

**OCCURRENCE, SEASONAL VARIATION AND MANAGEMENT OF TOMATO
LEAFMINER (*Tuta absoluta* MEYRICK.) IN TANZANIA**

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Life Sciences and Engineering of the Nelson Mandela African Institution of Science and
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ABSTRACT

Tomato leafminer [*Tuta absoluta*, Meyrick. (Lepidoptera: Gelechiidae)] is a notorious pest that causes massive losses in Solanaceae's crops including tomato (*Solanum lycopersicum* L.). This study was conducted to evaluate the occurrence, damage incidences, host range, population dynamics and developed bio-control strategies for managing *T. absoluta* in Tanzania. Field surveys were conducted in thirteen tomato production regions of Tanzania by purposively sampling one region and one district from each region then three villages were randomly selected for field survey. Optima lures was deployed per plot (0.5 acre) and number of adult moths trapped were counted after 7 days. To identify host of *T. absoluta*, in each selected village number and types of crops damaged per plot were recorded. Ten plants per plot were randomly selected to determine the rate of *T. absoluta* damage per plant by counting number of damaged leaves/fruits per plant in each plot and expressed as percentage damage. However, botanical extracts against *T. absoluta* larvae was conducted in laboratory by using four plant species extracted by using organic solvents. Different concentrations of extract were tested against 10 second instar larvae of *T. absoluta* that were selected randomly. Mortality rate, pupation rate and adult emergence rate were recorded at interval of 24h. Results indicated that *T. absoluta* was present in 13 regions surveyed and proliferates in 12 host plants including tomato, potato, nightshade, pepper and some weed species particularly *Solanum incanum*. Population density of *T. absoluta* was high in Iringa compared to Arusha and Morogoro and the dry season exhibited higher number of *T. absoluta* than in rainy season in all regions surveyed. The study on *T. absoluta* management revealed that, the pest can be controlled by using locally available entomopathogenic fungi (Patent No. TZ/P/2018/000035), plant extracts from *Bersama abyssinica* stem bark, *Commiphora africana* leaves, *Tergetes minuta* seeds and *Moringa oleifera* essential oil from seeds and cultivation of four accessions of tomato landraces namely BKM-22, KGM-5, HSK-4 and HSK-38. Application of the developed bio-control strategies caused 60% to 80% mortality of adults and larvae of *T. absoluta* in the laboratory and open field conditions. The identified bio-control strategies and resistance tomato landraces are recommended for further analysis and for consideration as remedies against *T. absoluta* in tomato in Tanzania.

DECLARATION

I, Never Zekeya do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Name and signature of candidate

Date

The above declaration is confirmed

Name and signature of supervisor 1

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CERTIFICATION

This is to certify that this thesis titled “Occurrence, Seasonal variation and development of biological control for sustainable management of tomato leafminer (*Tuta absoluta* Meyrick) in Tanzania” is written by Never Zekeya under supervision of Dr. Ernest R. Mbega, Prof. Patrick A. Ndakidemi and Dr. Musa Chacha at the NM-AIST. I approve the thesis for submission to the NM-AIST senate for award of the PhD degree in Life Science and Engineering (Biodiversity and Ecosystem Management).

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DEDICATION

This work is dedicated to my parents.

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LIST OF ABBREVIATIONS AND SYMBOLS

CA	-	<i>Capsicum annuum</i>
CL	-	<i>Citrullus lanatus</i>
CLE	-	<i>Commiphora africana</i> ethanol extract
CLP	-	<i>Commiphora africana</i> petroleum ether extract
CP	-	Chill pepper
CREATES	-	Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability
DAAD	-	Deutscher Akademischer Austausch Dienst
DMSO	-	Dimethyl sulphoxide
DS	-	<i>Datura stramonium</i>
IPM	-	Integrated Pest Management
IPR	-	Intellectual property right
LBAE	-	<i>Bersama abyssinica</i> ethanolic extract of leaves
LBAEA	-	<i>Bersama abyssinica</i> ethyl acetate extract of leaves
LBAP	-	<i>Bersama abyssinica</i> petroleum ether extract of leaves
SBAE	-	<i>Bersama abyssinica</i> ethanol extract of stem bark
SBAEA	-	<i>Bersama abyssinica</i> ethyl acetate extract of stem bark
SBAP	-	<i>Bersama abyssinica</i> petroleum ether extract of stem bark
MOE	-	<i>Moringa oleifera</i> ethanol extract
MOP	-	<i>Moringa oleifera</i> ethanol extract
NM-AIST	-	Nelson Mandela African Institution of Science and Technology
SA	-	<i>solanum aethiopicum</i>
SD	-	<i>Solanum dubium</i>
SI	-	<i>Solanum incanum</i>
SM	-	<i>Solanum melongena</i>
SN	-	<i>Solanum nigrum</i>
SL	-	<i>Solanum lycopersicum</i> ,
ST	-	<i>Solanum tuberosum</i>
TLM	-	Tomato leaf miner
TMA	-	Tanzania metrological data agency
TME	-	<i>Tergetes minuta</i> ethanol extract
TMP	-	<i>Tergetes minuta</i> petroleum ether extract

CHAPTER ONE

INTRODUCTION

1.1 Background

Tuta absoluta Meyrick. is a destructive moth belonging to the Lepidoptera: Gelechiidae family and native to South America (Desneux *et al.*, 2011; Urbaneja *et al.*, 2012). It is commonly known as tomato leafminer or South America pin worm and in Tanzania it is known as Kanitangaze. *Tuta absoluta* is currently an important insect pest in many countries leading to deterioration and losses of tomato (Arnó *et al.*, 2009; Urbaneja *et al.*, 2012; Zappala *et al.*, 2013). The pest can also infest several other plants including potato (*Solanum tuberosum*) (Pereyra and Sánchez, 2006), eggplant (*Solanum melongena*), night shades (*Solanum nigrum*) and weeds such as *Solanum incanum*, *Nicotiana glauca* and *Datura stramonium* (Desneux *et al.*, 2010; Polaszek *et al.*, 2012). Due to its aggressive nature and associated loss, it has become a key pest of concern in many countries including Tanzania. For instance, since its first report in the country in 2014 it has been reported to cause losses up to 100% in tomato (Chidege *et al.*, 2016). Management of *T. absoluta* is challenging due to concealed larval feeding nature that occurs within hidden plant parts such as stem, fruit or under leaf thus forefending it from contact managements and natural enemies (Kılıç, 2010; Guedes and Picanço, 2012; Zekeya *et al.*, 2016). The pest is also favoured by a very conducive tropical environmental conditions and a wide host range that all together lead to pest development and invasion throughout growing seasons (Salem and Abdel-Moniem, 2015).

Tuta absoluta is also capable of completing its life cycle in different plant parts under various environments, for instance; larvae develop inside plant tissue, pupa develops in the soils and adults develops on plant surfaces and can fly away. All these stages are difficult to manage by a single bullet option such as chemical sprays (Siqueira *et al.*, 2000b, Lietti *et al.*, 2005; Silva *et al.*, 2011; Guedes and Picanço, 2012; Tonnang *et al.*, 2015). The synthetic chemicals are associated with adverse impacts to humans, non targeted organisms, high cost to farmers, environmental pollution and can lead to resistance development by the pest (Abdel-Raheem *et al.*, 2015). Pest control using resistant varieties has been reported to be effective option in control of pest (Oliveira *et al.*, 2012). In some locations in the world, some tomato accessions have been reported to produce chemical cues and chemicals that unpleasant for *T. absoluta* oviposition and larvae feeding (Resende *et al.*, 2006; Rodrigues *et al.*, 2011). One of

study identified some wild tomato accessions for control of *T. absoluta* in Tanzania, but have deprived agronomic attributes for adoption by farmers (Rakha *et al.*, 2017). Some landraces, were collected from various regions of Tanzania for conservation (Hamisy *et al.*, 2016), however, none of the screening study was conducted their resistance against *T. absoluta* in Tanzania. Other efforts to reduce use of insecticides in tomato fields including; cultural control methods such as controlled irrigation, crop rotation and destruction of infested plant material from the farms have been tested in some locations but not effective after the pest has been established in the field (Abbes *et al.*, 2012; Karem, 2014). Other methods such as biological control including using natural enemies such as parasitoids, predators and entomopathogenic microbes have been reported to be very potential (Guedes and Picanço, 2012), however, there are no established biological control methodologies in Tanzania.

Pheromone traps have been recommended for minimizing *T. absoluta* populations in field, however, use of sex pheromone is of limited efficacy since females are able to reproduce parthenogenetically, can reproduce asexually and also due to polygenic behaviour of males, there is a strong implication for the efficiency of sex pheromone management strategies (Michereff Filho *et al.*, 2000; Silva, 2008; Abbes and Chermiti, 2011; Megido *et al.*, 2012). The pheromone traps have only been reported to perform well in greenhouses and are inefficient in open fields with high population of the pest (Cocco *et al.*, 2013). Although these methods are applied, they are not guaranteed to reduce this pest and may be not readily available especially to small holder farmers in Tanzania.

This study aimed at conducting a survey to study the population, to record host range of *T. absoluta*, developing bio-control agents and screening for resistance of landraces for sustainable management of *T. absoluta* in Tanzania.

1.2 Problem statement and justification

Tuta absoluta is a devastating insect pest that cause losses in tomato production amounting to 80- 100% in Tanzania (Chidege *et al.*, 2016). Synthetic chemical pesticides have been reported to be very prominent to farmers due to quick activity on pest; however, they are economically and environmentally unfriendly to poor rural tomato production farmers in Tanzania (Siqueira *et al.*, 2000a; Materu *et al.*, 2016). Due to difficulties in controlling the pest, tomato production farmers have been reported to duplicate pesticide doses and or mixing different chemicals to address *T. absoluta* (Consoli *et al.*, 1998; Arnó and Gabarra,

2011; Desneux *et al.*, 2011). As a result, the over application of synthetic chemical have been associated with disruption of Integrated Pest Management programs by killing biological active microbes and parasitoids (Consoli *et al.*, 1998). Due to this challenge, this study attempted to develop a sustainable bio-control strategy and identify resistant tomato accessions from locally available resources to equip farmers with affordable pest management options for effective and profitable production of tomato in Tanzania.

1.3 Research objectives

1.3.1 General objective

To study the occurrence, distribution, host range, screen for host resistance of landraces and to develop a sustainable managerial strategy against *T. absoluta*, for application by small holder farmers who depend on tomato for their income and nutrition in Tanzania.

1.3.2 Specific objectives

- (i) To study the occurrence, distribution and population dynamics of *T. absoluta* in different agro ecological zones of Tanzania.
- (ii) To identify host range and rate of *T. absoluta* damage in agro ecological zones of Tanzania.
- (iii) To evaluate the efficacy of plant-based biocontrol (PBB) against *T. absoluta*
- (iv) To screen and evaluate susceptibility of selected tomato landraces and Tengeru 97 to *T. absoluta* larval damage
- (v) To formulate a fungal based biopesticide for management of *T. absoluta*

1.4 Research questions

- (i) Is *Tuta absoluta* distributed in agroecological zones of Tanzania? What is the population density in each region?
- (ii) Is *Tuta absoluta* hosted by several plants other than tomato? What is the rate of damage in each host?
- (iii) Are there any plant extract effective for control of *T. absoluta* in Tanzania?
- (iv) Are the landrace varieties having superior agronomic features and resistant to *T. absoluta* damage than Tengeru 97?
- (v) Can local fungal isolate be effective and formulated for management of *T. absoluta* in Tanzania?

1.5 Significance of the study

Tuta absoluta is a destructive pest of tomato and several solanaceous crops. It is known for its losses reaching 100% in unmanaged fields. In Tanzania, farmers have raised an outcry due to effect of the pest. This study was conducted to determine the ditribution, host range, season variation, screened for host resistance in tomato landraces and developed an effective biocontrol strategies that can be used for management of the pest. Such information was very important to tomato growers, policy markers and other stakeholders towards effective management of *T.absoluta* in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of tomato

Tomato (*Solanum lycopersicum* L.) is a key vegetable crop for income, nutrition and industrial development globally (Mlozi *et al.*, 1992; Jacobi *et al.*, 2000; Adebooye *et al.*, 2006). It is a major source of dietary vitamins including vitamin E, K, ascorbic acid and lycopene (Franceschi *et al.*, 1994; Shi and Maguer, 2000; Giovannucci *et al.*, 2002; Adebooye *et al.*, 2006). In Tanzania, tomato crop play a significant role in transforming livelihood of rural societies (De Putter *et al.*, 2007; Kurosaki, 2007). It is a major horticultural crop that contribute to individual, household and national income in Tanzania. It contributes in alleviating malnutrition and reducing hanger gap as farmers spare tomato sells to purchase staple foods during offseasons (Zekeya *et al.*, 2017).

2.2 Distribution and management challenges of *Tuta absoluta*

Tuta absoluta is native to South America countries including Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela (Van Lenteren and Bueno, 2003; Dandria and Catania, 2009; Desneux *et al.*, 2011; Megido *et al.*, 2012; Chailleux *et al.*, 2013). A summary on distribution of *T. absoluta* across regions is provided (Table 1). It was identified for the first time in 1917 and documented as tomato pest in 1960s in Peru (Guedes and Picanço, 2012; Zappala *et al.*, 2013; Gabarra *et al.*, 2014). *Tuta absoluta* is rapidly spreading across regions including Europe, Mediterranean, Middle East, South Asia and Africa (Tropea Garzia *et al.*, 2012; Abbes *et al.*, 2014; Brévault *et al.*, 2014). In Europe, it was reported for the first time in 2006 in Spain (Desneux *et al.*, 2010) and thereafter in France, Italy, Greece, Malta and Bulgaria (Desneux *et al.*, 2011; Campos *et al.*, 2017). In Africa, *T. absoluta* was reported first in 2008 in the northern African countries including Algeria, Tunisia and Morocco and thereafter, it was reported in Libya in 2009 (Campos *et al.*, 2017). The tomato leafminer continued to spread in Africa and invaded Egypt in 2010 (Moussa *et al.*, 2013), then crossed to Sudan and South Sudan in 2011 (Brévault *et al.*, 2014). Later on in 2012, *T. absoluta* invaded Ethiopia (Tonnang *et al.*, 2015), then Kenya (2013), Senegal Uganda, Zambia, Malawi, Nigeria, Mozambique, South Africa and Zimbabwe (in 2014) (Tonnang *et al.*, 2015; Guimapi *et al.*, 2016; Tumuhaise *et al.*, 2016). In Tanzania, the pest was formerly reported in 2014 in tomato fields at Ngarenanyuki, Arumeru District, Arusha region (Chidege *et al.*, 2016). Since then, the pest has spread very fast and is

threatening tomato production in the country (Brévault *et al.*, 2014; Materu *et al.*, 2016; Zekeya *et al.*, 2016). *Tuta absoluta* has posed a great challenge to manage due to its strange feeding, reproduction behaviour and wider host range including *Solanum lycopersicum* (tomato), *S. tuberosum* (Irish potato) (Pereyra and Sánchez, 2006), *S. melongena* (eggplant), *S. americana* (black night shade), *S. nigrum* (night shade) and *Capsicum annuum* (pepper) (Megido *et al.*, 2014). Weedy hosts include *Datura stramonium* and *Lycium chilense* (Rehman *et al.*, 2012). Of the described plants, tomato is the most preference and more vulnerable to pest damage than other known hosts (Pereyra and Sánchez, 2006; Karadjova *et al.*, 2013; Megido *et al.*, 2014). A summary of host plants and pest status reported in different parts globally is as shown in Table 1. *Tuta absoluta* can spread through transportation of contaminated tomato and other solanaceous crops across different locations and also by wind dispersal (Desneux *et al.*, 2010; Cifuentes *et al.*, 2011; Tropea Garzia *et al.*, 2012; Tonnang *et al.*, 2015). Success of *T. absoluta* in new regions is greatly facilitated by presence of alternative or alternate crop and weed host plants during tomato offseason (Loni *et al.*, 2011; Megido *et al.*, 2014; Kutinkova *et al.*, 2016). Another factor contributing to the success of *T. absoluta* is its explosive reproductive potential where females lay more than 250 eggs within first few days after emerging (Abbes and Chermiti, 2014), resulting in a short lifecycle and several generations per year (Luna *et al.*, 2012; Abbes and Chermiti, 2014; Retta and Berhe, 2015).

Table 1: Global distribution and status of *Tuta absoluta* and host range.

Continent	Country	host	Status	Reference
South America	Peru, Chile, Ecuador, Argentina, Brazil, Bolivia, and Colombia	Tomato (<i>S. lycopersicum</i>) (up to 80-100% yield loss) Potato (<i>S. tuberosum</i>)	Persistent since 1914	(Lietti <i>et al.</i> , 2005; Desneux <i>et al.</i> , 2011; Luna <i>et al.</i> , 2015)
Central and North America	Canada, USA, Mexico, Panama, and Costa Rica	Tomato (<i>S. lycopersicum</i>) Potato (<i>S. tuberosum</i>).	moderate impact	(Pereyra and Sánchez, 2006; Parra, 2009; Desneux <i>et al.</i> , 2011; Proffit <i>et al.</i> , 2011b)
Europe	Spain, Italy, Malta, France, Portugal, Bulgaria, UK, Serbia Lithuania, Greece, and Netherlands	Tomato (<i>S. lycopersicum</i>) Potato (<i>S. tuberosum</i>). (up to 80-100% yield losses)	Problem since 2006 and continuing to increase	(Desneux <i>et al.</i> , 2010; Ostrauskas and Ivinskis, 2010; Karadjova <i>et al.</i> , 2013)
Asia	Turkey, India, Iran, Bangladesh, Saud Arabia, China, and Nepal	Tomato (<i>S. lycopersicum</i>) Eggplant (<i>S. melongena</i>) (50-100% crop damage)	Problem increasing since 2010	(Kılıç, 2010; Desneux <i>et al.</i> , 2011; Shashank <i>et al.</i> , 2015; Campos <i>et al.</i> , 2017; Rakha <i>et al.</i> , 2017)
Africa	Algeria, Morocco, Libya, Egypt, Senegal, Nigeria, Ethiopia, Kenya, Tunisia, Tanzania, and Zambia	Tomato (<i>S. lycopersicum</i>) (up to 100% yield loss), Potato (<i>S. tuberosum</i>) Night shade (<i>S. villosum</i>) Eggplant (<i>S. melongana</i>) Datura (<i>D. stramonium</i>).	Problem increasing since 2008	(Chermiti <i>et al.</i> , 2009; Desneux <i>et al.</i> , 2011; Tonnang <i>et al.</i> , 2015; Rakha <i>et al.</i> , 2017)
Australia	Not reported	However, is at risk due cross continental business	No problems reported	(Steiner and Goodwin, 2015)

2.3 Management options for *Tuta absoluta*

2.3.1 Synthetic chemical pesticides

Chemical pesticides such as pyrethroids (Guedes and Picanço, 2012), organophosphates, spinosad and emamectin benzoate (Campos *et al.*, 2014), chloride channel activators, benzoylureas (Haddi, 2012), diamide (Roditakis *et al.*, 2015), Cartap and Abamectin (Guedes and Siqueira, 2013; Campos *et al.*, 2015) have been reported to be widely used to control *T. absoluta*. Application of these chemicals against *T. absoluta* has been reported with little success, mainly because of a complex feeding and life cycle of the insect as previously discussed and due to pest resistance (Siqueira *et al.*, 2000; Campos *et al.*, 2015). As a consequence, farmers become stranded and try to increase application rates of chemicals without following recommended rates (Consoli 1998; Siqueira *et al.*, 2000; Lietti *et al.*, 2005). In Spain and Brazil for instance, farmers use about 15 and 30 applications, respectively to control the pest (Silva *et al.*, 2011; Guedes and Picanço, 2012). Studies show that resistance of *T. absoluta* to spinosad chemical reached to 180,000 folds within seven generations in Brazil (Campos *et al.*, 2014). In Tunisia, more than 18 chemicals were introduced during 2009-2011 for the control of *T. absoluta* but none of them seemed efficient in solving the pest problem (Abbes *et al.*, 2012). In Tanzania, chemical pesticides are applied intensively, and sometimes farmers duplicate doses of chemical pesticide to control *T. absoluta* (Zekeya *et al.*, 2016). This initiative has been reported to not only fail in managing *T. absoluta* but also to insignificant turnover and high cost of tomato production (Materu *et al.*, 2016). Failure of these chemicals in controlling *T. absoluta* has opened a gap for development of other methods including botanicals, biological control, pheromone traps, and host resistant varieties for managing *T. absoluta*.

2.3.2 Botanicals pesticides

Botanicals have been reported to play a great role in controlling pests including *T. absoluta* (Cork *et al.*, 2009; Sharma and Bhandari, 2014). Africa in particular has been reported to be a good source of botanical plants that possess insecticidal activities against pests (Shiberu and Getu, 2017). For instance, extracts from neem plant have been reported to be effective against *T. absoluta* under laboratory condition (Durmusoglu *et al.*, 2011; Yankova *et al.*, 2014). Other botanicals promising in controlling *T. absoluta* and other insect pests include piper (*Piper amalago*) (Brito *et al.*, 2015), toothache plant (*Acmella oleracea*) (Moreno *et al.*, 2012) and jojoba seeds (Abdel-Baky and Al-Soqeer, 2017) but they scarce in local environment. In this study a list of other locally available plants namely *Bersama abyssinica*,

Commiphora africana, *Tergetes minuta* and *Moringa oleifera* have been tested for their insecticidal activity against the tomato leafminer based on indigenous knowledge and preliminary information (Zekeya *et al.*, 2014b)

2.3.3 Biological control

a) Application of parasitoids as biocontrol agents against *Tuta absoluta*.

Natural enemies play a great ecological role in controlling pests in natural settings (Ghoneim, 2014c). A study showed that the larvae of *T. absoluta* search for and acquire some biological characteristics and thermal requirements from host plants that can attract parasitoids (Megido *et al.*, 2014). *Tuta absoluta* and host plants have the ability to emit volatile compounds that attract either pest predator or parasitoid towards it and this favors the parasitoid indirectly (Proffit *et al.*, 2011a). One of the successful used parasitoids include *Trichogramma pretiosum* which can parasitize a number of insect species including *T. absoluta* (Zappala, 2012). This natural enemy has been utilized in America and Europe. However there is scarce information regarding availability and application in Tanzania. More examples of parasitoids include *Necremnus* near *artytes* (Walker) and *Necremnus* near *tidius* (Walker) from order Hymenoptera (Ferracini *et al.*, 2012; Balzan and Wäckers, 2013), Trichogrammatidae, Encyrtidae, and Eupelmidae (Ghoneim, 2014b). Of the parasitoids, *Trichogramma bourarachae* has been cited to be very popular in biological control due to its efficacy in controlling pests and ability to be produced quickly and affordably relative to other parasitoids (Zouba *et al.*, 2013; Ghoneim, 2014a). *Trichogramma achaeae* Nagaraja and Nagarkatti have been reported as the most important and effective parasitoids of *T. absoluta*. This parasitoid has been reported to have a worldwide distribution and capable of parasitizing *T. absoluta* eggs and larvae (Hoffmann and Frodsham, 1993; Chailleux *et al.*, 2012). This parasitoid is genetically compatible with many pest biocontrols (Michel-Salzat and Whitfield, 2004). Some studies revealed that use of *Trichogramma* spp. is gaining popularity as biocontrol of several pest (Luna *et al.*, 2015). Other parasitoids reported to have potential for parasitizing *T. absoluta* include *Pseudapanteles dignus* (Hymenoptera) (Sánchez *et al.*, 2009), *Neochrysocharis Formosa*, *Pnigalio* (Ratzeburgiella) *cristatus* (Ratzeburg), *Pnigalio* sp. *soemius* complex (Hymenoptera: Eulophidae), *Diadegma ledicola* Horstmann (Hymenoptera: Ichneumonidae) and *Braconosculator* (Ferracini *et al.*, 2012).

