Rid	26 ^a	4 ^a	30 ^a
	Untreated control	5 ^b	3 ^a	8 ^b

* Means followed by the same superscript are not significantly different at 5% Tukey-Kramer HSD

¹MRK= Marketable yield

²UNMRK= Unmarketable yield

6.4. Discussion

The study revealed that phosphite generally works well to combat *P. infestans* infection both in susceptible and moderate resistant potato cultivars and in tomato in Ethiopian conditions.

However, phosphite alone seemed to be less effective than the fungicide and combination of phosphite and the fungicide in the susceptible cultivar, but it provided a better protection than no control measure was taken. In contrast, phosphite performed equally well with that of the fungicide and combined treatments in the moderate resistant cultivar, Belete, at least in two of the three experimental years when the infection pressure was somewhat lower. This indicated that there was cultivar dependent phosphite response to late blight in our experiment. This has previously been reported by Liljeroth et al., 2016 who found that potassium phosphite treatment performed better in the moderate resistant starch potato cultivars than in the susceptible cultivars. For example, in the moderate resistant cultivar Merano phosphite was equally efficient as the conventional fungicide, whereas in the susceptible cultivar Bintje it only gave 40% protection compared to the fungicide. An experiment conducted under greenhouse condition with artificial inoculation has shown a different result (Lobato et al., 2008). According to their result cv. Shepody that is susceptible to late blight was more protected than the moderate susceptible cv. Kennebec after a foliar application of potassium phosphite. Wang-Pruski et al., (2010) reported that the cv. Russet Burbank responded to phosphite substantially than cv. Shepody.

The combinations between the fungicide and phosphite were as effective as the recommended dose of the conventional fungicide. This Ridomil +phosphite combination treatment was effective in both of the cultivars. Consequently, the amount of conventional fungicide could be reduced with up to 75%. Our findings are consistent with the field study by Liljeroth et al., 2016 conducted for four years in Sweden. They found that food and starch potato plants treated with half dose of phosphite combined with half dose of the fungicide, Ranman Top, provided the same level of protection against late blight similar to the fungicide alone. Phosphite can also be alternated with mancozeb still getting outstanding suppression of late blight incidence (Njogu et

al., 2014). Phosphite works against late blight both directly and indirectly, the addition of fungicide to these modes of action might increase the pressure on the disease and offer better protection than the sole fungicide. Furthermore, reducing the amount of fungicide used as much as 75% is a good achievement in line with more environmentally friendly potato cultivation.

The phosphite and fungicide treatments had yield benefits over the untreated control. The yield of Jalene treated with full dose of phosphite was almost similar to the untreated control Belete in 2014. In the year 2015, even though the foliar infection in the untreated Belete was higher than Ridomil, phosphite and the combined treatments the yield was not affected. This might be due to the observed delay in infection where the chance of tuber formation and maturation could continue because photosynthesis was not interrupted. The reason behind the infection delay could be related to the level of resistance and the low rainfall observed during the season. Mitiku et al., (2014) showed Belete to be less susceptible to foliar late blight and yield higher than Jalene. The yield obtained in phosphite treated Jalene and Belete plants was significantly lower compared to other chemical treatments. However, it was higher than the untreated control and non-significant with each other. In congruence with the present study, Agha et al., (2016) reported that yield was affected both by foliar phosphite application and cultivar differences.

Phosphite treatment is known to induce a systemic defense in potato, however, defence response activation was not detrimental to yield (Lobato et al., 2011). Similarly, our study showed that no yield penalty due to phosphite treatment (See chapter 3 also). The yield penalty observed in phosphite treated Jalene as compared to the rest of the treatments was due to the effect of foliar late blight. This was further shown by the negative correlation between rAUDPC and yield. Rickard (2000) reviewed that potato yield increased thorough foliar phosphite application as

fertilizer. In our study the yield advantage in the treated plots was rather due to the treatments in reducing late blight infection pressure.