Although parasitoids are very promising in the management of pest, limited information is available regarding application of parasitoids against *T. absoluta* on tomato in Tanzania

b) Application of microbes

Use of microorganism as biopesticides for management of pests has increasingly gained popularity in recent years (Mollá *et al.*, 2011). Bacteria and fungi have been used for a long time in management of tomato borer in America and Europe (Van Lenteren and Bueno, 2003; Trottin-Caudal *et al.*, 2012). The microbes have been reported to attack pests by their pathogenic effects (Pires *et al.*, 2009; İnanli *et al.*, 2012). Currently there are many commercially available bacterial and fungal formulations for controlling pests including *T. absoluta* in America and Europe but no information for their use in the African continent (Sabbour, 2014). The formulations available are either by foliar spray or by drenching the roots (İnanli *et al.*, 2012). One of the best and successful formulations of microbial formulation include that of *Metarhizium anisopliae* (fungus) and *Bacillus subtilis* (bacteria) which have been reported to reduce the population of *T. absoluta* on tomato at all developmental stages in America and Europe (İnanli *et al.*, 2012). Microbial control of pests is increasing globally as a sustainable and cost-effective management approach (Urbaneja *et al.*, 2012). In Europe and America, application of bacteria such as *Bacillus thuringensis* for pest control has been commercialized and has become an essential component of development of biotechnology for pest management (Berg, 2009; Batalla-Carrera *et al.*, 2010; González-Cabrera *et al.*, 2011; Sabbour and Nayera, 2014). Efficacy of *B. thuringensis* on insect pests including tomato leafminer has been reported in Egypt (Desneux *et al.*, 2010; Sabbour *et al.* 2014). This biocontrol has a synergic effect when applied with other biocontrols that influence activity against pests (Molla *et al.*, 2012). Some bacteria organisms such as Rhizobacteria are known to balance and regulate nutrients in agricultural soils (Price, 2006; Robertson and Vitousek, 2009).

Other microbial formulations effective against *T. absoluta* include that of *Metarhizium anisopliae* and *Beauveria bassiana* (İnanli *et al.*, 2012). Most of these reports however have been demonstrated on screen house studies (González-Cabrera *et al.*, 2011; Sabbour and Nayera, 2014) and only a few have been tested on field conditions and thus they may not be readily available for small-holder farmers in Tanzania. Nematodes have been reported as biocontrols of *T. absoluta* in some countries and depicted high insect mortality (Batalla-Carrera *et al.*, 2010). The nematodes were reported to be effective against larvae, pupae and

adult *T. absoluta* (Garcia-del-Pino *et al.*, 2013). Unfortunately, none of these strategies have been reported in Sub-Saharan Africa including Tanzania.

Entomopathogenic fungi industry has grown up in such a way that it is promoted as an alternative to synthetic chemicals in suppressing or eliminating insect pests in a more safe way (Strasser *et al.*, 2000; Shah and Pell, 2003). They are currently used in other parts of the world as foliar or endophytes through drenching in many agricultural fields (Ekési *et al.*, 2007) but not reported intensively in Tanzania. One of the good examples of the fungus commonly used include *Beauveria bassiana* (Meyling and Eilenberg, 2007; Akello *et al.*, 2009) which can be applied for both endophytic and foliar spray applications. Entomopathogenic fungi such as *Metarhizium anisopliae* has been reported to be effective against tomato leafminer in Europe, America and Asia but not tested in Tanzania (Biondi *et al.*, 2018). Other study by Lakhdari (2016) revealed that *Aspergillus flavus* is efficacious on larvae, pupae and adult *T. absoluta* in Algeria. Generally, though entomopathogenic microbes have been applied for pest management in developed countries, little has been done on their application for managing *T. absoluta* in developing countries including Tanzania (Zekeya *et al.*, 2017; van Lenteren *et al.*, 2018). As a way forward, there is a need to focus on fungal based biocontrols due to existing information and benefits (Guedes and Picanço, 2012; Kaoud, 2014).

Fungi of the genus *Aspergillus*, have been reported to have great potential for pest control in tropical countries (Zhang *et al.*, 2015; Lakhdari *et al.*, 2016). The genus *Aspergillus* belongs to one of the oldest fungal groups of conidia that freely establishes in many environments (Beguin and Nolard, 1994) and comprises high numbers of potentially economically important species (Bennett, 2010). The genus comprises more than 250 species (Geiser *et al.*, 2007), and is xerophile or halophile. It is naturally widely distributed in soil, food remains, leather, and textile products (Hubka *et al.*, 2013). *Aspergillus* genus is characterized by the production of lenticular ascospores and uniseriate conidial heads with hyphae in shades of green, black, or yellow depending on species color (Hubka *et al.*, 2015). Few members of the genus are pathogenic to humans (Aspergillosis) (Karkowska-Kuleta *et al.*, 2009; Brown *et al.*, 2012) and their carcinogenic syndrome is indirectly caused by their metabolites (Gourama and Bullerman, 1995; Pitt *et al.*, 2000; Bayman *et al.*, 2002). Nevertheless, majority of *Aspergillus* have been reported to be beneficial in agricultural and industrial applications (Contesini *et al.*, 2010; Powell *et al.*, 2013). Some are important sources of

industrial biodegradable enzymes (de Vries and Visser, 2001). They also have medicinal components against infectious diseases (Khan *et al.*, 2010), and are involved in the formation and balancing of nutrient composition in soil (Schuster *et al.*, 2002; Verma *et al.*, 2011). Industrial effluent in urban and peri-urban environments is the principal source of entomopathogenic *Aspergillus* spp. in agricultural fields, due to its tolerance to heavy metals (Iram *et al.*, 2009), and some species that live as endophytes of plants support growth and defend against pathogens and insect pests (Khan *et al.*, 2011). The exploration of native fungal as biocontrol agents would suppress pest populations and reduce economic losses incurred in tomato production in Africa.

2.3.4 Use of pheromone traps

Sex pheromone traps have been cited as among environmentally accepted pest monitoring and management strategy (Harizanova *et al.*, 2009; Reddy and Guerrero, 2010; van der Straten *et al.*, 2011). The traps are used prior to other control strategies so as to determine the presence and abundance of insects so as to decide on appropriate control measure to apply (Witzgall *et al.*, 2010; Cocco *et al.*, 2013). Although these traps are designed to trap only adult male moth, they have been reported to be effective in managing *T. absoluta* (Vacas *et al.*, 2011; Abbes *et al.*, 2014). For effective application in the field, the sex pheromone traps are to be properly hanged at right positions depending on the height of tomato varieties and wind direction (Soliman *et al.*, 2013). Other factor reported to be important is color of the trap which, affect and influence the pest towards it thus enhancing trapping efficiency (Megido *et al.*, 2013b; Braham, 2014; Mwangi, 2015). Shining colors especially red has been reported to be the most attractive to *T. absoluta* (Taha *et al.*, 2012). Combination of these factors have been reported to improve traps efficiency in the field (Speranza and Sannino, 2012; Lobos *et al.*, 2013) especially when they are combined with insect killing ingredients. Use of killing agent in combination with sex pheromones has been reported to have minor effect however when the pheromone traps used are only for disrupting mating system they are effective (Gacemi and Guenaoui, 2012; Mafra-Neto *et al.*, 2013). Currently, no study has reported the efficacy of pheromone traps when synergized by botanicals in Tanzania. It is evident that a pheromone trap bitted with active compound could be effective for monitoring and control of *T. absoluta* in open field, hence botanicals have been evaluated so as to be incorporated in IPM with pheromone traps for monitoring and control of *T. absoluta* in Tanzania.

2.3.5 Application of host resistance

Wild tomato varieties including landraces possess important traits compared to improved varieties for defense (Terzopoulos *et al.*, 2009). Mechanism of resistance is either by physical deterrent to pest or through secondary metabolites such as acyl sugars particularly the zingiberene have been reported to kill various life stages of *Tuta absoluta* (de Azevedo *et al.*, 2003; Resende *et al.*, 2006; Maluf *et al.*, 2010). The physiology of wild tomato especially trichome protect it against invading pest (Simmons and Gurr, 2005). It is also reported that the leaf surface of most wild accession affects oviposition and kill eggs or young larvae of *T. absoluta* (Rakha *et al.*, 2017). This is because they embrace traits responsible for defense against sucking pests including aphids (*Aphis gossypii*) (Digilio *et al.*, 2010; Scintu *et al.*, 2015). They also resist against several plant pathogens and diseases (Acciarri *et al.*, 2010; Salim *et al.*, 2011) including viruses that are more prominent on tomato and several solanaceous crops (García-Martínez *et al.*, 2011, 2012). Landraces have many advantages over improved cultivar as they thrive under harsh environmental conditions such as drought, high salinity and heavy rains (Taffouo *et al.*, 2010; Moles *et al.*, 2016). They can be grown in limited nutrient soil and does not require fertilizer (Oloyede and Adebooye, 2005). Studies have revealed that landrace accessions possess resistant trait against *Tuta absoluta* (Pereyra and Sánchez, 2006; Oliveira *et al.*, 2009). Mechanism of resistance in tomato landraces has been reported to be due to either physical deterrent to pest or through secondary metabolites such as acyl sugars particularly the zingiberene, a compound which has been reported to kill various life stages of *Tuta absoluta* (de Azevedo *et al.*, 2003; Resende *et al.*, 2006; Maluf *et al.*, 2010). The physiology of tomato landraces especially trichome protect it against invading pest (Simmons and Gurr, 2005). It is also reported that the leaf surface of most wild accession affect oviposition and kill eggs or young larvae of *T. absoluta* (Rakha *et al.*, 2017). Despite merits of indigenous tomato, they are not accessible by farmers in agro-ecological zones of Tanzania due to low priority by farmers and breeders focusing on improved variety that are commercially available (Hamisy *et al.*, 2016). Cultivation and promotion of land races can save as antagonistic units of various insect pests challenges (Alba *et al.*, 2009). There is limited information on resistant tomato varieties against *T. absoluta* in Tanzania. However there is evidence that, tomato landraces could be source of resistance against pest in Tanzania (Hamisy *et al.*, 2016). Therefore, as part of this study, some land races have been screened for resistance against *T. absoluta* in in screen house and in field.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the study

Baseline survey on occurrence, distribution, host range and damage by *Tuta absoluta* was conducted on farmers' fields in thirteen regions namely; Arusha, Iringa, Morogoro, Tanga, Kilimanjaro, Manyara, Singida, Dodoma, Zanzibar, Dar es Salaam, Pwani, Mwanza and Mbeya from September to December 2016 (Table 3). Result from baseline survey led to section of three regions as representative of agro-zones; northern (Arusha), east-central (Morogoro) and southern (Iringa) were selected for studies on population dynamics of *T. absoluta*. Choice of these regions were due to occurrence of high incidences of *T. absoluta* recorded during the survey. Laboratory bioassay was done at Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania laboratory at the Tengeru, main campus. Screen house studies were conducted World Vegetable Centre, Tengeru, Arusha, Tanzania.

3.2 Tomato genotypes used in this study

Tomato seeds (variety Tengeru 97) were purchased from KIBO SEED CO.LTD, Arusha and landraces accessions (Table 5) seeds were offered by Tropical Pesticides Research Institute (TPRI), Arusha Tanzania. All collected seeds were stored at 4⁰C at the NM-AIST laboratory.

3.3 Pheromone traps used in this study

Delta traps (*Tuta absoluta*-Optima, Russell IPM Company, London, UK) containing synthetic female *T. absoluta* sex pheromone, which consists of two components: (3E, 8Z, 11Z)-3, 8, 11-tetradecatrien-1-yl acetate (TDTA) and (3E, 8Z)-3, 8-tetradecadien-1-yl acetate (TDDA) (0.5mg per lure) were purchased from ZAIDI AGRO LTD, Dar es Salaam.

3.4 Plant extracts used in this study

Leaves from three plant species namely; *Commiphora africana*, *Moringa oleifera* and *Tergetes minuta* were collected from farmer's fields in Arusha, and barks of *Bersama abyssinica* were obtained from Rungwe District, Mbeya based on indigenous knowledge and result from previous study. All collected plant materials were dried under shade and stored at room temperature until used.

3.5 *Tuta absoluta* insects used in laboratory and screen-house studies

Larvae of *T. absoluta* were collected from infested tomato leaves at Ngarenanyuki-Arusha in November 2015. Insects were reared and maintained on a 14 days old tomato plants var. Tengeru 97 in the screen house conditions ($23 \pm 2^\circ\text{C}$ and relative humidity of 80% RH) at the WorldVeg laboratory. The plants were in 2L plastic pots with diameter 14 cm and height (20 cm) containing sterile sand: compost soil (1:3 ratio, respectively) filled to a depth of about 12 cm.

During the rearing process, about 10 males and 20 females adult *T. absoluta* were introduced in each potted tomato plant and maintained in Perspex cages covered with fine polyester mesh (0.0132 mesh/cm) cages (75×45×35 cm) at $23 \pm 2^\circ\text{C}$ and 70% RH. Female were monitored for oviposition of eggs and plants were removed from cages after every 24 h for larvae to hatch. After 12 ± 3 days, when the majority of the larvae reached the 4th instars, the infested materials were collected and reared inside cages until adult emerged. The adults were transferred into other cages using a mechanical aspirator measuring (3x40cm) width and length respectively at the WorldVeg. To feed the *T. absoluta* adult, a cotton wool was immersed in 10% honey solution and placed on petri dishes (two petri dishes per 20 adult *T. absoluta*) placed under the tomato plants. After every six generation, new larvae were collected from field to activate the laboratory colonies. Experimental larvae and adults were collected from leaves using painting brush and adults were collected using mechanical aspirator respectively.

3.6 Field surveys

To assess occurrence and distribution of *T. absoluta* in Tanzania, thirteen regions (Table 1) were surveyed as follows: In each region, one main tomato growing district was purposively selected based on information available at the regional agricultural office and in each district three villages were randomly selected. To assess occurrence and distribution of *T. absoluta*, in each village, two pheromone traps (Russell IPM, UK) was randomly placed at distance of 100 m within tomato farm planted with variety Tengeru 97 and the distance between one farm and another was at least 1km to avoid movement of *T. absoluta* across fields. Number of *T. absoluta* caught per trap was recorded after seven days.

In addition, in each farm, damage levels by *T. absoluta* on tomato plant were established by counting *T. absoluta* larval mines as described by Kılıç, (2010). The percentage of tomato

loss was calculated by this formula: $(\text{Total Fruit} - \text{Marketable Fruit}) / \text{Total number of fruits} \times 100$ (Kilic, 2010).

To assess occurrence of *T. absoluta* in different host plants, three locations were randomly selected per village, and plants known to be hosts of *T. absoluta* (Megido *et al.*, 2012) were critically examined for mines on leaves as signs of infestation by the pest. Estimation of damage incidences by *T. absoluta* was calculated by counting number of plants with mines divided by total number of plants of similar species multiplied by 100% in a 5 m x 5 m plot in triplicates arranged in a zigzag format within each assessed farm. To ascertain whether or not the leaf mines were due to *T. absoluta*, set of pheromone trap was placed in the middle of the farm (farm size was approximately 0.5 to 1 acres).

Global Positioning System (GPS) coordinates points for regions covered in the survey were recorded using a mobile phone Bizz GPRS function (LBKC_GD0041_Samsung SM-A8001Z). The map of Tanzania was obtained from google maps (<https://www.google.com/maps/d/u/0/ed>). To indicate locations, GPS coordinates were entered and labeled accordingly.

3.7 Assessing population dynamics of *T. absoluta*

Using information collected from the surveyed regions, three regions (with the highest levels of *T. absoluta* record) namely Iringa, Morogoro and Arusha were selected to study the population dynamics of the pest. In each region, three villages per district were selected and in each village three farms each with a size of about 0.5 acre planted with tomato variety Tengeru 97 were used for the study. In each farm, one pheromone trap was placed at the farm terminal as a general rule of using the traps. The trap was monitored on number of catches per week for eight weeks. New trap card was replaced weekly on a specific selected time frame within each season. In Season I (dry), lure was placed once, and sticky cards were replaced weekly from 1st September 2015 to 30th October 2015 and repeated from 1st September 2016 to 30 December 2016. Also, in season II (rainy) lure was placed once after seven weeks and sticky cards were replaced weekly from 1 January 2016 to 1th March 2016 and repeated from 1st January 2017 to 1th March 2017. Trapped *T. absoluta* were preserved in alcohol (99%) and samples of the insects were submitted to the National Herbarium of Tanzania (NHT), in Arusha, Tanzania.

3.8 Assessing efficacy of botanical against *Tuta absoluta*

3.8.1 Preparation and extraction of botanical extracts

Aerial plant parts from species namely; *Commiphora africana*, *Moringa oleifera* and *Tergetes minuta* were collected from farmers' fields in Arusha based on indigenous knowledge. Each sample was dried under shade, then samples of 100g from each plant was macerated using a blender (Ken wood, Japan) to obtain fine particles of about 2 -10 mm. The obtained particles were soaked in 1000mL of Petroleum ether (40-60°C, supplied by Carlo Erba, Strada-Rivoltana) for 48h. Then, the content was filtered using a filter paper (Whatman No1, Camlab UK.). The filtrated was collected on a round bottom flask and pellets were further soaked on analytical ethanol (analytical supplied by Scharlab S.L, Spain. Laboratory) for 48h. Filtrates from petroleum ether and that from ethanol were separately evaporated in a vacuum using a rotary evaporator under low pressure and reduced temperature (<50°C). The resulting extracts for each solvent were used separately for bioassays against *T. absoluta* in vitro. For each extract, 0.1g (10%) was dissolved in 10ml of sterile distilled water containing 1% dimethyl sulphoxide (DMSO) (RFCL, Haryan, India) and served as a stock solution. From each stock solutions, three different concentrations; 2%, 5% and 10% were prepared.

To prepare working solutions for *B. abyssinica*, 500g of stem bark or leaves granules were soaked in 1500ml petroleum ether for 48 h then, the pellets were then soaked on 1000ml ethyl acetate (analytical supplied by Scharlab S.L, Spain. Laboratory) for 48h and finally after filtering the pellets were also soaked in 1000ml ethanol (analytical supplied by Scharlab S.L, Spain. Laboratory) for 48h. The filtrates were separately evaporated in vacuum using a rotary evaporator as described previously. Six extracts; three from stem bark and three from leaves (SBAP, SBAEA, SBAE and LBAP, LBAEA, LBAE) respectively, were weighed and preserved in air tight bottles in a refrigerator at -4° C until used for adult *T. absoluta* bioassays using 4% and 8% concentrations for each and control solutions containing 4% and 8% of DMSO.

3.8.2 Testing botanical extracts against *Tuta absoluta* larvae

Second instar larval stage was used for larval bioassay using 2% 5% and 10% concentrations each from *C. africana*, *M. oleifera* and *T. minuta* and 4% and 8% concentrations from *B. abyssinica* bark or leaf and respective controls as previously described were sprayed on five weeks old tomato plant and left to air dry for 1 h, thereafter three fresh tomato leaflets were picked and placed into dry petri dish before inoculation of larva. Then 10 *T. absoluta* larvae

were randomly selected and inoculated into treated tomato leaflets on one-layer filter paper in a petri-dish (16cm diameter, 3cm height) in triplicates. Number of live and dead larvae in both treated and control units was recorded at interval of 48h. Fresh untreated tomato leaflets were placed regularly if the leaf has been consumed by *T. absoluta* or overstayed beyond 48h. Monitoring was extended for live larvae until pupation and or adult emergence. Percent of larvae mortality, pupation and adult emergence was recorded.

3.8.3 Testing botanical extracts against *Tuta absoluta* adult

Newly emerged adults (1 day old) were collected using a mechanical aspirator and inoculated in the rearing cages before bioassay. Then plastic vials (4.5cm width and 15cm height) were separately preloaded by 2%, 5% and 10 % of *C. africana*, *T. minuta* and *M. oleifera* extracts whereas 4 and 8% of *B. abyssinica* extracts were used for this bioassay and control containing 4% and % of DMSO. Excess water was removed from the vials followed by inoculation of 10 adult *T. absoluta* in each treated vial which was then covered by mesh net on top to protect insect from escaping. Treated and control adult *T. absoluta* were fed on honey solution by placing on top of vial to keep them active. Number of dead insects was recorded after every 24 h and treatments were replicated thrice.

3.9 Testing resistance of tomato genotypes against *Tuta absoluta*

3.9.1 Screening for resistant accessions against *T. absoluta* in screen house

Twenty-one tomato landrace (Table 5) and Tengeru 97 (check) were sown separately in 66 well trays (two seeds were sown per hole) in sterile soil in which each tray contained a total of 132 seeds. Seedlings were raised in screen house ($28.4 \pm 2.1^{\circ}\text{C}$ and 70%RH) and were watered once a day. In each accession, number of seedlings emerged was recorded and expressed as percentage of germination and emergence duration was recorded as well. Seedlings were then thinned to one seedling two weeks after planting. Three weeks after transplanting, trays with all tomato accessions were transferred in closed transparent cages of about 6x4x3.5 cm length, width and height respectively. Then, 46 newly (2 days) emerged adults female *T. absoluta* were introduced and symptoms of infestation (mines/accession) were recorded for 7 days after inoculation of insect.

3.9.2 Evaluation of resistant accessions in field

Only accessions that lower number of mines (<2.0) per plant were selected for further screening for resistance in field. Thus, accessions; BKM-22, KGM-5, HSK-43, HSK 38 and

improved variety (Tengeru 97) as check/control were used for this study. All seeds were sown in 72-pit (4 cm diameter) trays each filled with heat sterilized forest soil. Trays with seeds were sprinkled with water until wet and covered by white Agro net (0.4m hole) (A to Z Company Ltd) maintained at the temperature of about $23.7 \pm 2.4^{\circ}\text{C}$, $27.2 \pm 1.3^{\circ}\text{C}$ and $21.1 \pm 2.2^{\circ}\text{C}$, in northern, east-central and southern zone of Tanzania, respectively (Shenge *et al.*, 2010). Each tray was watered once a day during the evening for 21 days, then seedlings were transferred to the field for transplanting and grown with no any synthetic input such as fertilizers or pesticides as described in literature (Hamisy *et al.*, 2016). Experimental plots were 20 m x 20 m each and seedlings were sown at the spacing of 60 cm and 40 cm between ridges and between plants, respectively. One day prior to transplanting, pits were prepared and watered using furrow irrigation scheme. One seedling was planted per hole of about 15 cm deep. Plots were weeded and pruned biweekly. All plots were watered after every three days by furrow irrigation until all plants were moist enough. Insect pests other than *T. absoluta* such as aphids (*Aphis gossypii*), mites (*Aculops lycopersici*), leaf miner (*Lyriomyza sativae*) and whitefly (*Bemisia tabaci*) were left uncontrolled in all plot to avoid interference with host plants.

3.9.3 Identification of morphological traits for *Tuta absoluta* resistance

Three weeks after transplanting in fields, number of leaves per plant was counted from fully expanded plant. Number of mines by *T. absoluta* larvae per plant in each tomato variety was recorded once per week for six weeks consecutively. Number and percentage of fruit damage per plant was calculated by subtracting marketable fruit from total yield per each accession in each location. In additional, harvest frequency per genotype was recorded to evaluate the accession with the shortest maturity rate.

3.9.4 Assessment for post-harvest longevity and resilience

After harvest, 100 mature and firm fruits per genotype were exposed at room temperature (20°C and 75% RH) on benches to evaluate stability. Changes in shape, color and spoilage and microbial contamination was observed and recorded daily until all fruits degraded in containers.

3.10 Assessing efficacy of fungal based biopesticides

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3.11 Data analysis

All data were subjected to analysis of variance (ANOVA) with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA). All data were checked for normality, for those that were not equally distributed were log transformed to obtain normally distributed data set. Tukey's Honest Significant Difference (HSD) was used to separate mean difference between treatments in normal data set while Bonferroni was used to separate mean in non-parametric data at 5% significance level of probability.

Pairwise differences were evaluated with Tukey's HSD at $p < 5\%$. For the distribution survey, the variables in the trap catch ANOVA were number of adult *T. absoluta* trapped/trap/week. For the population dynamics study, the variables were number of *T. absoluta* /trap/ per; zone, weather, lure duration (week/month) and season. To evaluate level of damage by *T. absoluta* larvae, number and percentage of fruit damage was the variable whereas for host range data number of damaged.

For botanical bioassay, larval survival duration, adult survival duration, percentage larval mortality, pupation and adult emergence were the variable for ANOVA analysis. However, number of fruits, weight of fruits, percentage of damaged fruits and fruit resilience were the variables for ANOVA in a host resistance of tomato landraces trial.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Occurrence and damage levels by *Tuta absoluta* on tomato in different regions of Tanzania covered during this study.

Survey results showed that *T. absoluta* exist in all 13 surveyed regions of Tanzania with varying densities (Illustration 1) and the number of adult *T. absoluta* trapped differed significantly ($p < .0001$) between regions (Table 2). Of the surveyed regions Iringa appeared to have the highest density 299.3 ± 22.7 moths per trap, whereas the lowest density of *T. absoluta* averaging 13.3 ± 1.0 moths per trap was recorded in Mwanza (Table 2). The damage by *T. absoluta* was observed in premature green to full ripen tomato fruits (plate 1) By levels, the current study found that the incidence of *T. absoluta* damage was significantly ($p < .0001$) different between regions (Table 2). The highest damage averaged $90 \pm 0\%$, $86.6 \pm 3.3\%$, $83.3 \pm 5.7\%$ and 80 ± 0 in Iringa, Morogoro Arusha, Dar es salaam and Tanga respectively. The lowest damage by *T. absoluta* averaging $30 \pm 5.7\%$ was recorded in Mwanza (Table 3).

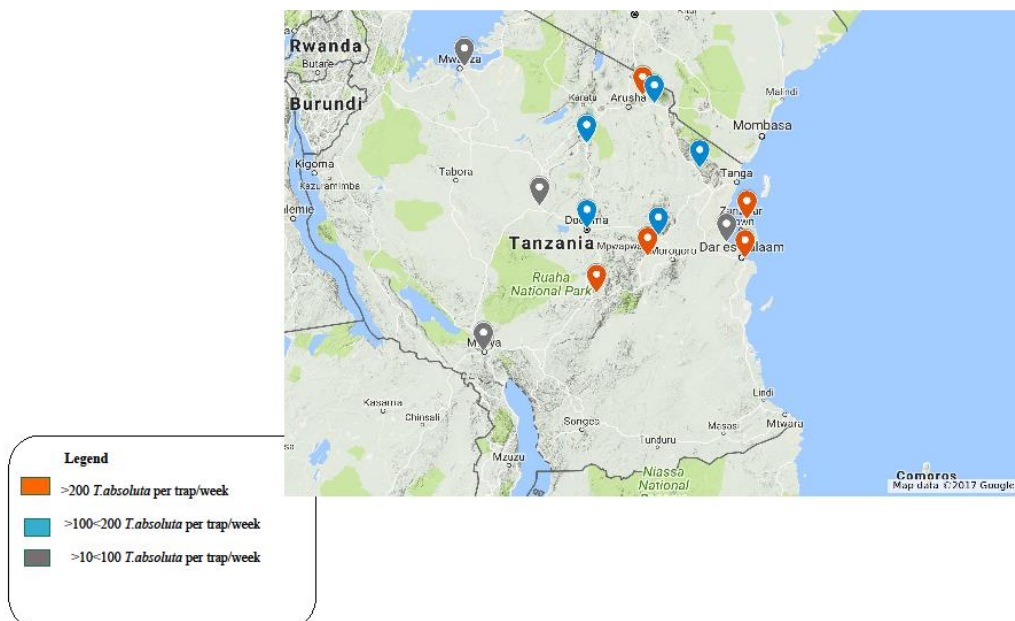


Illustration 1: Map of survey sites for the study of *T. absoluta* occurrence and distribution

Table 2: Occurrence, density and damage level by *T. absoluta* on tomato in the surveyed regions

Region	Number of adult <i>Tuta absoluta</i>/ trap	Level of damage by <i>T. absoluta</i> larvae
Arusha	192.1 ± 12.1b	83.3 ± 6.6a
Dar es salaam	135.7 ± 11.1b	80 .0 ± 0.0a
Dodoma	16.2 ± 1.2c	43.3 ± 3.3de
Iringa	299.3 ± 22.7a	90 .0 ± 0.0a
Kilimanjaro	27.1 ± 2.2c	53.3 ± 3.7 cd
Mbeya	22.0 ± 1.2c	46.6 ± 6.6 de
Manyara	137.8 ± 11.3b	70 ± 5.7bc
Morogoro	173.1 ± 10. 1b	86.7 ± 3. 3a
Mwanza	13.3 ± 1.0c	30 ± 5.7e
Pwani	20.8 ± 1.6c	43.3 ± 3.3de
Singida	32.1 ± 3.6c	43.3 ± 12.0de
Tanga	169.7 ± 14.9b	80 .0 ± 5.70a
Zanzibar	121.9 ± 11.9b	76.6± 3.3ab
P value	<.0001	<.0001



Plate 1: *T. absoluta* larvae damage on prevarvest green (a) and mature harvest red (b) tomato fruits in Ilula, Iringa region during the 2016 tomato growing season

4.1.2 Host range and incidence of damage by *T. absoluta* larvae

Results showed that *T. absoluta* larvae were associated with ten (10) hosts out of 11 plants evaluated during the survey (Table 3). The highest rate of infestation by *T. absoluta* on these hosts was on tomato, potato, night shade, African eggplant, eggplant and weed species identified as *Solanum dubium*. Of all these plants, tomato was found to be the most preferred host as it also appeared to have the highest number of mines by *T. absoluta* compared to other hosts in all surveyed regions (Table. 3 and Table 4). In all locations covered in the current study, the *T. absoluta* larvae were found associated with the different host plants damaging leaves, shoots, fruits and whole plant (e.g. tomato, plate 2). More results summary of infestation at each village in region and host evaluated in this study (Table 3).

The results also showed that, potato was the second most preferred host in all surveyed regions. The highest damage rate reached $60 \pm 0\%$ in Arusha and the lowest incidence was $0.0 \pm 0.0 \%$ in Morogoro, Tanga, Dodoma, Pwani and Mwanza (Table 4).

The results also showed that the damage by *T. absoluta* larvae on night shade plant varied significantly ($p < .0001$) between regions in which Arusha exhibited the highest score averaging 33.3 ± 3.3 compared to other regions (Table 4). It was only in Zanzibar where night shade seemed not to be infested by *T. absoluta* (Table 4). The damage by *Tuta absoluta*

larvae on weed plant identified as *S. incanum* differed significantly ($p < .0001$) between surveyed regions with Arusha, Iringa Manyara and Kilimanjaro exhibiting the highest damage level compared to other regions (Table 4). The levels of damage by *T. absoluta* on other host plants in each region are as shown in Table 4.

Table 3: Occurrence and number of *Tuta absoluta* mines in different host in surveyed villages

Region	Ward/Villages	Host and number of <i>T. absoluta</i> mines											
		Lat (S°), Long(E°)	SL	ST	SN	SA	SI	DS	CP	CA	SL	SD	CA
Arusha	Ngarenanyuki -1	03.2136, 36.8613	+++	++	+	+	++	+	+	+	+	+	-
	Ngarenanyuki -2	03.1896, 36.9042	++	+	+	+	++	+	+	+	+	+	-
	Ngarenanyuki -3	03.2130, 36.9003	+++	++	+	+	+	+	+	+	+	+	-
Morogoro	Dumila-1	06.4225, 37.3583	+++	+	+	+	+	+	+	+	+	+	-
	Dumila-2	06.4104, 37.3431	++	+	+	+	+	+	+	+	+	+	-
	Magole	06.3749, 37.3724	+++	+	+	+	+	+	+	+	+	+	-
Iringa	Ilula-1	07.6957, 36.0234	++	+	+	+	+	+	+	+	+	+	-
	Ilula-2	07.6709,36.0308	+++	+	+	+	++	+	+	+	+	+	-
	Ilula-3	07.6995, 37.3593	++	+	+	+	+	+	+	+	+	+	-
Tanga	Magamba	04.4427, 38.1753	+++	+	+	+	++	+	+	+	+	+	-
	Shume	04.3958, 38.1455	++	+	+	+	+	+	+	+	+	+	-
	Soni	04.5030, 38.2229	++	+	+	+	+	+	+	+	+	+	-
Zanzibar	Bambi	06.0831, 39.3501	+	-	-	-	-	+	+	-	-	+	-
	Mzuri	06.1697, 39.1997	+	-	-	-	-	+	+	-	-	-	-
	Fuoni	06.1898, 39.2458	+	-	-	-	-	+	+	-	-	+	-
Dar es salaam	Kibamba	06.8066, 39.0618	+	-	+	+	+	+	+	-	-	-	-
	Kariakoo	06.8199, 39.2751	+++	-	+	+	+	+	+	+	+	+	-
	Jangwani	06.8128, 39.2913	++	-	+	+	+	+	+	+	+	+	-
Manyara	Gallapo	04.2552, 35.8508	+	+	+	+	+	+	+	+	+	+	-
	Magugu	03.9949, 35.7684	++	+	+	+	+	+	+	+	+	+	-

	Dareda	04.2419,35.4979	++	+	+	+	+	+	+	+	+	+	-
Kilimanjaro	Weruweru	03.3278, 37.2575	++	+	+	+	+	+	+	+	+	+	-
	Mabogini	03.4188, 37.3663	++	+	+	+	+	+	+	+	+	+	-
	Njiapanda	03.4099,37.5283	++	+	+	+	+	+	+	+	+	+	-
Singida	Itigi-1	05.7325, 34.5123	+	-	+	+	+	+	+	+	+	+	-
	Itigi-2	05.7476, 34.8513	++	-	+	+	+	+	+	+	+	+	-
	Itigi-3	05.7639, 34.8497	+	-	+	+	+	+	+	+	+	+	-
Pwani	Msoga	06.5713, 38.3065	++	-	+	+	+	+	+	+	+	+	-
	Lugoba	06.4683, 38.3265	++	-	+	+	+	+	+	-	-	-	-
	Msata	06.3518, 38.3786	+	-	+	+	+	+	+	-	-	-	-
Mbeya	Mbalizi	08.9407, 33.3416	++	+	+	+	+	+	+	-	-	-	-
	Igawilo	08.9007, 33.5527	+	+	+	+	+	+	+	-	-	+	-
	Inyala	08.8643, 33.6310	++	+	+	+	+	+	+	-	-	+	-
Dodoma	Mlali	06.1402, 36.8645	++	-	+	+	+	+	+	-	-	-	-
	Iduo	06.2815, 36.6477	++	-	+	+	+	+	+	-	-	+	-
	Sagara	06.2511, 36.6477	+	-	+	+	+	+	+	-	-	+	-
Mwanza	Nyegezi	02.5938, 32.9044	++	-	+	+	+	+	+	-	-	+	-
	Nsumba	02.6021, 32.8995	+	-	+	+	+	+	+	-	-	-	-
	Feta	02.5850, 32.8982	+	-	+	+	+	+	+	-	-	-	-

Legend: - = adult *T. absoluta* not present; += 1 to 9 adult *T. absoluta* present; ++= 10 to 20 adult *T. absoluta* present; +++= more than 20 adult *T. absoluta*

Table 4: Percentage of *T. absoluta* larvae damage on different host plants

Region	SL	ST	SN	SI	SA	SM	CP	CL	DS	SD	CA
Iringa	90.0 ± 0.0 a	36.6 ± 3.3b	26.6 ± 3.7ab	30.0 ± 0.0a	23.3± 3.7a	10.0 ±0.0bc	3.3 ± 3.3a	6.6 ±3.3a	3.3 ± 3.3a	3.3 ± 3.3a	0.0 ± 0.0a
Morogoro	86.6 ± 5.7a	0.0 ± 0.0c	20.0 ±0.0ab	23.3± 5.7ab	16.6 ±3.3a	16.6 ±3.3ab	10.0 ±0.0a	3.3 ± 3.3a	3.3±3.3a	3.3 ±3.3a	0.0 ± 0.0a
Arusha	83.3 ± 5.7a	60.0 ±0.0a	33.3 ± 3.3a	30.0 ±0.0a	23.3±3.3ab	16.6 ± 3.7ab	13.3 ± 3.3a	10.0 ±0.0a	3.3 ±3.3a	3.3±3.3a	0.0 ± 0.0a
Dar es salaam	80.0±0.0a	23.3±3.3b	26.6± 3.3 ab	23.3 ± 3.3ab	20.0±0.0abc	13.3 ±3.3bc	6.6 ± 3.3a	10.0±0.0a	6.6 ± 3.3a	6.6±3.7a	0.0 ± 0.0a
Tanga	80.0±0.0a	0.0 ±0.0c	23.3 ± 3.3 ab	20.0±0.0abc	20.0±0.0abc	20.0 ±0.0a	6.6 ± 3.3a	10.0±0.0a	6.6 ± 3.3a	3.3 ±3.3a	0.0 ± 0.0a
Zanzibar	76.6 ± 2.7ab	0.0 ± 0.0c	0.0 ± 0.0 d	16.6 ± 3.3abc	6.6 ± 0.3bc	0.0 ± 0.0d	0.0±0.0a	0.0 ± 0.0 a	0.0±0.0a	0.0±0.0a	0.0 ± 0.0a
Manyara	70.0 ±0.0ab	26.6 ±3.3b	26.6 ± 3.3 ab	30.0 ± 0.0a	20.0 ±0.0ab	6.6 ± 3.3cd	10.0±0.0a	6.6 ± 3.3a	6.6 ± 3.3 a	3.3 ± 3.3a	0.0 ± 0.0a
Kilimanjaro	53.3 ± 3.3ab	36.6 ± 3.3b	30.0 ± 0.0ab	30.0 ±0.0a	23.3 ±3.3 ab	13.3 ±0.3bc	10.0 ± 0.0a	10.0 ± 0.0a	6.6 ± 3.7a	3.3 ±3.3a	0.0 ± 0.0a
Mbeya	46.6±2.7bc	33.3 ±3.3b	23.3 ± 3.3bc	13.3 ± 3.3bc	20.0 ± 0.0 ab	6.6±3.3cd	10.0±0.0a	3.3 ±3.3a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Dodoma	43.3 ± 3.3bc	0.0 ± 0.0c	10.0 ±0.0d	6.6 ± 3.3c	10.0 ± 0.0bc	3.3 ±3.3cd	0.0± 0.0ab	3.3 ± 3.3a	0.0 ± 0.0a	0.0 ± 0.0a	0.0±0.0a
Pwani	43.3± 3.7bc	0.0 ± 0.0c	16.6 ± 3.3 cd	10.0 ± 0.0bc	10.0 ± 0.0bc	13.3 ± 3.3bc	0.0± 0.0ab	3.3 ± 3.3a	3.3 ± 3.3a	0.0 ± 0.0a	0.0±0.0a
Singida	43.3 ± 3.3bc	0.0 ± 0.0c	16.6 ± 3.3 cd	13.3 ± 3.3bc	13.3±3.3bc	10.0±0.0bc	3.3 ± 3.3a	3.3 ± 3.3a	0.0 ± 0.0a	0.0 ± 0.0a	0.0± 0.0a
Mwanza	30.0 ± 0.0 c	0.0 ± 0.0c	16.6 ± 3.3 cd	10.0± 0.0bc	16.6 ±3.3bc	6.6 ± 0.3cd	6.6 ± 3.3a	3.3 ± 3.3a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.03	0.10	0.08	0.32	-

Legend: SL-*Solanum lycopersicum*, ST-potato (*Solanum tuberosum*), SN- nightshade (*Solanum nigrum*), SM-eggplant (*Solanum melongena*), SA-African eggplant (*solanum aethiopicum*), CP-Chill pepper (*Capsicum*), SD-weed (*Solanum dubium*), SL-watermelon (*Citrullus lanatus*), DS- jimson weed (*Datura stramonium*), SI- weed (*Solanum incanum*) and CA- sweet pepper (*Capsicum annuum*).

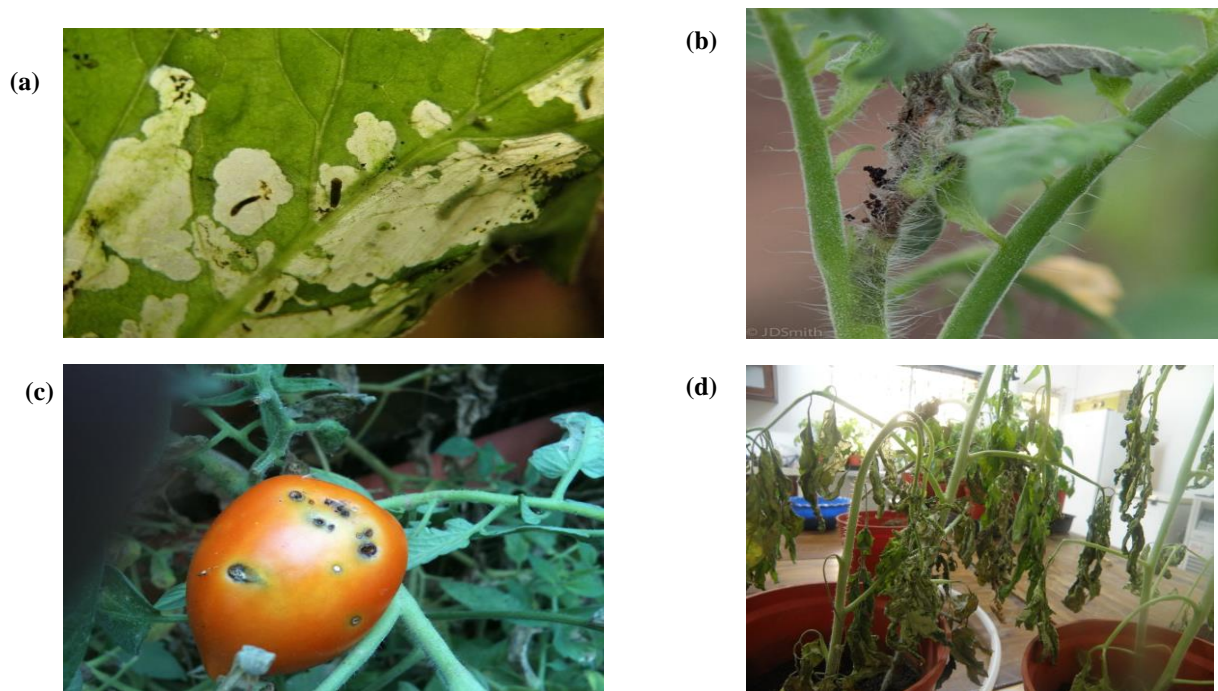


Plate 2: Damage by *T. absoluta* larvae infesting tomato in all aerial parts including leaves (a), shoot (b), fruits (c) stem and whole plant (d).

4.1.3 Population dynamics of *Tuta absoluta*

The population of *T. absoluta* varied significantly ($p < .0001$) between seasons and between the regions of Iringa, Arusha and Morogoro (Table. 5). The highest (250.25 ± 21.30 moth/trap) number of adult *T. absoluta* trapped was recorded in Iringa during the September-December 2015 dry season and the lowest (120.93 ± 10.32 moth/trap) was recorded in Morogoro during the January –April 2016 rain season (Table 5). The results trend shows that in seasons with low rains record, higher number of adult *T. absoluta* was trapped. For instance, this highest count of adult *T. absoluta* (250.25 ± 21.30) was recorded at 0.00mm rain and at a temperature of while the average 20°C and the described lowest count (120.93 ± 10.32) was recorded at 180.9 mm rain and 27°C temperature. Despite the seasons, Iringa exhibited the highest moth density in both dry and wet season across three years of field study (Table 5). The results also showed that the duration of the lure (Plate 3) significantly ($p < .0001$) affected catches of *T. absoluta*. Iringa region exhibited the highest count of adult *T. absoluta* in week 1 (493.50 ± 27.54 moth/trap) compared with other regions. The last three weeks; 14, 15, 16 exhibited the lowest moth count in all three regions, however the lowest moth count/trap (51.4 ± 8.1) was trapped in Morogoro (Fig. 1).

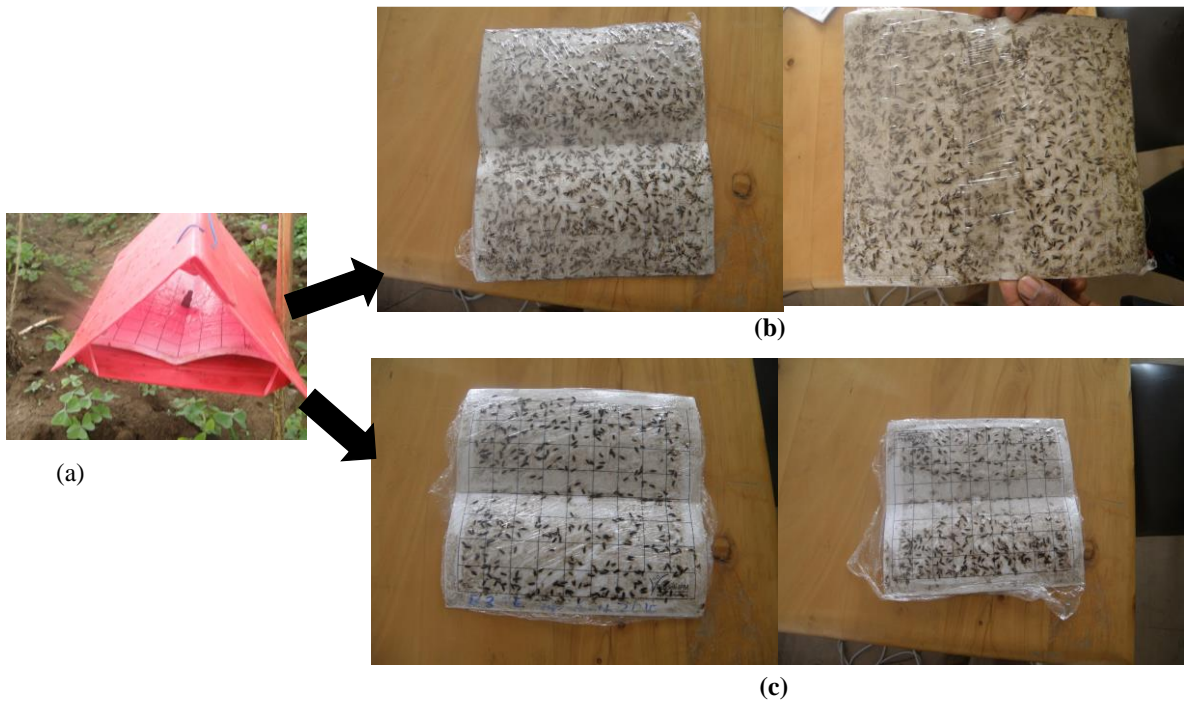


Plate 3: Delta trap with pheromone lure (a) for attraction of adult *T. absoluta*, a dense card with moth in dry season, November 2016 (b) and less dense cards in rainy season, April 2016 (c).