Liljeroth et al., 2016 showed that potato tuber blight at harvest was highly reduced due to phosphite treatment. Resistance against foliage late blight is different from resistance against tuber blight. Cultivars which are resistant to foliage blight might be susceptible for tuber blight (Flier et al., 2003a; Nowicki et al., 2012). For instance, Lacey (1967) reported incidence of tuber blight at harvest in the absence of foliar blight. In other cases, low incidence of tuber blight has been reported after high levels of foliar blight (Nyankanga et al., 2007). In our study, no potato tuber blight was observed at harvest in all the experimental years. Therefore it was not possible to draw any conclusion the effect of phosphite on tuber blight. The carryover effect of phosphite to natural late blight infection was investigated by storing phosphite treated and untreated tubers for 6 months, but there was no tuber infection in both treated and untreated tubers. The combined half dose of phosphite and half dose of Ridomil applied at 21 days interval on a susceptible potato cultivar under farmers' field condition reduced late blight incidence and yielded a similar to the full Ridomil treatment applied under the same time interval. This could be due to unfavorable conditions such as the low precipitation in 2015, which limited *P.infestans* infections. Further investigation is needed particularly under more favourable conditions for late blight infection. The implementation of this method could benefit farmers in saving their time and money; however it should be assisted with weather forecasting.

Similar to potato, phosphite and their combination with fungicide reduced foliar late blight infection and yield loss in tomato. Untreated plants had the highest late blight infection than the other treatments. However, no variation was detected between the chemical treatments. In an early study, Forster et al., (1998) reported that phosphite treated tomato plants showed low

Phytophthora crown rot incidence. Combination of phosphite and Ridomil was also as effective as the recommended dose of the fungicide Ridomil. Double dose of phosphite had similar effect with the rest of the treatments without any visually observed phytotoxicity. In potato, however, a double dose of phosphite led to phytotoxicity late in the season, but did not seem to affect yield, maybe because of its lateness.

6.5. Conclusion

In this study we found an apparent effect of the inorganic salt phosphite and its combination with fungicides on foliar late blight under tropical cool highland in Ethiopia. It has been witnessed that late blight races developed resistance towards the conventional fungicides. However, using alternatives like phosphite, which works both directly and indirectly, could delay resistance development. Therefore phosphite could be used against potato and tomato late blight alone or combined with reduced dose of fungicides in Ethiopia. The amount of fungicide use could also be reduced by 75 percent.

Chapter 7. General Conclusions and Recommendations

7.1. Conclusions

- Foliar application of potassium phosphite on potato plants reduced the larval population density of PTM.
- Intercropped potato plots had higher yield over monocropped plots at Holetta
- Adult female PTM preferred untreated potato plant more than phosphite treated potato in a laboratory Y-tube bioassay
- PTM preferred potato plants more than tomato plants both in field and laboratory study
- Potassium phosphite directly affects mycelial growth of different *P. infestans* isolates collected from different areas
- The effects of phosphite on mycelial growth of *P. infestans* isolates was highly dependent on the concentration of phosphite used
- Phosphite treatment reduced sporangia germination of different *P. infestans* isolates *in vitro* test.
- Phosphite treatment reduced late blight severity in potato and tomato plants as compared to untreated plants
- Late blight severity reduction obtained by phosphite and fungicide synergism was as effective as sole fungicide treatment
- Fungicide use was reduced by 75% by mixing or alternating with phosphite

7.2. Recommendation

- Phosphite could be combined with other control options to improve PTM control or can be part of Integrated Pest Management of PTM.

- The yield reward of intercropping in potato plants, at Holetta, and tomato yield quality at Melkassa could be increased by considering other possible companion crops or crop proportion/ratio.
- Future work should investigate the change in the volatile profile of potato plants after phosphite treatment and further investigate the possible reasons for larval density reduction in phosphite treated potato plants observed under field condition and the communication between phosphite treated and the untreated neighboring plant of the same species.
- The reason behind the recorded difference in susceptibility between isolates should be investigated and the variations under field conditions should be studied to decide on phosphite use based on the sensitivity of the isolates in a specific location.
- The response of different *P. infestans* isolates to concentration based phosphite induced resistance of potato/tomato should be further investigated.
- Further work should investigate reduced amount of fungicide synergism with phosphite and the reason behind high efficiency of phosphite on less susceptible cultivars.

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