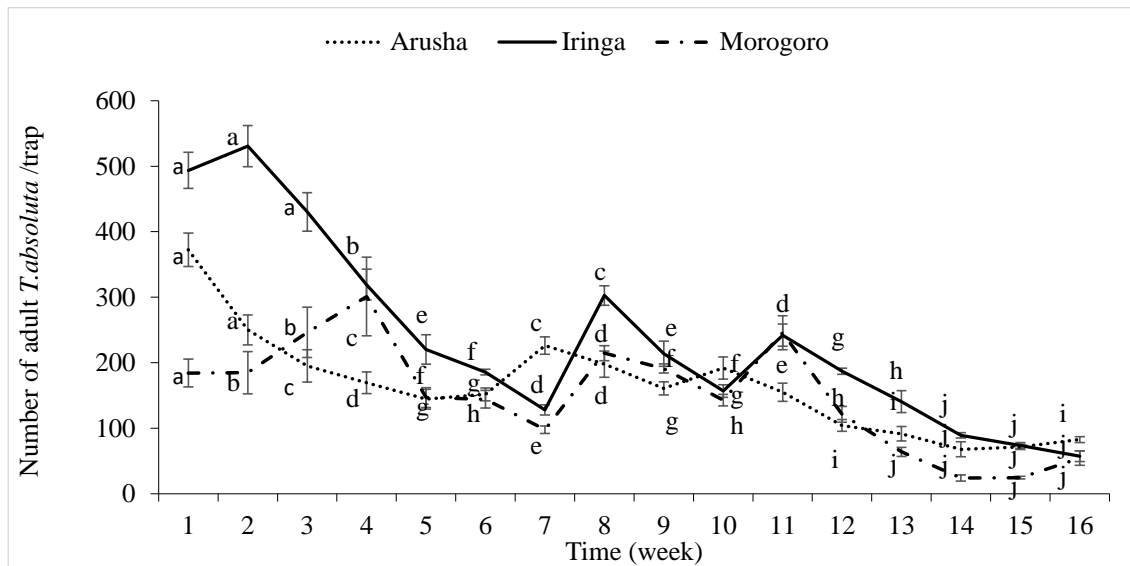


Figure 1: Population of *T. absoluta* in 16 different weeks in Iringa, Morogoro and Arusha regions recorded during this study

Table 5: Number of *T. absoluta*/trap under different conditions in Arusha, Morogoro and Iringa

Season of year	Region	Temperature (°C)	Rainfall (mm)	<i>T. absoluta</i> density
Sept-Dec2015	Arusha	20.35	0.875	198.39 ±15.09abc
	Iringa	20.23	0	250.25±21.30a
	Morogoro	24.12	8.67	180.70±19.59bcd
Sept-Dec 2016	Arusha	22.11	89.67	136.72±11.18abc
	Iringa	23.7	67.72	230.81±23.39b
	Morogoro	27.93	113.22	151.5±19.02de
Jan-Apr2016	Arusha	19.57	9.32	183.3±13.62cde
	Iringa	20.63	0.15	244.27±20.77ab
	Morogoro	23.86	48.2	143.81±11.48de
Jan-Apr2017	Arusha	22.56	157.92	139.79±10.73de
	Iringa	22.47	169.07	217.31±22.98ab
	Morogoro	27.13	180.9	120.93±10.32e
P value				<.0001

4.1.4 Effect of plant extracts on *Tuta absoluta*

Using five weeks old potted tomato plants (plate 4) for *T. absoluta* rearing (Plate 5) and studying the effects different plant extracts under invitro experiments (Illustration 2), the results showed that *B. abyssinica* leaves were effective against larvae of *T. absoluta* by

exhibiting $86.6 \pm 3.8\%$ mortality compared to ethyl acetate and ethanolic extracts and water control (Fig 2). The petroleum ether extract from *B. abyssinica* stem bark extract also exerted high activity on larvae by causing up to $83.3 \pm 4.8\%$ than control that exhibited only $6.6 \pm 0.9\%$ larvae mortality (Fig. 2). The ethanolic extract of *B. abyssinica* stem bark and leaves extracts were significantly ($p < .0001$) effective, affecting survival duration of adult *T. absoluta* compared to control. The *B. abyssinica* ethanolic extracts of stem barks were more active than ethyl acetate and petroleum ether extract (Fig. 3). The petroleum ether and ethanol extracts from *C. africana*, *T. minuta* and *M. oleifera* were significantly ($p = .0033$) effective against *T. absoluta* by causing high mortality on larvae that reached $80.00 \pm 5.77\%$ mortality in 10% *C. africana* leaf petroleum ether extract (CLP), *M. oleifera* ethanolic extract (MOE) *C. africana* ethanolic extract (CLE) and *T. minuta* ethanolic extract (TME) treated larvae compared to control that caused only $23.33 \pm 0.33\%$ mortality after 9 days of exposure (Fig. 4). Botanical extracts from *C. africana*, *T. minuta* and *M. oleifera* significantly ($p = .0033$) inhibited pupation compared to control that had higher pupation rate averaging $76.66 \pm 3.33\%$. The pupation rate in 10% CLP, MOE, CLE and TME treated larvae was low amounting $20.00 \pm 5.77\%$ only (Fig. 5). For the successful pupae placed in cages (plate 6) for emergence of adult, result showed that extracts from *C. africana*, *T. minuta* and *M. oleifera* significantly ($p = .0033$) inhibited adult development from treated pupae compared to control that had high rate of adult emergence averaging $70.00 \pm 5.77\%$. Treating larvae with CLE and MOE resulted in low number ($16.66 \pm 6.66\%$) of adults emerged (Fig. 6). The effect of plant extracts was significant ($p < .0001$) in reducing survival duration of adult *T. absoluta* especially in 10% extracts treatments where survival duration was 9.00 ± 0.42 day shorter than in 5%, 2% extracts and control, where in the later adult survived for 17.00 ± 0.78 days (Fig. 7). The effect of treatment was significant ($p < .0001$) on survival duration of adults *T. absoluta*. In the CLP treated insect, survival duration reached 10.9 days compared to control that survived longer up to 27.5 days (Fig. 8). Of all extracts, the ethanolic extracts of *C. africana* leaves and *M. oleifera* seeds at 10% concentrations had higher activity against larvae compared to *T. minuta* leaves, petroleum of all extracts and control. However, petroleum ether extracts of *C. africana* and *T. minuta* depicted the highest mortality rate against adult *T. absoluta* compared to ethanol and control (Fig. 7). All extracts were effective in controlling different stages of *T. absoluta* under laboratory conditions (Illustration 2).



Plate 4: Young (5 weeks old) potted tomato plant with three fully expanded leaves used for *T. absoluta* inoculation and botanical bioassay against *T. absoluta* larvae



Plate 5: Colony of *T. absoluta* reared in meshed Perspex cages at 20-28⁰C and 70-80%RH for collection of moths for botanical bioassays.

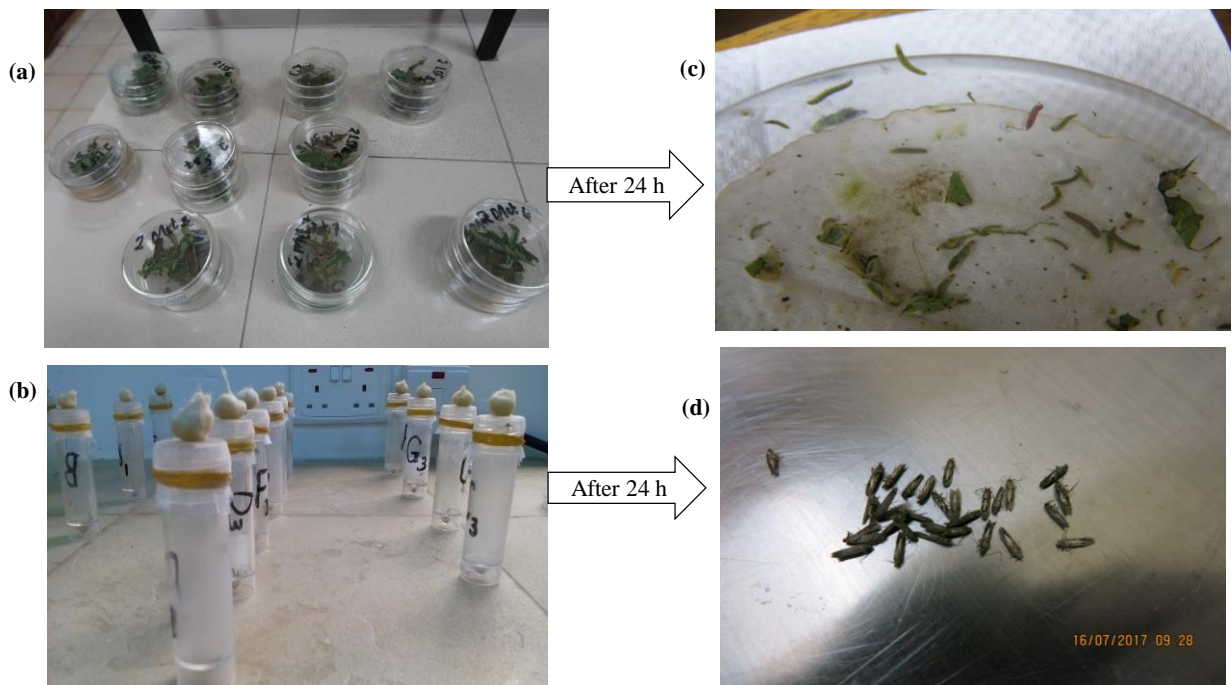


Illustration 2: Effect of botanical extracts on *T. absoluta* larvae in petri dishes (a) and adults into plastic vials (b) mortality on larvae after 24 h (c) mortality on adults after 24 h (d)

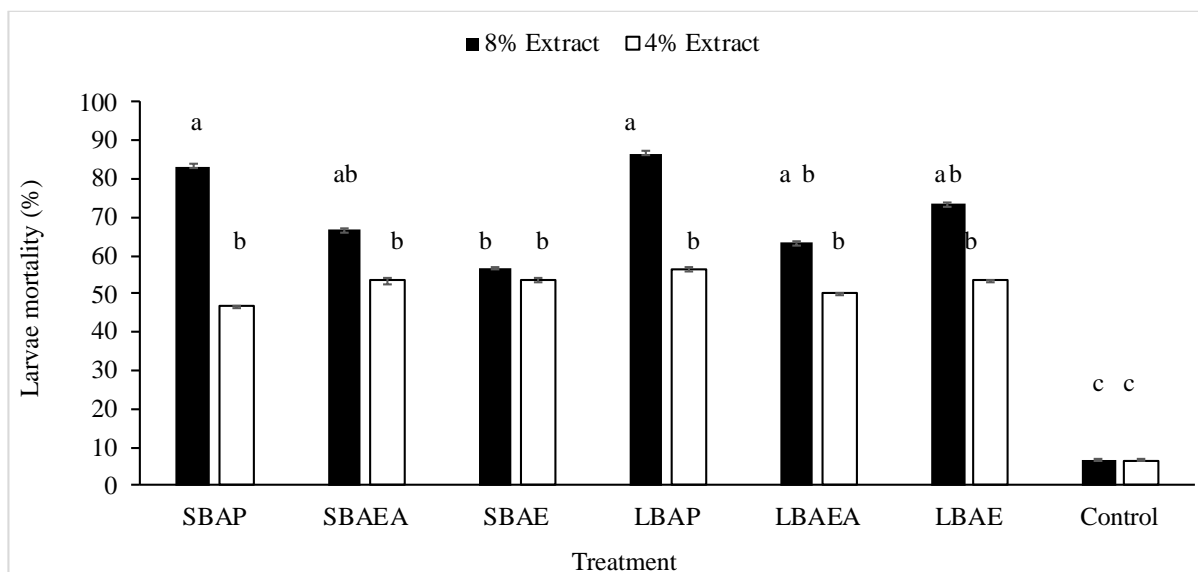


Figure 2: Effect of *B. abyssinica* extract on *T. absoluta* larvae

Legend: BAE-*Bersama abyssinica* ethanolic extract of leaves, LBAEA- *Bersama abyssinica* ethyl acetate extract of leaves, LBAP- *Bersama abyssinica* petroleum ether extract of leaves, SBAE-*Bersama abyssinica* ethanol extract of stem bark, SBAEA- *Bersama abyssinica* ethyl acetate extract of stem bark and SBAP- *Bersama abyssinica* petroleum ether extract of stem bark.

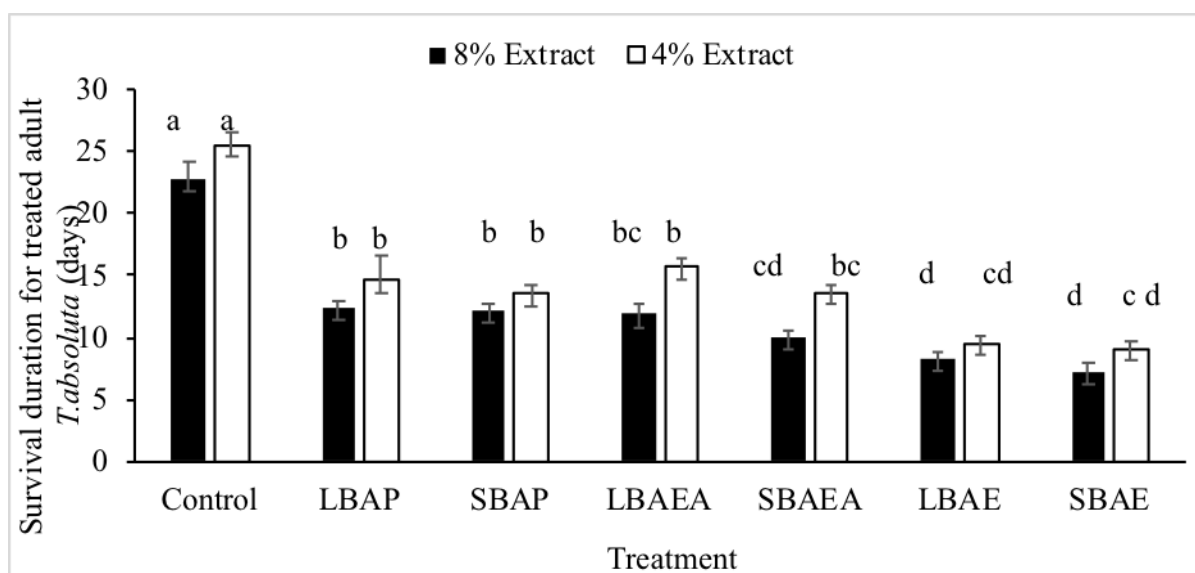


Figure 3: Effect of *B. abyssinica* extract on survival duration of adult *T. absoluta*

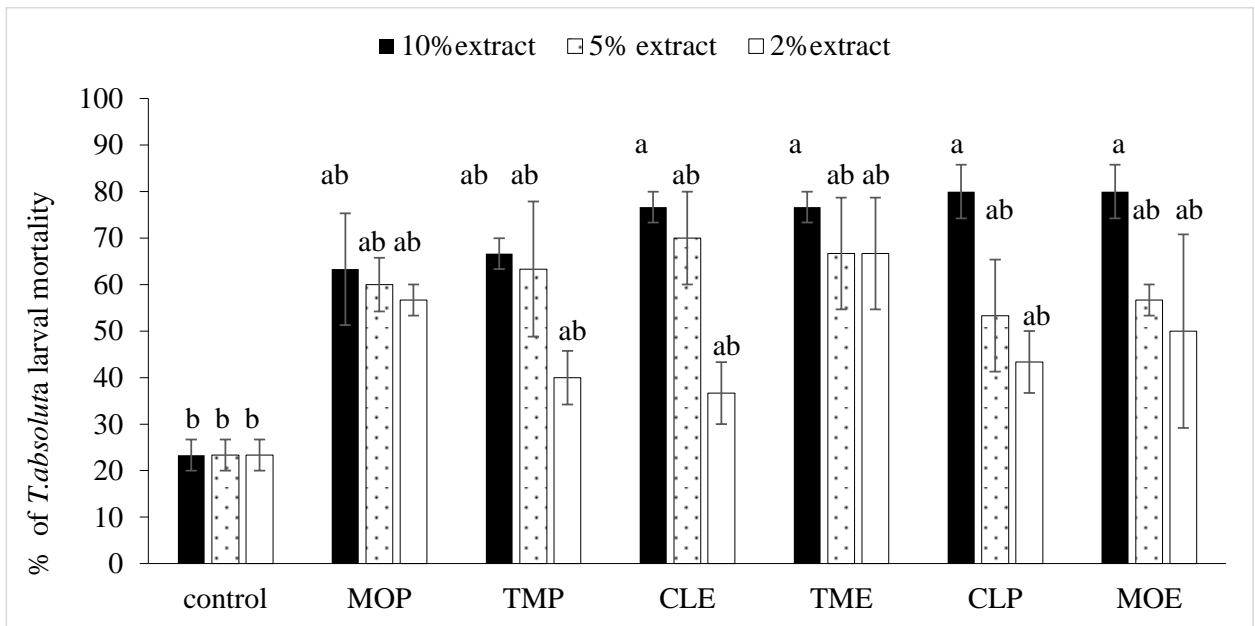


Figure 4: Effect of botanical extracts on larvae of *T. absoluta*

Legend: TME- *Tergetes minuta* ethanol extract, TMP- *Tergetes minuta* petroleum ether extract, CLE-*Commiphora africana* ethanol extract, CLP-*Commiphora africana* petroleum ether extract, MOE-*Moringa oleifera* ethanol extract and MOP- *Moringa oleifera* ethanol extract.

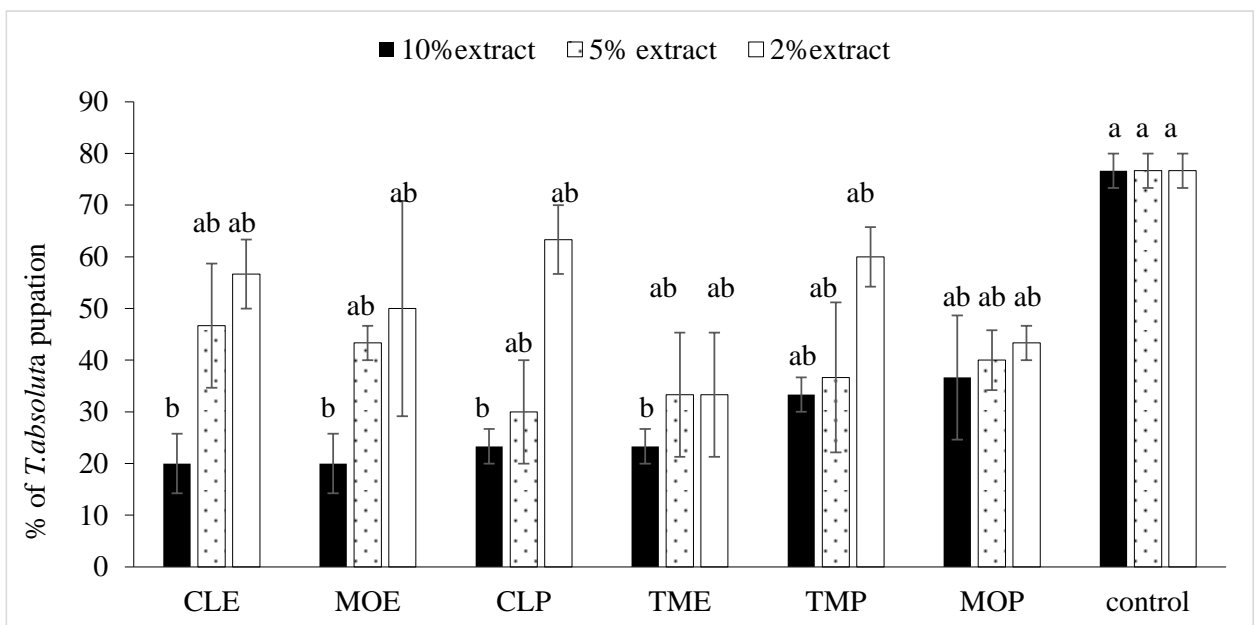


Figure 5: Effect of botanical extracts on pupation of *T. absoluta*. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

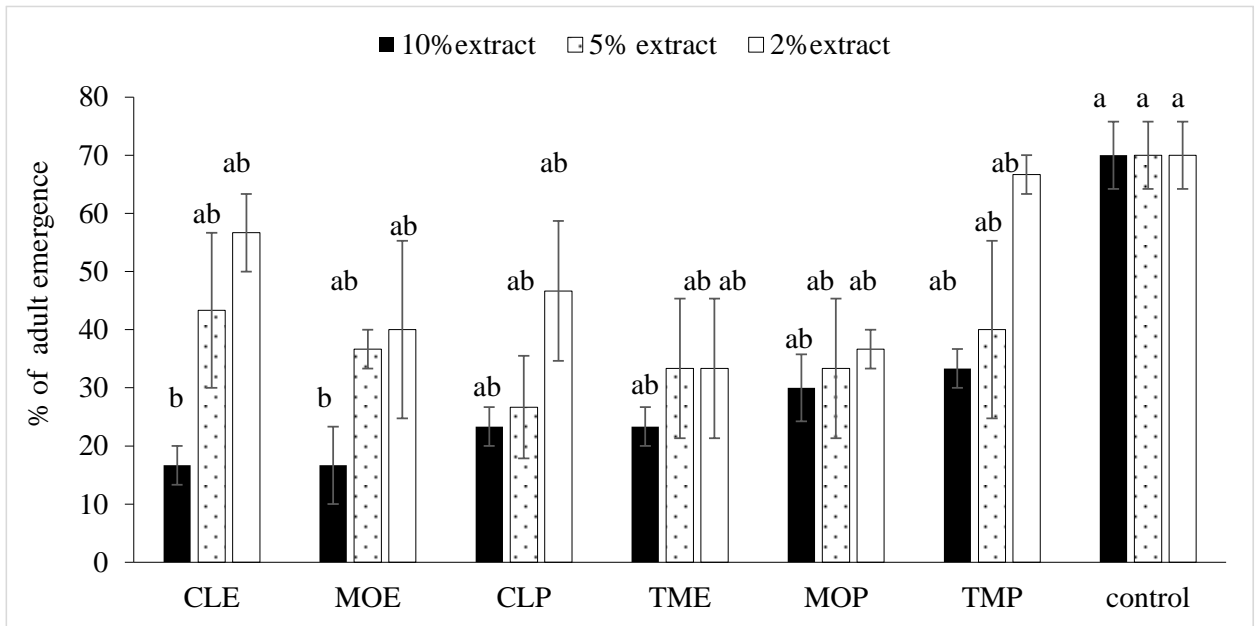


Figure 6: Effect of botanical extracts on emergence of adult *T. absoluta*. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

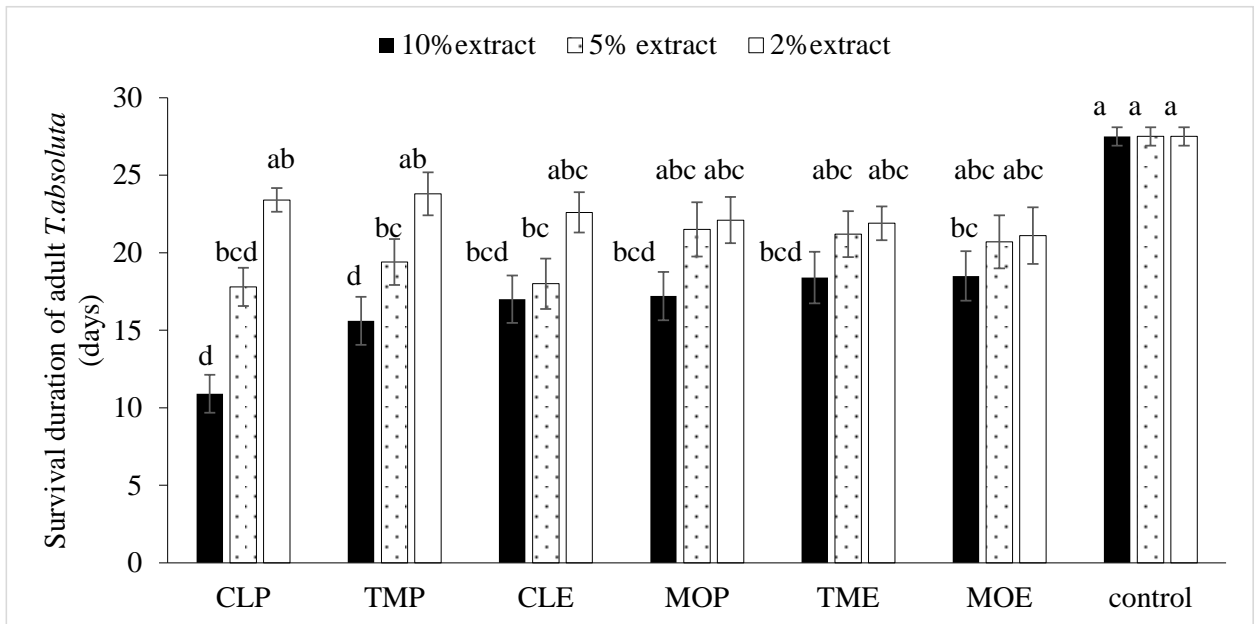


Figure 7: Effect of Botanical extract on survival of adult *T. absoluta*. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

4.1.5 Seedlings emergence rate and susceptibility to *Tuta absoluta* in screenhouse conditions

Result showed that landrace accession had rate of seedlings emergence compared to check. In accession BKB-22 the seedlings emerged reached 93.93% within 5.42 ± 0.71 days whereas Tengeru 97 emergence rate reached 86.36% after 8.19 ± 0.22 days (Table 5). Landrace accessions exhibited significant ($p < .0001$) resistance against *T. absoluta* larvae damage in averaging 5.38 ± 0.85 mines/accession compared with the control Tengeru 97 exhibited 6.84 ± 0.03 mines/plant (Table 5).

For field trial, number of leaves/plant and height from seedling stage was a determinant factor for growth and vigor (plate 7). There was significant ($p < .0001$) variation in number of leaves/plants between tomato genotypes in which most land races exhibited higher number of leaves/plants compared with the control cultivar (Table 5). Accession HSK-38 possessed significantly the highest number of leaves that averaged 29.04 ± 1.12 leaves/plant than accession KGM-5 and control where the later possessed few leaves averaging 18.75 ± 0.62 leaves/plant (Fig. 8).

The number of *T. absoluta* mines varied significantly ($p < .0001$) between tomato genotypes and most landraces exhibited few mines compared to the control that was more susceptible to larvae damage. Accession BKB-22 exhibited a significant low number of mines averaging 7.94 ± 0.74 per plant compared to the control that possessed higher number of mines averaging 12.98 ± 0.98 mines/plant (Fig. 9).

The harvest frequency varied significantly ($p < .0001$) between tomato accessions and growing zones. Landrace accessions exhibited several harvest frequencies with short harvest intervals in which the east-central zone had more harvest frequency/week than southern and northern zone. In central zone accession KGM-5 had high harvest frequencies that reached 3.61 ± 0.32 /week compared to accession BKB-22, HSK-43, HSK-38 and the control, where the later had only 0.5 ± 0.09 harvest/week. In northern zone accession 3 had more harvest frequency which averaged 3.12 ± 0.35 than accession KGM-5, HSK-43, HSK-38 and control that had only 0.48 ± 0.08 frequency/week (Fig. 10). The southern zone exhibited the lowest fruit harvest frequency in which accession 2 had more harvest rate averaging 2.90 ± 0.21 in contrast to check which only 0.71 ± 0.10 harvest/week was achieved (Fig. 10).

Fruit damage by *T. absoluta* larvae is indicated by presence mines or pin holes on fruits (Kilic, 2010). Variation of fruit damage/plant was significant ($p < .0001$) different between tomato varieties and zone where up to 118.66 ± 21.18 g/plant was damaged due to *T. absoluta* larvae in check plants than in landrace accession KGM-5, HSK-43, HSK-38, BKB-22 the later exhibiting only 22.69 ± 3.23 g/plant of damaged fruits in northern zone, followed by northern zone that averaged 66.44 ± 11.54 g/plant in check compared to accession 4 which was damaged by 29.85 ± 4.01 g/plant. The east-central zone exhibited the lowest damage with the check being most damaged with an average damage of 51.81 ± 5.00 g/plant compared to accession KGM-5, HSK-43, BKB-22 and HSK-38, where the later exhibited the lowest damage that averaged 34.62 ± 4.44 g/plant (Fig. 11).

Tomato yield varied significantly ($p < .0001$) between tomato accessions and zones in which landrace accessions yielded more fruits (t/ha) than control in all zones. The east-central zone yielded highest fruits than southern and northern where accession HSK-43 yielded the highest fruits that averaged 18.82 ± 2.67 t/ha compared to check that yielded 1.96 ± 1.08 t/ha. In the southern zone, the highest yield was achieved by accession BKB-22 that averaged 18.82 ± 1.93 t/ha in contrast to control that yielded the lowest fruits averaging 8.60 ± 3.50 t/ha. On other hand in the northern zone accession 3 yielded the highest weight that averaged 17.15 ± 3.45 t/ha than accession KGM-5, HSK-43, HSK-38 and the control that yielded the lowest fruits averaging 0.51 ± 0.08 t/ha (Fig. 12).

Percentage of tomato fruits damaged by *T. absoluta* larvae differed significantly ($p < .0001$) between accessions and growing zone. The control tomato plants in the southern zone exhibited the highest percentage of damage compared to landraces accession in the northern and east-central zone. The southern zone exhibited the lowest fruit damage where only 8.34 ± 1.35 % damage incidences was recorded in accession 1 compared to the control which had the highest damage incidence that averaged 80.27 ± 12.14 %. High yield loss due to *T. absoluta* damage reached 65.07 ± 11.46 % and 65.07 ± 11.46 % damage in the control compared to accession HSK-38 that averaged 9.54 ± 1.2 % and 11.39 ± 1.24 % in tomato damage in the southern zone and east-central zone respectively (Fig. 13).

The results also showed that four landraces accessions persisted longer for more than 35.53 ± 1.81 days before deterioration than check that lasted for shorter 16.65 ± 0.36 days (Fig. 14). The landraces showed physiological, agronomic and nutritional attributes that

superseded improved tomato cultivar Tengeru 97 that was used as control in this experiment. All the four landrace accessions lasted longer in 20⁰ C and below and remained firm (Plate 7).

Table 6: Seedlings emergence rate and susceptibility of tomato accessions to *T. absoluta* damage

Source	Genotype	Seedlings emerged	% Seedlings emerged	Emergence time (days)	No. of mines/plants
Bukoba	BKB-22	124	93.939	5.42±0.71	0.14±0.01a
Kigoma	KGM-5	118	89.39	5.85±0.33	0.5±0.05a
Mbeya	HSK-43	124	93.939	6.11±0.18	0.66±0.10a
Ruvuma	HSK-38	122	92.42	7.14±0.67	0.66±0.12a
Control	Tengeru 97	114	86.36	8.19±0.22	6.84±0.03b
Singida	SGD-1	122	92.42	6.19±0.30	4.28±0.84b
Ruvuma	HSK-29	114	86.363	6.22 ±0.28	4.33±0.54b
Singida	SGD-7	112	84.84	6.28±0.26	4.57±0.21b
Mbeya	HSK-39	124	93.93	6.33±0.32	4.66±0.18b
Kigoma	KGM-9	122	92.42	6.52±0.22	4.71±0.25b
Bukoba	BKB-1	118	89.39	6.52±0.04	4.80±0.58b
Njombe	HSK-17	116	87.87	6.57±0.24	4.85±0.88b
Njombe	HSK-18	114	86.36	6.70±0.28	4.90±0.29b
Njombe	HSK-19	112	84.84	7.10±0.03	4.95±0.19b
Singida	SGD-2	120	90.90	7.04±0.07	5.04±0.75b
Kigoma	KGM-8	118	89.3	7.14±0.35	5.09±0.21b
Ruvuma	HSK-31	114	86.36	7.23±0.44	5.12±0.22b
Mbeya	HSK-41	120	90.90	8.129±0.46	5.07±0.65b
Iringa	HSK-1	122	92.42	8.14±0.29	5.14±0.91b
Iringa	HSK-7	118	89.39	8.12±0.74	5.14±0.62b
Iringa	HSK-09	116	87.87	8.22±0.87	5.38±0.85b
Bukoba	BKB-25	118	89.39	8.80±0.32	5.31±0.65b
	Total seeds	132			
P value					<.0001



(a)



(b)

Plate 6: Tomato seedlings (3 weeks old) raised in plastic trays (66 holes) in screen house condition (a) increased in length and vigour 3 weeks after transplanting in the field in Arusha (b).

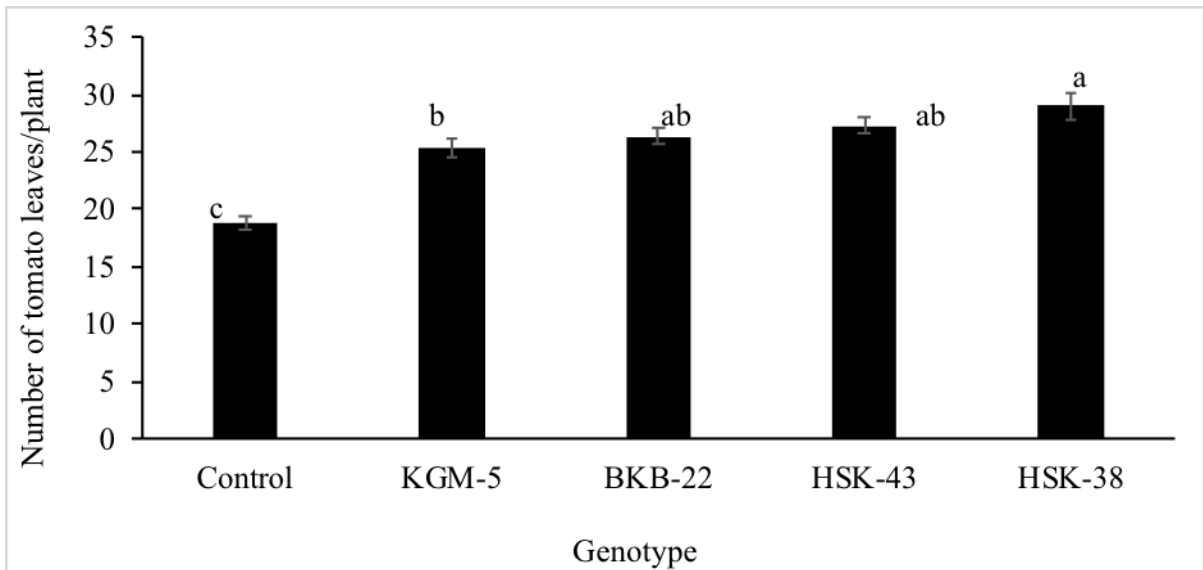


Figure 8: Number of tomato leaves/ selected tomato genotypes plant. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

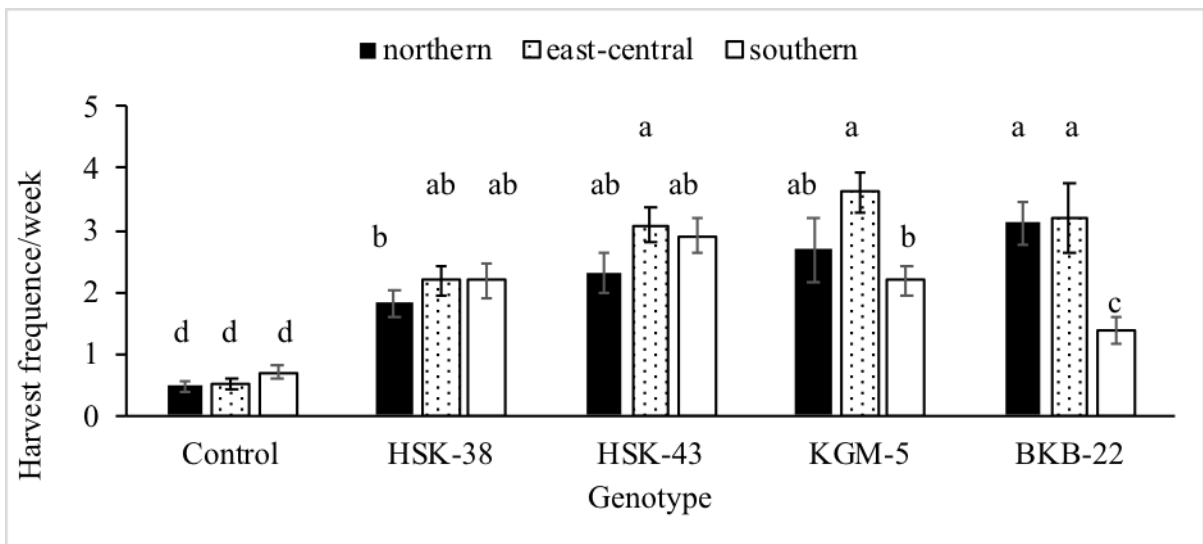


Figure 9: Weekly tomato harvest on selected tomato genotypes in different zones. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

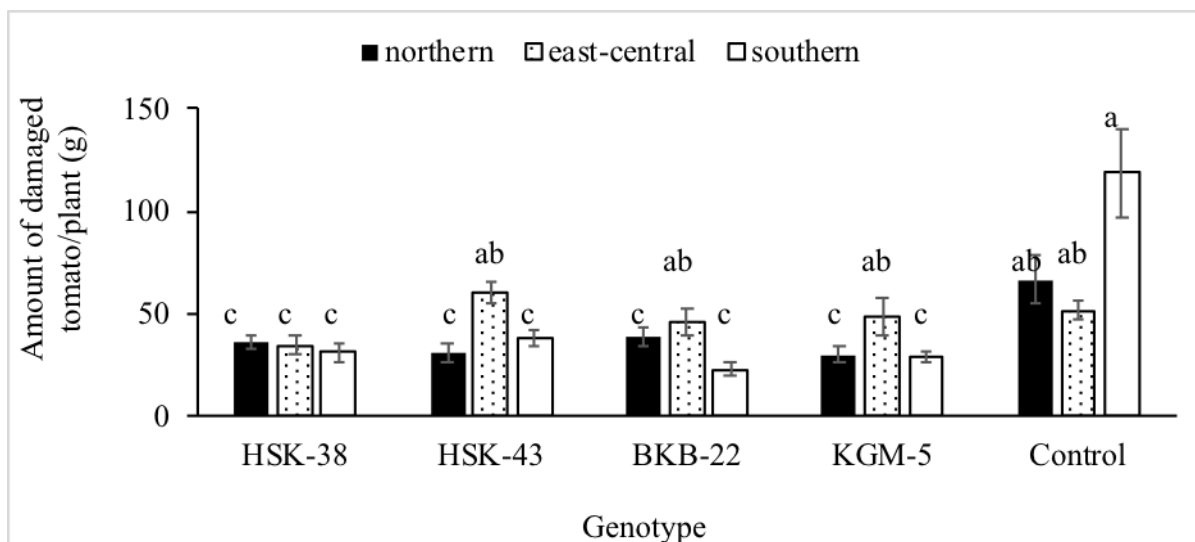


Figure 10: Level of fruits damaged by *T. absoluta* larvae on selected tomato genotypes. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

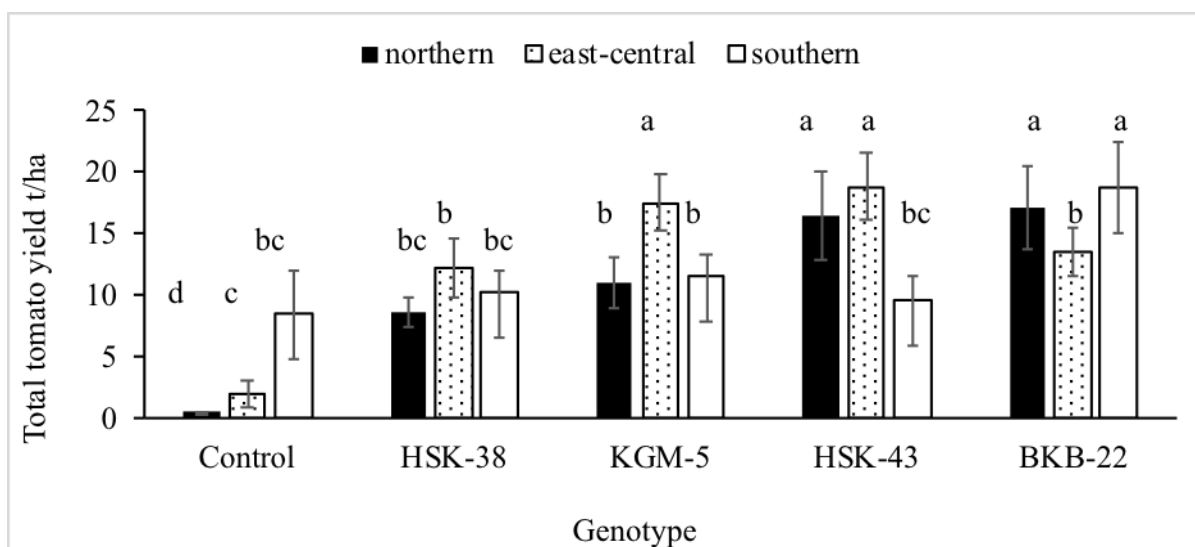


Figure 11: Yield of selected tomato genotypes (yield/ha) in northern, eastern and southern zones. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

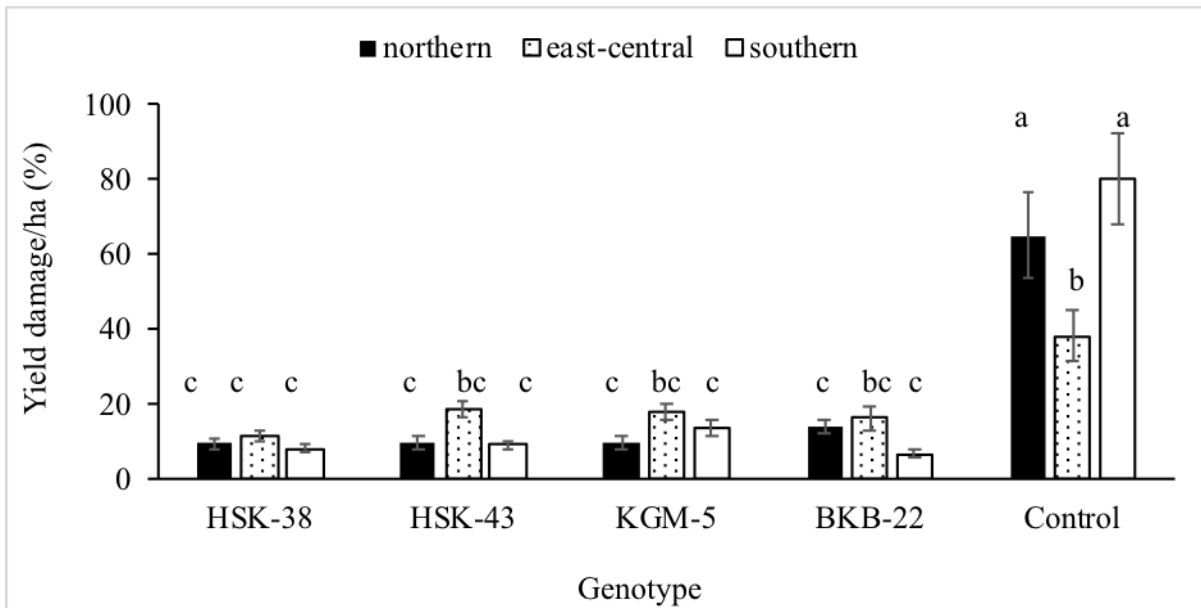


Figure 12: Percentage of yield damage/ha due to *T. absoluta* larvae. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

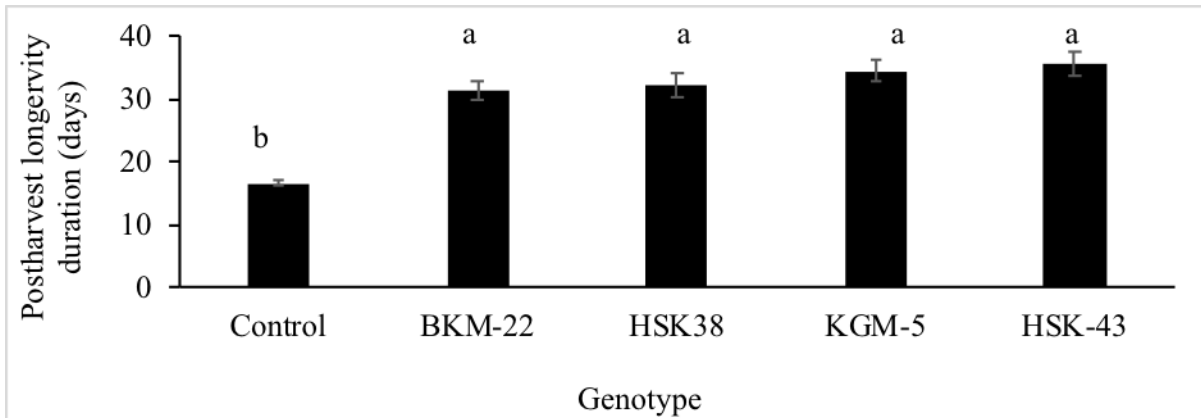


Figure 13: Post-harvest longevity of fruits from selected tomato genotypes stored at 20°C in the laboratory at the NM-AIST. Turkey's HSD was used to separate means at 5% probability level of significance. Error bars indicates standard error and different letters mean statistically different between treatments.

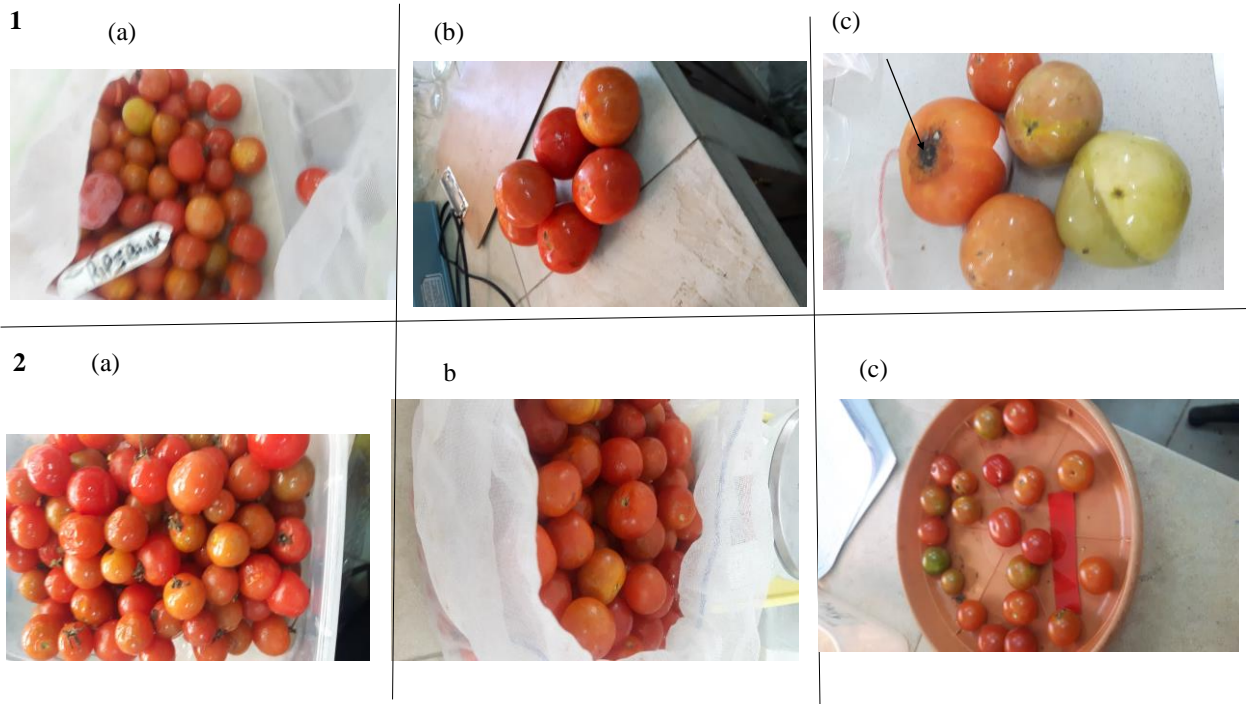


Plate 7: Resilience variation between Tengeru 97 (1) and landraces (2) fruits; Three hours after harvest Tengeru 97 (1a, after seven days decay (1b) and microbial spoilage after 14 on tomato (1c), landrace fruits 3 h post-harvest (2a), 7 days of landrace ripening (2b) and 14 days green ripened and retained firmness (2c).

4.1.6 Effect of entomopathogenic fungi on *T. absoluta*.

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4.2 Discussion

The survey conducted in thirteen regions of Tanzania revealed that *Tuta absoluta* is present and is associated with about ten host plants. The presence of a wide host range, infestation mechanism and favorable climatic conditions give the *T. absoluta* the power to invade new areas in high densities as per support by Desneux *et al.*, 2011 and Megido *et al.*, 2013a. In the surveyed areas, the speed of dispersion and establishment of *T. absoluta* is highly influenced by the presence of the highly preferred tomato host particularly among others, Tengeru 97 as noted in this study (Megido *et al.*, 2013a; Urbaneja *et al.*, 2013). In 2014, *T. absoluta* was only reported at Ngarenanyuki, northern Tanzania (Chidege *et al.*, 2016), however in this study *T. absoluta* had been found to be present in all 13 surveyed regions of Tanzania alarming a possibility that it is most likely to be present in other regions of the country and beyond to eventually in Sub Saharan Africa (Zekeya *et al.*, 2017). The rapid spread of *T. absoluta* from northern (Arusha) to southern (Iringa) has previously been described as a “north-south movement” pattern of *T. absoluta* which is causing high damage in tomato (Guimapi *et al.*, 2016). The main carriers for the *T. absoluta* has been reported to be associated with the availability of tomato and other favorable host across seasons and movement of infected tomato fruits and containers especially to and from big markets such as Kariakoo in Dar es Salaam where major whole sellers of tomato and other solanaceous are marketing produces (Braham and Nefzaouil, 2016) The study on population dynamics of *T. absoluta* in the selected three regions revealed that the southern region i.e. Iringa exhibited a higher population density than the Arusha and Morogoro regions. Such observations were due to not only continuous cultivation of preferred tomato hosts but also presence of favorable climate conditions including an ideal temperature below 25°C and rainfall below 8.0 mm. It has been clearly supported that weather conditions, particularly temperature and rainfall are important factors for development of *T. absoluta* (Karadjova *et al.*, 2013; Megido *et al.*, 2013a). In this study the population of *T. absoluta* was adversely affected by high temperatures and heavy rains above 87mm. Although *T. absoluta* can survive under adverse weather conditions, adults seemed to be less tolerant to temperatures above 27°C and heavy rains (Cuthbertson *et al.*, 2013; Van Damme *et al.*, 2015). This study revealed that, the population of *T. absoluta* was high during the dry seasons from September to December and this was due to two major reasons; the pest was still new and not widely spread in the country that is supported by other studies (Silva *et al.*, 2011). Secondly, low cultivation of tomato

during the rainy and farmers usually plant main staple food crops thus there was scarcity of tomato plants as main host of *T. absoluta* subsequently lowering the population of the pest.

In this study that the population of *T. absoluta* was high during the dry than rainy season (January to April), due to favorable low rains and temperature of below 25°C as well as the abundance of tomato host. However, the first month of each season exhibited higher trap catches than subsequent months, due to reduced pesticide use in newly-planted crops and/or the heightened attractiveness of newly-deployed pheromone lures which can draw moths from great distances (Kılıç, 2010; Balzan and Moonen, 2012; Karadjova *et al.*, 2013). It was noted that during the first three weeks after lure deployment, higher numbers of moths were trapped per week than in the next five weeks as the lure's pheromone emission rate slowly waned and also the pest was in other forms such as pupae, larvae and eggs that are not trapped. Understanding the occurrence, population dynamics of *T. absoluta* and factors influencing its distribution in different agro-ecological locations is of economic importance for tomato growers and policy makers to plan for better management below economic injury level. These findings inform farmers about the seasonal variation in populations of *T. absoluta* in different regions of Tanzania for suggesting proper management strategies based on pest distribution and abundance.

Despite a wide range of hosts of *T. absoluta* identified in this study, tomato plant seemed to be the preferred host possibly due to chemical cues and terpenes secreted by tomato which has been reported to attract *T. absoluta* for oviposition and feeding (Rakha *et al.*, 2017). Other Solanaceae crops especially potato were second leading host of the *T. absoluta* particularly in Arusha region which could be influenced by attractive physiology and cues produced by potato leaves that is closely related to tomato plant (Rakha *et al.*, 2017). Weed (*S. incanum*) was found to harbor *T. absoluta* in all thirteen regions making *T. absoluta* present across seasons and maintain population in crop off seasons. This has a great impact on insect reproduction potential and could influence leaf and fruit damage when crops are planted. However, none of *T. absoluta* larvae or moths was reported in *Capsicum annum* (green sweet pepper) thus there was no damage and loss of pepper due to *T. absoluta* larvae.

In the current study, ethanolic extract of *B. abyssinica* stem bark and leaves had high activity on moths showing that polar compounds can be extracted using water and sprayed in the fields to control *T. absoluta*. Additionally, extracts of *C. africana*, *T. minuta* and *M. oleifera* were

effective against *T. absoluta* larva and inhibited pupation and delayed adult development. This study is in line with other studies which revealed that plant extracts can be effective in controlling insect pest (Moreno *et al.*, 2012; Ghanim and Abdel 2014). Other studies revealed that plant compounds are effective in controlling eggs and larvae of *T. absoluta* (Kona *et al.*, 2014). The botanical extracts from *B. abyssinica* have been revealed to possess high insecticidal activity against insect pests (Zekeya *et al.*, 2014). This study revealed that ethanolic extracts of *B. abyssinica* were effective on *T. absoluta* larvae than petroleum ether showing that plant materials have active polar compounds that can be dissolved in water and filtrate could be used by farmers to control pest locally. However, some petroleum ether extracts were effective on adult *T. absoluta* indicating that non-polar metabolites easily attack insect cuticle and penetrate into insect's tissue and finally cause mortality. Several studies show that extract and essential oils are best biocontrol agents of *T. absoluta* (Moawad *et al.*, 2013). Studies shows that *C. africana* and *M. oleifera* and *T. minuta* possess active metabolites against insect pests (Cestari *et al.*, 2004; De Oliveira *et al.*, 2011). The current study revealed that *B. abyssinica* have high activity against adult *T. absoluta* by reducing survival duration whereas *C. africana*, *M. oleifera* and *T. minuta* have high insecticidal activity against all stages of *T. absoluta* hence could be potential source of organic pesticide for the management of *T. absoluta* in Tanzania.

Landraces have additional physiological, agronomic and nutritional attributes that supersede improved tomato varieties (Louette, 2000). All tomato landraces showed high resistance to *T. absoluta* damage by exhibiting lower number of larval amines and low amount of tomato fruit damage. This was due to the fact that the landraces are capable of producing chemical cues that are unattractive to *T. absoluta* for feeding and oviposition (Rakha *et al.*, 2017). These indigenous tomato materials have unique growth-related attributes including high germination rate, early maturation and long harvest duration with attractive colour. They can thrive under harsh conditions and have short harvest cycles as a result of higher yield than improved tomato varieties (Hamisy *et al.*, 2016). In this study neither pesticide nor fertilizer was applied in plants to improve yield, yet landraces yielded more than variety Tengeru 97 that was highly susceptible to damage by *T. absoluta* and other pests. Although landrace bear small fruits than improve varieties this study showed that they can reside longer in room temperature before deterioration than improved cultivars. In addition, the small size of landraces fruits can facilitate its easy consumption as the whole fruit can be eaten entirely with its skin and seeds which, have been reported to reserve high amount of nutrients

including protein, vitamin C and phosphorus (Adebooye *et al.*, 2006). This study revealed that of all landraces performed better in east-central regions than in southern and northern regions and this might be due to warm temperature from 25°C favouring tomato growth. Accession HSK-38 performed better by yielding more fruits in east-central regions with warm temperature than northern and southern regions that are chiller, and accession HSK-43 and KGM-5 lasted longer in 20°C and below showing that in cool temperature these tomato materials can maintain quality than in warm temperatures.

CHAPTER FIVE

CONCLUSSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study revealed that *T. absoluta* has spread in the entire surveyed regions of the country, with high densities in Iringa, Arusha and Morogoro causing massive damage and loss in more than ten host plants beside tomato plant.

Biological control including application of microbes and parasitoids are effective in parasitizing eggs and larvae only with limited information in Tanzania, thus application of local plants and microbial control will reduce the problem in the country.

Additionally, utilizing locally available tomato landraces identified by this study; HSK-38, BKM-22, KGM-5 and HSK-4 would reduce the problem of pest resistance and accumulation chemicals in environment.

This study also evaluated the efficacy of botanical pesticides that all yielded a considerable output including use of extracts from *B. abyssinica*, *M. oleifera*, *T. minuta* and *C. africana*, that could be potential source of plant-based bio-insecticide.

Bio-pesticide developed from this study (Patent No. TZ/P/2018/000035) was very efficacy against the pest and is the strongest milestone in this study, thus intensely recommended for registration and application by tomato farmers for control of *T. absoluta* in Tanzania.

5.2 Recommendations

Information regarding occurrence, distribution and host range of *T. absoluta* should be disseminated in all regions to create awareness and proper management of the pest.

The developed bio-pesticide should be registered and promoted for application by tomato farmers against *T. absoluta* in Tanzania.

Information regarding efficacy of botanicals against *T. absoluta* developed in this study should be used to lay grounds for further research on developing plant-based bio pesticides.

Tomato landraces identified to be resistant to *T. absoluta* are recommended for breeding programs in searching for resistant tomato plants against *T. absoluta* in Tanzania.

REFERENCE

- Abbes, K., Biondi, A., Zappalà, L. and Chermiti, B. (2014). Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica*. **42**(1): 85-92.
- Abbes, K. and Chermiti, B. (2011). Comparison of two marks of sex pheromone dispensers commercialized in Tunisia for their efficiency to monitor and to control by mass-trapping *Tuta absoluta* under greenhouses. *Tunis. J. Plant Prot.* **6**(2): 133-148.
- Abbes, K. and Chermiti, B. (2014). Propensity of three Tunisian populations of the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) for deuterotokous parthenogenetic reproduction. *African Entomology*. **22**(3): 538-544.
- Abdel-Baky, F. and Al-Soqeer, A. A. (2017). Controlling the 2nd instars larvae of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) by simmondsin extracted from Jojoba seeds in KSA. *J. Entomol.* **14**:73-80.
- Abdel-Raheem, M., Ismail, I., Abdel-Rahman, R., Abdel-Rhman, I. and Naglaa, F. (2015). *Swift Journals* **12**: 2062-2684.
- Acciarri, N., Sabatini, E., Ciriaci, T., Rotino, L., Valentine, D. and Tamietti, G. (2010). The presence of genes for resistance against *Verticillium dahliae* in Italian tomato landraces. *European Journal of Horticultural Science*. **75**(1): 8-14.
- Adebooye, O., Oloyede, F. and Onagoruwa, O. (2006). Fruit characteristics and nutrient composition of landrace morphotypes of snake tomato. *Journal of vegetable science*. **11**(4): 5-16.
- Akello, J., Dubois, T., Coyne, D. and Kyamanywa, S. (2009). The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (*Musa* sp.) plants. *Biological control*. **49**(1): 6-10.
- Alba, J. M., Montserrat, M. and Fernández-Muñoz, R. (2009). Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. *Experimental and Applied Acarology*. **47**(1): 35-47.
- Arnó, J. and Gabarra, R. (2011). Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *Journal of Pest Science*. **84**(4): 513-520.

- Arnó, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodríguez, D., Garreta, A., Gómez, A. and Gabarra, R. (2009). Tuta absoluta, a new pest in IPM tomatoes in the northeast of Spain. *IOBC/wprs Bulletin* 49:203-208.
- Balzan, M. and Moonen, A. C. (2012). Management strategies for the control of Tuta absoluta (Lepidoptera: Gelechiidae) damage in open-field cultivations of processing tomato in Tuscany (Italy). *EPPO bulletin*. **42**(2): 217-225.
- Balzan, M. V. and Wäckers, F. L. (2013). Flowers to selectively enhance the fitness of a host-feeding parasitoid: adult feeding by Tuta absoluta and its parasitoid Necremnus artynes. *Biological Control*. **67**(1): 21-31.
- Batalla-Carrera, L., Morton, A. and García-del-Pino, F. (2010). Efficacy of entomopathogenic nematodes against the tomato leafminer Tuta absoluta in laboratory and greenhouse conditions. *BioControl*. **55**(4): 523-530.
- Bayman, P., Baker, J. L., Doster, M. A., Michailides, T. J. and Mahoney, N. E. (2002). Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. *Applied and Environmental Microbiology*. **68**(5): 2326-2329.
- Beguín, H. and Nolard, N. (1994). Mould biodiversity in homes I. Air and surface analysis of 130 dwellings. *Aerobiologia*. **10**(2-3): 157.
- Bennett, J. W. (2010). An overview of the genus Aspergillus. *Aspergillus: Molecular Biology and Genomics*: 1-17-23.
- Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*. **84**(1): 11-18.
- Biondi, A., Guedes, R. N. C., Wan, F. H. and Desneux, N. (2018). Ecology, worldwide spread, and management of the invasive South American tomato pinworm, Tuta absoluta: past, present, and future. *Annual Review of Entomology*. **63**(239-258).
- Braham, M. (2014). Role of trap colors and exposure time of pheromone on trapping efficacy of males of the tomato leafminer, Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae). *African Journal of Agricultural Research*. **9**(29): 2263-2271.

- Braham, M. and Nefzaouil, F. (2016). Study of the effectiveness of two methods: mass trapping and leaf and fruits removal for the control of the tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) in Tunisia. *Journal of Global Agricultural Ecology* **4**(3): 114-122.
- Brévault, T., Sylla, S., Diatte, M., Bernadas, G. and Diarra, K. (2014). *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): a new threat to tomato production in Sub-Saharan Africa. *African Entomology*. **22**(2): 441-444.
- Brito, E. F. D., Baldin, E. L. L., Silva, R. D. C. M., Ribeiro, L. D. P. and Vendramim, J. D. (2015). Bioactivity of Piper extracts on *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato. *Pesquisa Agropecuária Brasileira*. **50**(3): 196-202.
- Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G. and White, T. C. (2012). Hidden killers: human fungal infections. *Science Translational Medicine*. **4**(165): 165rv13-165rv13.
- Campos, M. R., Biondi, A., Adiga, A., Guedes, R. N. and Desneux, N. (2017). From the Western Palaearctic region to beyond: *Tuta absoluta* 10 years after invading Europe. *Journal of Pest Science*. **90**(3): 787-796.
- Campos, M. R., Rodrigues, A. R. S., Silva, W. M., Silva, T. B. M., Silva, V. R. F., Guedes, R. N. C. and Siqueira, H. A. A. (2014). Spinosad and the tomato borer *Tuta absoluta*: a bioinsecticide, an invasive pest threat, and high insecticide resistance. *PLoS ONE*. **9**(8): e103235.
- Campos, M. R., Silva, T. B., Silva, W. M., Silva, J. E. and Siqueira, H. A. (2015). Spinosyn resistance in the tomato borer *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Journal of Pest Science*. **88**(2): 405-412.
- Cestari, I. M., Sarti, S. J., Waib, C. M. and Branco Jr, A. C. (2004). Evaluation of the potential insecticide activity of *Tagetes minuta* (Asteraceae) essential oil against the head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Neotropical Entomology*. **33**(6): 805-807.

- Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R. and Desneux, N. (2013). Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. *Journal of Pest Science*. **86**(3): 533-541.
- Chailleux, A., Desneux, N., Seguret, J., Khanh, H. D. T., Maignet, P. and Tabone, E. (2012). Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. *PLoS ONE*. **7**(10): e48068.
- Chermiti, B., Abbes, K., Aoun, M., Ben Othmane, S., Ouhibi, M., Gamoon, W. and Kacem, S. (2009). First estimate of the damage of *Tuta absoluta* (Povolny)(Lepidoptera: Gelechiidae) and evaluation of the efficiency of sex pheromone traps in greenhouses of tomato crops in the Bekalta region, Tunisia. *African Journal of Plant Science and Biotechnology*. **3**(49-52).
- Chidege, M., Al-zaidi, S., Hassan, N., Julie, A., Kaaya, E. and Mrogoro, S. (2016). First record of tomato leaf miner *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Tanzania. *Agriculture & Food Security*. **5**(1): 17-21
- Cifuentes, D., Chynoweth, R. and Bielza, P. (2011). Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Management Science*. **67**(9): 1155-1162.
- Cocco, A., Deliperi, S. and Delrio, G. (2013). Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. *Journal of Applied Entomology*. **137**(1-2): 16-28.
- Consoli, F., Parra, J. and Hassan, S. (1998). Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *Journal of Applied Entomology*. **122**(1-5): 43-47.
- Contesini, F. J., Lopes, D. B., Macedo, G. A., da Graça Nascimento, M. and de Oliveira Carvalho, P. (2010). *Aspergillus* sp. lipase: potential biocatalyst for industrial use. *Journal of Molecular Catalysis B: Enzymatic*. **67**(3-4): 163-171.

- Cork, A., Dobson, H., Grzywacz, D., Hodges, R., Orr, A. and Stevenson, P. (2009). Review of pre-and post-harvest pest management for pulses with special reference to East and Southern Africa. Natural Resources Institute, University of Greenwich.
- Cuthbertson, A. G., Mathers, J. J., Blackburn, L. F., Korycinska, A., Luo, W., Jacobson, R.J. and Northing, P. (2013). Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under simulated UK glasshouse conditions. *Insects*. **4**(2): 185-197.
- de Azevedo, S. M., Faria, M. V., Maluf, W. R., De Oliveira, A. C. B. and de Freitas, J. A. (2003). Zingiberene-mediated resistance to the South American tomato pinworm derived from *Lycopersicon hirsutum* var. *hirsutum*. *Euphytica*. **134**(3): 347-351.
- De Oliveira, C. F. R., Luz, L. A., Paiva, P. M. G., Coelho, L. C. B. B., Marangoni, S. and Macedo, M. L. R. (2011). Evaluation of seed coagulant *Moringa oleifera* lectin (cMoL) as a bioinsecticidal tool with potential for the control of insects. *Process Biochemistry*. **46**(2): 498-504.
- De Putter, H., Van Koesveld, M. and De Visser, C. (2007). Overview of the vegetable sector in Tanzania. Wageningen UR. 122-128
- de Vries, R. P. and Visser, J. (2001). Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*. **65**(4): 497-522.
- Desneux, N., Luna, M.G., Guillemaud, T. and Urbaneja, A. (2011). The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *Journal of Pest Science*. **84**(4): 403-408.
- Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Ruescas, D. C., Tabone, E. and Frandon, J. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science*. **83**(3): 197-215.

- Digilio, M. C., Corrado, G., Sasso, R., Coppola, V., Iodice, L., Pasquariello, M., Bossi, S., Maffei, M.E., Coppola, M. and Pennacchio, F. (2010). Molecular and chemical mechanisms involved in aphid resistance in cultivated tomato. *New Phytologist*. **187**(4): 1089-1101.
- Durmusoglu, E., Hatipoglu, A. and Balci, H. (2011). Efficiency of some plant extracts against *Tuta absoluta* (Meyrick, 1917)(Lepidoptera: Gelechiidae) under laboratory conditions. *Turkiye Entomoloj Dergisi-Turkish Journal of Entomology*. **35**(4): 651-663.
- Ekesi, S., Dimbi, S. and Maniania, N. (2007). The role of entomopathogenic fungi in the integrated management of fruit flies (Diptera: Tephritidae) with emphasis on species occurring in Africa. *Research SignPost: Kerala, India*, pp. 239-274.
- Ferracini, C., Ingegno, B.L., Navone, P., Ferrari, E., Mosti, M., Tavella, L. and Alma, A. (2012). Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *Journal of Economic Entomology*. **105**(4): 1311-1319.
- Franceschi, S., Bidoli, E., Vecchia, C. L., Talamini, R., D'Avanzo, B. and Negri, E. (1994). Tomatoes and risk of digestive-tract cancers. *International Journal of Cancer*. **59**(2): 181-184.
- Gabarra, R., Arnó, J., Lara, L., Verdú, M. J., Ribes, A., Beitia, F., Urbaneja, A., del Mar Téllez, M., Mollá, O. and Riudavets, J. (2014). Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish Mediterranean Coast. *BioControl*. **59**(1): 45-54.
- Gacemi, A. and Guenaoui, Y. (2012). Efficacy of emamectin benzoate on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting a protected tomato crop in Algeria. *Academic Journal of Entomology*. **5**(1): 37-40.
- García-del-Pino, F., Alabern, X. and Morton, A. (2013). Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. *BioControl*. **58**(6): 723-731.
- García-Martínez, S., Grau, A., Alonso, A., Rubio, F., Valero, M. and Ruiz, J. J. (2011). UMH 1200, a breeding line within the Muchamiel tomato type resistant to three viruses. *HortScience*. **46**(7): 1054-1055.

- García-Martínez, S., Grau, A., Alonso, A., Rubio, F., Valero, M. and Ruiz, J. J. (2012). UMH 1203, a multiple virus-resistant fresh-market tomato breeding line for open-field conditions. *HortScience*. **47**(1): 124-125.
- Geiser, D., Klich, M., Frisvad, J. C., Peterson, S., Varga, J. and Samson, R. A. (2007). The current status of species recognition and identification in *Aspergillus*. *Studies in Mycology*. **59**: 1-10.
- Ghoneim, K. (2014a). Parasitic insects and mites as potential biocontrol agents for a devastating pest of tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the world: a review. *International Journal*. **2**(8): 81-115.
- Ghoneim, K. (2014b). Parasitic insects and mites as potential biocontrol agents for a devastating pest of tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the World: A review. *International Journal of Research and Reviews in Applied Sciences*. **19**(1): 1.
- Ghoneim, K. (2014c). Predatory insects and arachnids as potential biological control agents against the invasive tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), in perspective and prospective. *Journal of Entomology Zoological Study*. **2**(52-71).
- Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J. and Willett, W. C. (2002). A prospective study of tomato products, lycopene, and prostate cancer risk. *Journal of the National Cancer Institute*. **94**(5): 391-398.
- González-Cabrera, J., Mollá, O., Montón, H. and Urbaneja, A. (2011). Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Bio Control*. **56**(1): 71-80.
- Gourama, H. and Bullerman, L. B. (1995). *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food Protection*. **58**(12): 1395-1404.
- Guedes, R. and Picanço, M. (2012). The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. *EPPO Bulletin*. **42**(2): 211-216.
- Guedes, R. and Siqueira, H. (2013). The tomato borer *Tuta absoluta*: insecticide resistance and control failure. *Plant Sciences Reviews* 2012: 245-251.

- Guimapi, R. Y., Mohamed, S. A., Okeyo, G. O., Ndjomatchoua, F. T., Ekesi, S. and Tonnang, H. E. (2016). Modeling the risk of invasion and spread of *Tuta absoluta* in Africa. *Ecological Complexity*. 28: 77-93.
- Haddi, K. (2012). Studies on insecticide resistance in *tuta absoluta* (Meyrick), with special emphasis on characterisation of two target site mechanisms. *International Journal* 13:24-28
- Hamisy, W. C., Mpina, M.H. and Shilla, O. (2016). Eco-geographic Distribution of Tomato (*Solanum lycopersicon* l.) Landraces in Tanzania: Implications for Conservation and Sustainable use of Plant Genetic Resources. *International Journal*. 14: 1-6.
- Harizanova, V., Stoeva, A. and Mohamedova, M. (2009). Tomato leaf miner, *Tuta absoluta* (Povolny, Lepidoptera: Gelechiidae)–first record in Bulgaria. *Agricultural Science and Technology*. 1(3): 95-98.
- Hoffmann, M. P. and Frodsham, A. (1993). Natural enemies of vegetable insect pests.
- Hubka, V., Kolařík, M., Kubátová, A. and Peterson, S. W. (2013). Taxonomic revision of *Eurotium* and transfer of species to *Aspergillus*. *Mycologia*. 105(4): 912-937.
- Hubka, V., Nováková, A., Kolařík, M., Jurjević, Ž. and Peterson, S. W. (2015). Revision of *Aspergillus* section *Flavipedes*: seven new species and proposal of section *Jani* sect. nov. *Mycologia*. 107(1): 169-208.
- İnanlı, C., Yoldaş, Z. and Birgücü, A. (2012). Effects of entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) on larvae and egg stages of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Ege Üniversitesi Ziraat Fakültesi Dergisi*. 49(3): 239-242.
- Iram, S., Ahmad, I. and Stuben, D. (2009). Analysis of mines and contaminated agricultural soil samples for fungal diversity and tolerance to heavy metals. *Pakistan Journal of Botany* 41(2): 885-895.
- Jacobi, P., Amend, J. and Kiango, S. (2000). Urban agriculture in Dar es Salaam: providing an indispensable part of the diet. *Growing cities, growing food: Urban Agriculture on the Policy Agenda*: 257-283.
- Kaoud, H. (2014). Alternative methods for the control of *Tuta absoluta*. *GJMAS J*. 2(41-46).

- Karadjova, O., Ilieva, Z., Krumov, V., Petrova, E. and Ventsislavov, V. (2013). *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): Potential for entry, establishment and spread in Bulgaria. *Bulgarian Journal of Agricultural Science*. **19**(3): 563-571.
- Karkowska-Kuleta, J., Rapala-Kozik, M. and Kozik, A. (2009). Fungi pathogenic to humans: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochimica Polonica*. **56**(2): 211.
- Khan, A. L., Hamayun, M., Kim, Y. H., Kang, S. M., Lee, J. H. and Lee, I. J. (2011). Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. *Process Biochemistry*. **46**(2): 440-447.
- Khan, R., Shahzad, S., Choudhary, M. I., Khan, S. A. and Ahmad, A. (2010). Communities of endophytic fungi in medicinal plant *Withania somnifera*. *Pakistan Journal of Botany* **42**(2): 1281-1287.
- Kılıç, T. (2010). First record of *Tuta absoluta* in Turkey. *Phytoparasitica*. **38**(3): 243-244.
- Kona, N. E. M., Taha, A. K. and Mahmoud, M. E. (2014). Effects of Botanical Extracts of Neem (*Azadirachta indica*) and *Jatropha* (*Jatropha curcus*) on Eggs and Larvae of Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Persian Gulf Crop Protection*. **3**(3): 41-46.
- Kurosaki, R. (2007). Multiple uses of small-scale valley bottom land: Case study of the Matengo in Southern Tanzania.
- Kutinkova, H., Caicedo, F. and Lingren, B. (2016). The Main Pests on Solanacea Crops in Zona 1 of Ecuador. *New Knowledge Journal of Science*. **5**(1):26-31
- Lakhdari, W., Dehliz, A., Acheuk, F., Mlik, R., Hammi, H., Matallah, S. and Doumandji-Mitiche, B. (2016). Biocontrol test against the leaf miner of tomato *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) by using entomopathogenic fungi in the Algerian Sahara. (*Abstract in Algeria Conference*).
- Leger, R. J. S., Staples, R. C. and Roberts, D. W. (1993). Entomopathogenic isolates of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Aspergillus flavus* produce multiple extracellular chitinase isozymes. *Journal of Invertebrate Pathology*. **61**(1): 81-84.

- Lietti, M. M., Botto, E. and Alzogaray, R. A. (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Neotropical Entomology*. **34**(1): 113-119.
- Lobos, E., Occhionero, M., Werenitzky, D., Fernandez, J., Gonzalez, L., Rodriguez, C., Calvo, C., Lopez, G. and Oehlschlager, A. (2013). Optimization of a trap for *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and trials to determine the effectiveness of mass trapping. *Neotropical Entomology*. **42**(5): 448-457.
- Loni, A., Rossi, E. and van Achterberg, K. (2011). First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta*. *Bulletin of Insectology*. **64**(1): 115-117.
- Louette, D. (2000). Traditional management of seed and genetic diversity: what is a landrace. *Genes in the field: On-Farm Conservation of Crop Diversity*. 9:109-142.
- Luna, M., Sánchez, N. E., Pereyra, P. C., Nieves, E., Savino, V., Luft, E., Virla, E. and Speranza, S. (2012). Biological control of *Tuta absoluta* in Argentina and Italy: evaluation of indigenous insects as natural enemies. *EPPO Bulletin*. **42**(2): 260-267.
- Luna, M. G., Pereyra, P. C., Coviella, C. E., Nieves, E., Savino, V., Gervassio, N. G. S., Luft, E., Virla, E. and Sánchez, N.E. (2015). Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina. *Florida Entomologist*. **98**(2): 489-494.
- Mafra-Neto, A., De Lame, F., Fettig, C. J., Munson, A. S., Perring, T. M., Stelinski, L. L., Stoltman, L. L., Mafra, L. E., Borges, R. and Vargas, R. I. (Ed.) (2013). Manipulation of insect behavior with specialized pheromone and lure application technology (SPLAT®). In: *Pest Management with Natural Products. ACS Symposium Series. ACS Publications*, 2013.
- Maluf, W. R., de Fátima Silva, V., das Graças Cardoso, M., Gomes, L. A. A., Neto, Á. C. G., Maciel, G. M. and Nízio, D. A. C. (2010). Resistance to the South American tomato pinworm *Tuta absoluta* in high acylsugar and/or high zingiberene tomato genotypes. *Euphytica*. **176**(1): 113-123.

- Materu, C. L., Shao, E. A., Losujaki, E., Chidege, M. and Mwambela, N. (2016). Farmer's perception knowledge and practices on management of Tuta Absoluta Meyerick (Lepidoptera Gelechiidae) in tomato growing areas in Tanzania. *International Journal*. **3**(1): 15-20.
- Megido, R. C., Brostaux, Y., Haubruge, E. and Verheggen, F. (2013a). Propensity of the tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae), to develop on four potato plant varieties. *American Journal of Potato Research*. **90**(3): 255-260.
- Megido, R. C., De Backer, L., Ettaïb, R., Brostaux, Y., Fauconnier, M. L., Delaplace, P., Lognay, G., Belkadhi, M., Haubruge, E. and Francis, F. (2014). Role of larval host plant experience and solanaceous plant volatile emissions in Tuta absoluta (Lepidoptera: Gelechiidae) host finding behavior. *Arthropod-Plant Interactions*. **8**(4): 293-304.
- Megido, R. C., Haubruge, E. and Verheggen, F. (2012). First evidence of deuterotokous parthenogenesis in the tomato leafminer, Tuta absoluta (Meyrick)(Lepidoptera: Gelechiidae). *Journal of Pest Science*. **85**(4): 409-412.
- Megido, R. C., Haubruge, E. and Verheggen, F. J. (2013b). Pheromone-based management strategies to control the tomato leafminer, Tuta absoluta (Lepidoptera: Gelechiidae). A review/Synthèse bibliographique: les stratégies de lutte phéromonale utilisées pour contrôler la mineuse de la tomate, Tuta absoluta (Lepidoptera: Gelechiidae). *Biotechnologie, Agronomie, Société et Environnement*. **17**(3): 475-481.
- Meyling, N. V. and Eilenberg, J. (2007). Ecology of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: potential for conservation biological control. *Biological Control*. **43**(2): 145-155.
- Michel-Salzat, A. and Whitfield, J. B. (2004). Preliminary evolutionary relationships within the parasitoid wasp genus Cotesia (Hymenoptera: Braconidae: Microgastrinae): combined analysis of four genes. *Systematic Entomology*. **29**(3): 371-382.
- Michereff Filho, M., Vilela, E. F., Attygalle, A. B., Meinwald, J., Svatoš, A. and Jham, G. N. (2000). Field trapping of tomato moth, Tuta absoluta with pheromone traps. *Journal of Chemical Ecology*. **26**(4): 875-881.

- Mlozi, M. R., Lupanga, I. and Mvena, Z. (1992). Urban agriculture as a survival strategy in Tanzania. *The rural-urban interface in Africa: Expansion and Adaptation*. 12:284-294.
- Moawad, S. S., Ebadah, I. and Mahmoud, Y. (2013). Biological and Histological studies on the efficacy of some Botanical and Commercial Oils on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Egyptian Journal of Biological Pest Control*. **23**(2): 301-309.
- Moles, T. M., Pompeiano, A., Reyes, T. H., Scartazza, A. and Guglielminetti, L. (2016). The efficient physiological strategy of a tomato landrace in response to short-term salinity stress. *Plant Physiology and Biochemistry*. **109**(262-272).
- Mollá, O., González-Cabrera, J. and Urbaneja, A. (2011). The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer *Tuta absoluta*. *BioControl*. **56**(6): 883-891.
- Moreno, S. C., Carvalho, G. A., Picanço, M. C., Morais, E. G. and Pereira, R. M. (2012). Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species. *Pest Management Science*. **68**(3): 386-393.
- Moussa, S., Sharma, A., Baiomy, F. and El-Adl, F. E. (2013). The Status of Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt and potential effective pesticides. *Academic Journal of Entomology*. **6**(3): 110-115.
- Mwangi, F. N. (2015). Evaluation Of Botanical Pesticides and Coloured Sticky Insect Traps For Management Of Insect Pests (Thrips, Whiteflies and Aphids) In French Beans (*Phaseolus Vulgaris* L.), Dissertation for Award of at University of Nairobi.
- Oliveira, F. A., da Silva, D. J. H., Leite, G. L. D., Jham, G. N. and Picanço, M. (2009). Resistance of 57 greenhouse-grown accessions of *Lycopersicon esculentum* and three cultivars to *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Scientia Horticulturae*. **119**(2): 182-187.
- Oloyede, F. and Adebooye, O. (2005). Effect of season on growth, fruit yield and nutrient profile of two landraces of *Trichosanthes cucumerina* L. *African Journal of Biotechnology*. **4**(10):57-62

- Ostrauskas, H. and Ivinskis, P. (2010). Records of the tomato pinworm (*Tuta absoluta* (Meyrick, 1917))–Lepidoptera: Gelechiidae–in Lithuania. *Acta Zoologica Lituanica*. **20**(2): 151-155.
- Parra, J. R. P. (2009). Egg parasitoids commercialization in the new world, Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. *Progress in Biocontrol*: Springer, Netherlands, pp. 373-388.
- Pereyra, P. C. and Sánchez, N. E. (2006). Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology*. **35**(5): 671-676.
- Pires, L. M., Marques, E. J., Wanderley-Teixeira, V., Teixeira, Á. A., Alves, L. C. and Alves, E. S. B. (2009). Ultrastructure of *Tuta absoluta* parasitized eggs and the reproductive potential of females after parasitism by *Metarhizium anisopliae*. *Micron*. **40**(2): 255-261.
- Pitt, J., Basilico, J., Abarca, M. and Lopez, C. (2000). Mycotoxins and toxigenic fungi. *Medical Mycology*. **38**(1): 41-46.
- Polaszek, A., Rugman-Jones, P. F., Stouthamer, R., Hernandez-Suarez, E., Cabello, T. and del Pino Pérez, M. (2012). Molecular and morphological diagnoses of five species of *Trichogramma*: biological control agents of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae) in the Canary Islands. *BioControl*. **57**(1): 21-35.
- Powell, K. A., Renwick, A. and Peberdy, J. F. (2013). The genus *Aspergillus*: from taxonomy and genetics to industrial application. Springer Science & Business Mediapp.
- Price, P. B. (2006). Microbial life in glacial ice and implications for a cold origin of life. *FEMS Microbiology Ecology*. **59**(2): 217-231.
- Proffit, M., Birgersson, G., Bengtsson, M., Reis Jr, R., Witzgall, P. and Lima, E. (2011a). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. *Journal of Chemical Ecology*. **37**(6): 565-574.
- Proffit, M., Birgersson, G., Bengtsson, M., Reis, R., Witzgall, P. and Lima, E. (2011b). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. *Journal of Chemical Ecology*. **37**(6): 565-574.

- Rakha, M., Zekeya, N., Sevgan, S., Musembi, M., Ramasamy, S. and Hanson, P. (2017). Screening recently identified whitefly/spider mite-resistant wild tomato accessions for resistance to *Tuta absoluta*. *Plant Breeding*. **136**(4): 562-568.
- Reddy, G. V. and Guerrero, A. (2010). Chapter Twenty-New Pheromones and Insect Control Strategies. *Vitamins and Hormones*. **83**(493-519).
- Rehman, A., Saeed, A., Khan, A., Rafique, A. and Ashraf, M. (2012). Potato: a new host plant of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) in Turkey. *Pakistan Journal Zoology* **44**(4): 1183-1184.
- Resende, J. T. V. D., Maluf, W. R., Faria, M. V., Pfann, A. Z. and Nascimento, I. R. D. (2006). Acylsugars in tomato leaflets confer resistance to the South American tomato pinworm, *Tuta absoluta* Meyr. *Scientia Agricola*. **63**(1): 20-25.
- Retta, A. N. and Berhe, D. H. (2015). Tomato leaf miner–*Tuta absoluta* (Meyrick), a devastating pest of tomatoes in the highlands of Northern Ethiopia: A call for attention and action. *Research Journal of Agriculture and Environmental Management*. **4**(6): 264-269.
- Robertson, G. P. and Vitousek, P. M. (2009). Nitrogen in agriculture: balancing the cost of an essential resource. *Annual Review of Environment and Resources*. **34**(97-125).
- Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M. and Bassi, A. (2015). First report of *Tuta absoluta* resistance to diamide insecticides. *Journal of Pest Science*. **88**(1): 9-16.
- Rodrigues, G. B., Elsayed, A.Y. and da Silva, D. J. (2011). Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill.). *Revista Brasileira de Ciências Agrárias*. **6**(2): 243-252.
- Sabbour, M. (2014). Biocontrol of the tomato pinworm *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Egypt. *Middle East Journal of Agriculture Research*. **3**(3): 499-503.
- Sabbour, M. and Nayera, Y. S. (2014). Evaluations of three *Bacillus thuringiensis* against *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Egypt. *Egyptian Journal of Biocontrol*. **3**(8): 2319-7064.

- Salem, S. and Abdel-Moniem, A. (2015). Evaluation of non-traditional approaches for controlling tomato moth, *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae), a new invasive pest in Egypt. *Archives of Phytopathology and Plant Protection*. **48**(4): 319-326.
- Salim, A. P., Saminaidu, K., Marimuthu, M., Perumal, Y., Rethinasamy, V., Palanisami, J. R. and Vadivel, K. (2011). Defense responses in tomato landrace and wild genotypes to early blight pathogen *Alternaria solani* infection and accumulation of pathogenesis-related proteins. *Archives of Phytopathology and Plant Protection*. **44**(12): 1147-1164.
- Sánchez, N. E., Pereyra, P. C. and Luna, M. G. (2009). Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environmental Entomology*. **38**(2): 365-374.
- Schuster, E., Dunn-Coleman, N., Frisvad, J. and Van Dijck, P. (2002). On the safety of *Aspergillus niger*—a review. *Applied Microbiology and Biotechnology*. **59**(4-5): 426-435.
- Scintu, A., Rodriguez, M., Rau, D., Giovannoni, J. and Attene, G. (2015). Characterization of a wide collection of tomato (*Solanum lycopersicum* L.) for morpho-phenological, quality and resistance traits. *Journal of Agriculture* 21:38-43
- Shah, P. and Pell, J. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*. **61**(5-6): 413-423.
- Sharma, N. and Bhandari, A. S. (2014). Management of pathogens of stored cereal grains, Sustainable Agriculture Reviews. Springer, Netherlands. pp. 87-107.
- Shashank, P., Chandrashekar, K., Meshram, N. M. and Sreedevi, K. (2015). Occurrence of *Tuta absoluta* (Lepidoptera: Gelechiidae) an invasive pest from India. *Indian Journal of Entomology*. **77**(4): 323-329.
- Shenge, K., Mabagala, R. and Mortensen, C. N. (2010). Current status of bacterial-speck and-spot diseases of tomato in three tomato-growing regions of Tanzania. *Journal of Agricultural Extension and Rural Development*. **2**(5): 84-88.

- Shi, J. and Maguer, M. L. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*. **40**(1): 1-42.
- Shiberu, T. and Getu, E. (2017). Effects of crude extracts of medicinal plants in the management of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory and glasshouse conditions in Ethiopia. *Journal of Entomology and Nematology*. **9**(2): 9-13.
- Silva, G. A., Picanço, M. C., Bacci, L., Crespo, A. L. B., Rosado, J. F. and Guedes, R. N. C. (2011). Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Management Science*. **67**(8): 913-920.
- Simmons, A. T. and Gurr, G. M. (2005). Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. *Agricultural and Forest Entomology*. **7**(4): 265-276.
- Siqueira, H., Guedes, R. and Picanço, M. (2000a). Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae). *Journal of Applied Entomology*. **124**(5-6): 233-238.
- Siqueira, H. Á. A., Guedes, R. N. C. and Picanço, M. C. (2000b). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology*. **2**(2): 147-153.
- Soliman, S., Hegazi, E., Attaia, A. and Imam, A. (2013). Evaluating the Role of Sex Pheromone in Monitoring and Controlling Tomato Leaf Miner, *Tuta absoluta* (Meyrick), Under the Egyptian North Western Coast Conditions. *Egyptian Journal of Biological Pest Control*. **23**(1): 169.
- Speranza, S. and Sannino, L. (2012). The current status of *Tuta absoluta* in Italy. *EPPO Bulletin*. **42**(2): 328-332.
- Steiner, M. and Goodwin, S. (2015). *Tuta absoluta*. *Practical Hydroponics and Greenhouses* 153: 38-42.

- Strasser, H., Vey, A. and Butt, T. M. (2000). Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology*. **10**(6): 717-735.
- Taffouo, V., Nouck, A., Dibong, S. and Amougou, A. (2010). Effects of salinity stress on seedlings growth, mineral nutrients and total chlorophyll of some tomato (*Lycopersicon esculentum* L.) cultivars. *African Journal of Biotechnology*. **9**(33):22-27.
- Taha, A., Homam, B., Afsah, A. and EL-Sharkawy, F. M. (2012). Effect of trap color on captures of *Tuta absoluta* moths (Lepidoptera: Gelechiidae). *International Journal of Environmental Science and Engineering* 3:43-48.
- Terzopoulos, P., Walters, S. and Bebeli, P. (2009). Evaluation of Greek tomato landrace populations for heterogeneity of horticultural traits. *European Journal of Horticultural Science*. **74**(1): 24-24
- Tonnang, H. E., Mohamed, S. F., Khamis, F. and Ekesi, S. (2015). Identification and risk assessment for worldwide invasion and spread of *Tuta absoluta* with a focus on Sub-Saharan Africa: implications for phytosanitary measures and management. *PLoS ONE*. **10**(8): e0135283.
- Tropea Garzia, G., Siscaro, G., Biondi, A. and Zappalà, L. (2012). *Tuta absoluta*, a South American pest of tomato now in the EPPO region: biology, distribution and damage. *EPPO Bulletin*. **42**(2): 205-210.
- Trottin-Caudal, Y., Baffert, V., Leyre, J. M. and Hulas, N. (2012). Experimental studies on *Tuta absoluta* (Meyrick) in protected tomato crops in France: biological control and integrated crop protection. *EPPO Bulletin*. **42**(2): 234-240.
- Tumuhaise, V., Khamis, F. M., Agona, A., Sseruwu, G. and Mohamed, S. A. (2016). First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Uganda. *International Journal of Tropical Insect Science*. **36**(3): 135-139.

- Urbaneja, A., Desneux, N., Gabarra, R., Arnó, J., González-Cabrera, J., Mafra Neto, A., Stoltman, L., Pinto, A. D. S. and Parra, J. R. (2013). Biology, ecology and management of the South American tomato pinworm, *Tuta absoluta*. *Potential Invasive Pests Agricultural Crops*. 3:98-107.
- Urbaneja, A., González-Cabrera, J., Arnó, J. and Gabarra, R. (2012). Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest Management Science*. **68**(9): 1215-1222.
- Vacas, S., Alfaro, C., Primo, J. and Navarro-Llopis, V. (2011). Studies on the development of a mating disruption system to control the tomato leafminer, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae). *Pest Management Science*. **67**(11): 1473-1480.
- Van Damme, V., Berkvens, N., Moerkens, R., Berckmoes, E., Wittemans, L., De Vis, R., Casteels, H., Tirry, L. and De Clercq, P. (2015). Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. *Journal of Pest Science*. **88**(3): 533-541.
- van der Straten, M. J., Potting, R. P. and van der Linden, A. (Ed.) (2011). Introduction of the tomato leafminer *Tuta absoluta* into Europe. *In: Proceedings Netherlands Entomological Society Meetings*, pp. 23-30, 2011.
- van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J. and Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl*. **63**(1): 39-59.
- Van Lenteren, J. C. and Bueno, V. H. (2003). Augmentative biological control of arthropods in Latin America. *BioControl*. **48**(2): 123-139.
- Verma, V. C., Singh, S. K., Solanki, R. and Prakash, S. (2011). Biofabrication of anisotropic gold nanotriangles using extract of endophytic *Aspergillus clavatus* as a dual functional reductant and stabilizer. *Nanoscale Res Letter*. **6**(1): 16.
- Witzgall, P., Kirsch, P. and Cork, A. (2010). Sex pheromones and their impact on pest management. *Journal of Chemical Ecology*. **36**(1): 80-100.

- Yankova, V., Valchev, N. and Markova, D. (2014). Effectiveness of phytopesticide Ne m Azal T/S® against tomato leaf miner *Tuta absoluta* Meyrick in greenhouse tomato. *Bulgarian Journal of Agricultural Science (Bulgaria)*. **20** (5) 1116-1118
- Zappala, L., Biondi, A., Alma, A., Al-Jboory, I. J., Arno, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D. and Guenaoui, Y. (2013). Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *Journal of Pest Science*. **86**(4): 635-647.
- Zekeya, N., Chacha, M., Ndakidemi, P. A., Materu, C., Chidege, M. and Mbega, E. R. (2016). Tomato Leafminer (*Tuta absoluta* Meyrick 1917): A Threat to Tomato Production in Africa. *Journal of Agriculture and Ecology Research International*. **10**(1): 1-10.
- Zekeya, N., Chacha, M., Shahada, F. and Kidukuli, A. (2014). Analysis of phytochemical composition of *Bersama abyssinica* by gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytochemistry*. **3**(4): 246-252.
- Zekeya, N., Ndakidemi, P. A., Chacha, M. and Mbega, E. (2017). Tomato Leafminer, *Tuta absoluta* (Meyrick 1917), an emerging agricultural pest in Sub-Saharan Africa: Current and prospective management strategies. *African Journal of Agricultural Research*. **12**(6): 389-396.
- Zhang, P., You, Y., Song, Y., Wang, Y. and Zhang, L. (2015). First record of *Aspergillus oryzae* (Eurotiales: Trichocomaceae) as an entomopathogenic fungus of the locust, *Locusta migratoria* (Orthoptera: Acrididae). *Biocontrol Science and Technology*. **25**(11): 1285-1298.
- Zouba, A., Chermiti, B., Kadri, K. and Fattouch, S. (2013). Molecular characterization of *Trichogramma bourarachae* strains (Hymenoptera: Trichogrammatidae) from open field tomato crops in the South West of Tunisia. *Biomirror*. **4**(08): 05-11.

APPENDICES

Appendix 1: Evidence of oral presentation and award winner in the 50TH SIP Meeting - USA



Appendix 2: Evidence of a patent

THE UNITED REPUBLIC OF TANZANIA



Form No 12

THE PATENT (REGISTRATIONS) ACT CAP 217 R.E. 2002.

CERTIFICATE OF GRANT OF PATENT

Regulations 29(3)

To: **NEVER DAUDI ZEKEYA**
P.O. BOX 447
ARUSHA

In accordance with Sections 28(1) of the Patents Act, 1987, it is hereby certified that a Patent having number **TZ/P/2018/000035** has been granted to:

Name: **NEVER ZEKEYA, ERNEST MBEGA, PATRICK NDAKIDEMI AND MUSA CHACHA**

Address: **P.O. BOX 447**
ARUSHA

In respect of an invention described in an application for that Patent Certificate Filed on the **22ND** day of **JUNE, 2018**.

Being an invention for: **BIONHANCER AND BIOPESTICIDE METHODS FOR PESTS CONTROL**

A handwritten signature in blue ink, appearing to read 'V. Hando'.

DEPUTY REGISTRAR OF PATENTS



8th February, 2019

RESEARCH OUTPUTS

Journal Papers

Zekeya, N., Ndakidemi, P. A., Chacha, M., & Mbega, E. First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania. *Biocontrol Science and Technology* doi.org/10.1080/09583157.2019.1573972

Zekeya, N., Ndakidemi, P. A., Chacha, M., & Mbega, E. (2017). Tomato Leafminer, *Tuta absoluta* (Meyrick 1917), an emerging agricultural pest in Sub-Saharan Africa: Current and prospective management strategies. *African Journal of Agricultural Research*, 12(6), 389-396.

Zekeya, N., Ndakidemi, P. A., Chacha, M., & Mbega, E (2017). Tomato Leafminer (*Tuta absoluta* Meyrick 1917): A Threat to Tomato Production in Africa. *Journal of Agriculture and Ecology Research International* 10(1).

Patent

Never Zekeya, Ernest Mbega, Patrick Ndakidemi, Musa Chacha (2018). Patent NO. TZ/P/2018/000035: **Bioenhancer and biopesticide methods for controlling pests.** BRELA

Conference Paper

Zekeya, N., Ndakidemi, P. A., Chacha, Mbega, E. R., (2017). Entomopathogenic activity of *Aspergillus flavus* against tomato leaf miner, *Tuta absoluta* (Meyrick; 1917) in Tanzania. 50th Society for Invertebrate Pathology (SIP) meeting in San Diego, California, USA.

Poster Presentations

Zekeya, N., Ndakidemi, P. A., Mbega, E. R., Chacha, M., (2018). Occurrence and Distribution of *Tuta absoluta* (Gelechiidae) in agro ecological zones of Tanzania. In the 6th RUFORUM Biannual Conference, Nairobi, Kenya.

Full Length Research Paper

Tomato Leafminer, *Tuta absoluta* (Meyrick 1917), an emerging agricultural pest in Sub-Saharan Africa: Current and prospective management strategies

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Tomato (*Solanum lycopersicum* L.) is an important vegetable crop for income and nutrition of small-holder farmers in sub-Saharan Africa. However, it is attacked by many insect pests that cause high economic losses. This review focuses on one insect pest, namely *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae). Many studies have shown that chemical pesticides have failed to control tomato leafminer in many parts of the world including America, Europe, Asian and Sub-Saharan Africa, where the pest is impacting significantly the tomato value chains as farmers were unaware of the pest and unprepared to control it. The review has also evaluated current approaches used to manage *T. absoluta* in different countries and proposes areas for future investment in research for effective and affordable management to prevent further losses caused by *T. absoluta* in tomato production in Sub-Saharan Africa.

Key words: *Tuta absoluta*, agricultural pest, pesticides resistance, pheromone trap, biocontrols

INTRODUCTION

Tomato leaf miner, *Tuta absoluta* (Meyrick, 1917; Lepidoptera: Gelechiidae) is a devastating pest of tomato and other Solanaceous crops in many areas of the world causing severe damage and yield loss (Cifuentes et al., 2011; Zappala et al., 2012, 2013; El-Arnaouty et al., 2014; Tonnang et al., 2015; Bawin et al., 2015). It is a native of South America and known to cause substantial losses (Urbaneja et al., 2013; Zucchi et al., 2009). *T. absoluta* has been reported to be common in tomatoes growing in altitudes not exceeding 1000 m above sea level (Tonnang et al., 2015). It spreads mainly by natural

dispersal means, such as through winds (Gontijo et al., 2013; Sridhar et al., 2014). Tomato leafminer was recorded first in 1917 and as tomato pest in 1960s in Peru (Seplyarsky et al., 2010; Guedes and Picanço, 2012). Then, it crossed borders to Europe, where for the first time it was reported in 2006 in Spain (Desneux et al., 2011). Later on, the pest extended its invasion to France, Italy, Greece, Malta and Bulgaria (Harizanova et al., 2009; Roditakis et al., 2010; Braham et al., 2012). In Africa, *T. absoluta* was reported first in Algeria and Morocco in 2008 and in 2009, it was reported in Libya

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(Harbi et al., 2012). *T. absoluta* continued to spread in Africa and invaded Egypt in 2010 (Moussa et al., 2013) then reached Sudan and South Sudan in 2011 (Pfeiffer et al., 2013; Brevault et al., 2014), then Ethiopia in 2012 (Goftishu et al., 2014). Other countries in Africa reported to be invaded by tomato leafminer are Kenya (2013) (Mohamed et al., 2015), Tanzania (2014) (Biondi et al., 2015) and Senegal (2014) (Tonnang et al., 2015). *T. absoluta* cannot easily be controlled by chemical sprays due to the fact that contact toxicity cannot reach larvae inside the leaves (Ayalew, 2015). The pest has a physiological ability to adapt and survive in harsh environments such as cold temperate and hot tropical regions (Cuthbertson et al., 2013; Ponti et al., 2015). Tonnang et al. (2015) reported that the pest can survive temperature as high as 49°C in summer in Sudan. In another report by Van Damme et al. (2015), *T. absoluta* adults have been reported to survive at temperatures below 5°C. The pest can also tolerate dryness, making it flourish well in hot and dry areas (Miranda et al., 1998). *T. absoluta* larvae feed by mining leaves, stems and fruits. It can attack tomato plants at all developmental stages causing up to 100% loss in tomato fruits, if not controlled (Desneux et al., 2010). It has high reproductive rate within a short period of time and capable of producing up to 12 generations per year at favorable temperature (Mollá et al., 2011).

Several chemical pesticides are used to control the pest, but none is suitably adapted for control of the tomato borer due to larvae feeding strategy inside plant tissues and foliar spray easily washed out by wind and rain (Abbes and Chermiti, 2011; Guedes and Picanço, 2012; Guedes and Siqueira, 2013). Additionally, most chemical pesticides have adverse impacts to both humans, non-targeted organisms and environment as well (Abdel-Raheem et al., 2015). It is always agreed that pest control using resistant tomato varieties is the best and sustainable option (Oliveira et al., 2012). Many accessions have been reported to be good source of germplasm resistant to pests including *T. absoluta* (Rodrigues et al., 2011). The resistance by host tomato plant can be attributed to acylsugar, a substance that was reported to provide resistance against *T. absoluta* (Resende et al., 2006). However, use of resistant variety is a long term approach for that would take time to attain a suitable one for Sub-Saharan Africa. Hence there is a need to develop a short and long term pest control strategies that will avert losses caused by *T. absoluta* in Sub-Saharan Africa.

There are efforts to reduce use of insecticides in tomato fields, including cultural control methods such as controlled irrigation, crop rotation argumentative biological control (Van Lenteren and Bueno, 2003) and destruction of infested plant material the environment (Abbes et al., 2012). Cultivation of resistance tomato varieties has been reported in other countries (Guedes and Siqueira, 2013).

but not common to Sub-Saharan Africa. Other methods include biological control, such as use of natural enemies including parasitoids predators and entomopathogenic microbes (Guedes and Picanço, 2012). Abbes and Chermiti (2011) reported that use of insect's sex pheromones to control *T. absoluta* in open fields. Similarly, the pheromones are reported to perform well in greenhouses (Cocco et al., 2013). Although these methods are applied, they are not guaranteed to reduce this pest and may be costly and not readily available, especially for small holder tomato farmers in sub-Saharan Africa. There is need to propose a systematic, sustainable and integrated pest management strategy to control this invasive pest in Sub-Saharan Africa. Therefore, this review comprehensively describes the economic importance of *T. absoluta*, control methods that exist and finally proposes an IPM strategy that can be applied to manage the pest in Sub-Saharan Africa.

Economic importance of *Tuta absoluta*

Tomato is the major horticultural crop in Tanzania where it is estimated that 17.5 Mt/ha is produced per year (Materu et al., 2016). However, invasion of tomato borer has declined production by 50% (Materu et al., 2016). *T. absoluta* is a new pest in sub-Saharan Africa. It is greatly damaging tomato crop to levels that farmers are giving up production due to costs and losses it causes in tomato production (Muniappan and Heinrichs, 2015). It is well known that small-holder farmers rely on tomato for income in many parts of sub-Saharan Africa (Oerke et al., 2012), due to its high nutritive value and role in small-scale trade (Cetin and Vardar, 2008). Tomato is cultivated throughout the year in varied range of environments from valley, mountains, in arid and semi-arid areas as long as environmental conditions favors (Calatrava et al., 2011; Laube and Awo, 2012). During rainy season, farmers work out to control diseases whereas dry season is highly susceptible to pest including tomato borer. The two varying seasons increases infestation pressure to the pest causing economic losses of up to 100% in some countries in sub-Saharan Africa (Ayalew, 2015). It has been reported that, farmers in Tanzania increase pesticides use by misusing, doubling doses and wrong application for the purpose of protecting their crops from damage (Materu et al., 2016). However, little success has been achieved, but would increase the problem such as development resistance among pest populations (Muniappan and Heinrichs, 2015). Moreover, in Tanzania where the pest was noticed in farmers' fields recently, it seemed doubtlessly that tomato growers were not prepared for the pest thus they never had any appropriate control against it (Materu et al., 2016). Considering time of entry to Africa of *T. absoluta* being less than ten years, it is again probably that tomato growers in the sub-Saharan Africa are unable to grow the

crop due to fear of loses. There is need therefore to impose immediate attention and practical solutions in favor of tomato production within its value chain in sub-Saharan Africa.

Use of chemical pesticide

Chemical pesticides are common in pest control. Common chemicals are pyrethroids (Guedes and Picanço, 2012), organophosphates, spinosad, emamectin benzoate and abamectin (Campos et al., 2014), chloride channel activators, benzoylureas (Haddi 2012) and diamide (Roditakis et al., 2015). Application of these chemicals against *T. absoluta* has been reported with little success, mainly because of pest resistance and to some point could be utilized by plant as well (Siqueira et al., 2000). Tomato borer resistance has been reported widely used chemicals such as spinosad Cartap and Abamectin, creating further threat to farmers (Siqueira et al., 2000; Reyes et al., 2011; Haddi et al., 2012; Campos et al., 2015; Guedes and Siqueira, 2013).

Pest resistance has been reported to cause increased use of chemical pesticides applications against *T. absoluta* in many parts of the world (Consoli et al., 1998; Siqueira et al., 2000; Lietti et al., 2005). In Spain, about 15 applications and in Brazil up to 30 applications have been reported (Guedes and Picanço, 2012; Silva et al., 2011). The pest resistance against spinosad chemical reached up to 180,000 resistances within seven further generations in Brazil (Campos et al., 2014). In countries such as Tunisia, more than 18 chemicals were introduced during 2009-2011 for the control of tomato borer but none of them seemed efficient in solving the pest problem (Abbes et al., 2012). Failure of these chemicals in controlling *T. absoluta* opened a new window for development of other methods including biopesticides, pheromone traps, and parasitoids (Regnault-Roger, 2012; Cherifet et al., 2013; Zappala et al., 2013). Though chemical pesticides are economically and environmentally unaffordable, farmers still seek them for their agricultural uses because is the only easily accessible option. Thus introduction of IPM strategies in Sub-Saharan Africa will promote sustainable horticultural farming.

Bioactive compounds from plant against *T. absoluta*

Botanicals have been reported to play a great role in controlling pests (Isman, 2006; Sharma and Bhandari, 2014; Zekeya et al., 2014). Many laboratory studies revealed the efficacy of plant compounds against insect pests including *T. absoluta* (Castillo et al., 2010; Senthil-Nathan, 2013). For instance, extracts from neem plants were reported to be efficient against *T. absoluta* under laboratory condition (Durmusoglu et al., 2011). Valchev

and Markova (2014) reported that Neem plant contains a number of active metabolites such as alkaloids which can control insect pests. These compounds have been reported to have control efficacy against tomato borer (Yankova et al., 2014). Other plants which are promising in management of *T. absoluta* include Piper (Brito et al., 2015) whereas compounds from *Acmella oleracea* were revealed to be active against *Tuta absoluta* (Moreno et al., 2012). Though biochemical pesticides have been cited as promising for pest control, their application in the sub-Saharan Africa is limited and none of the compounds have been registered commercially to help farmers. Hence more researches and validation of these natural resources is highly demanded to protect crop damage and loss including those by *T. absoluta* (Cork et al., 2009). Plant based pesticides have been documented to be better than synthetic chemical pesticides as they are biodegradable, naturally available and environmentally friend to none targeted organisms.

Management *T. absoluta* by entomopathogenic microbes

Use of microorganism as biopesticides for management of pests has increasingly gained popularity in recent years (Mollá et al., 2011). Bacteria and fungi have been used for a long time in management of tomato borer in America and Europe (Trottin-Caudal et al., 2012; Parra, 2009). The microbes have been reported to attack pests by their pathogenic effects (Inanli et al., 2012; Pires et al., 2009). Currently there are many commercially available bacterial and fungal formulations for controlling pests including *T. absoluta* in America and Europe (Sabbour, 2014). The formulations are either by foliar spray or by drenching the roots (Amizadeh et al., 2015). One of the best and successful formulations was that of *Metarhizium anisopliae* (fungus) and *Bacillus subtilis* (bacteria) which have been reported to reduce the population of *T. absoluta* on tomato at all developmental stages in America and Europe (Inanli et al., 2012). Other formulations reported to be tested against include that of *Metarhizium anisopliae* and *Beauveria bassiana* (Inanli et al., 2012; Kaoud, 2014). Most of these reports however were all based on screen house studies (González-Cabrera et al., 2011; Sabbour and Nayera, 2014) and only a few have been tested on field conditions and thus they may not be readily available for small-holder farmers. Nematodes have been reported as biocontrols of *T. absoluta* in some countries and depicted high insect mortality (Batalla-Carrera et al., 2010). The nematodes were reported to be effective against larvae, pupae and adult *T. absoluta* (Garcia-del-Pino et al., 2013). Unfortunately, none of these strategies have been reported to be effective in Sub-Saharan Africa, thus this review highlight the potential of native entomopathogens and endophytes in management of *T. absoluta* with

respect to environmental conservation in Sub-Saharan Africa.

Pheromone traps for scheming *T. absoluta*

Sex pheromone traps have been cited as among environmentally accepted pest management strategy (Kılıç, 2010; Witzgall et al., 2010; Gacemi and Guenaoui, 2012). They have been reported to play a significant role in monitoring *T. absoluta* abundance (Harizanova et al., 2009; Reddy and Guerrero, 2010; Van der Straten et al., 2011). The traps are used prior to other control strategies so as to determine the presence and abundance of insects so as to decide on appropriate control measure to apply (Cocco et al., 2013; Witzgall and Cork, 2010). Although these traps are designed to control only adult male moth, they have been reported to be effective in managing tomato borer (Cocco et al., 2013; Reddy and Guerrero, 2010; Braham, 2014b; Cocco et al., 2012; Vacas et al., 2011). For effective application in the field, the sex pheromone traps are to be properly hanged at right positions depending on the height of tomato varieties and wind direction (Kılıç, 2010; Soliman et al., 2013). Another factor reported to be important is the color of the trap which, affects and influences the movement of the pest towards it, thus enhancing trapping efficiency (Braham, 2014a; Megido et al., 2013; Mwangi, 2015). Shining colors especially red has been reported to be the most attractive to *T. absoluta* (Taha et al., 2012). Combination of these factors have been reported to improve traps efficiency in the field (Kılıç, 2010; Lobos et al., 2013; Speranza and Sannino, 2012), especially when they are combined with insect killing ingredients. Use of killing agent in combination with sex pheromones is has minor effect however when the pheromone traps used are only for disrupting mating system (Mafra-Neto et al., 2013; Gacemi and Guenaoui, 2012).

Although pheromone traps in combination with active insect killing agent is reported to be used against *T. absoluta*, no study has reported the efficacy of pheromone traps when synergized by active plant compounds. Due to the current *T. absoluta* situation in sub-Saharan Africa, in particular Tanzania, it is evident that a pheromone trap baited with active compound could be developed and deployed in fields to improve monitoring and control of *T. absoluta*.

Parasitoids for management of *T. absoluta*

Natural enemies play a great ecological role in controlling pests in natural settings (Chailleux et al., 2013; Ferracini et al., 2012; Sánchez et al., 2009; Ghoneim, 2014). A study by Megido et al. (2014) showed that the larvae of *T. absoluta* search for and acquires some biological characteristics and thermal requirements from host plants that can attract a parasitoid as well. *T. absoluta* host plants have ability to emit volatile compounds that

attract either pest predator or parasitoid toward it that also favor the parasitoid indirectly (Proffit et al., 2011; De Backer et al., 2015). One of successful used parasitoids include *Trichogramma pretiosum* which, can parasitise a number of species including *T. absoluta* (Abbes et al., 2014; Zappala, 2012).

Other reports showing application of parasitoid principles in controlling *T. absoluta* are for instance from order Hymenoptera (Balzan and Wäckers, 2013; Ferracini et al., 2012). The most important *T. absoluta* egg parasitoids are originated in the family Trichogrammatidae, Encyrtidae and Eupelmidae. Several Trichogrammatid species parasitizes eggs of different insects orders, especially Hymenoptera, Neuroptera, Diptera and *Trichogramma* (Ghoneim2014a). There are about 210 species of *Trichogramma* that have been signaled as natural enemies of a variety of agricultural and forest pests in many regions of the world and some species are used commercially in biological control programs (Ghoneim, 2014a; Zouba et al., 2013). This is due to their good records in controlling pests and ability to be produced quickly and affordably relative to other parasitoids (Zouba et al., 2013). Of practical use, *Trichogramma achaeae* Nagaraja and Nagarkatti, has a worldwide distribution and was reported to parasitize on *T. absoluta* eggs (Chailleux et al., 2012; Steiner and Goodwin, 2015). This parasitoid especially female has high ability in parasitising both eggs and larvae of the pests (Jervis et al., 2008). *T. achaeae* is known to be genetically compatible with many pest insects as successful parasitoids (Michel and Whitfield, 2004). Further investigation on use of *Trichogramma* parasitoids in insect pest management is drawing an attention of some authors for other insect pests to date (Cabello et al., 2015; Luna et al., 2015). One of other successful parasitoid in the literature include; *Pseudapanteles dignus* (Hymenoptera) which, has been reported have ability to parasitize *T. absoluta* larvae (Sánchez et al., 2009). Larvae of other species reported to parasitize *T. absoluta* include *Neochrysocharis Formosa*, *Pnigalio* (Ratzeburgiela) *cristatus* (Ratzeburg), and *Braconosculator* (Ferracini et al., 2012). However little or none of native microbes have been used for management of the pest in the region. Hence screening and application natural enemies such as microbial parasitoids could be a potential management strategy in Sub-Saharan Africa.

Although parasitoids are very promising in management of pest, there are no any parasitoids of *T. absoluta* that have been reported from Sub-Saharan African countries including Tanzania. Hence, there is an urgent need to identify and assess parasitism rate in sub-Saharan Africa and include in IPM programs.

Cultivation of resistant tomato varieties

Plant and herbivore pests have coexisted since ancient

time and each part played a role in development of resistance in order to survive (VanDoorn and de Vos, 2013). Plant resistance to pests is influenced by genetic and phenotypic traits such as morphological and chemical released (Antonio et al., 2011). Tomato is known to have narrow genetic base due severe breeding and domestication of wild varieties (Do et al., 2009; Melo et al., 2008; Gharekhani and Salek-Ebrahimi, 2014). However, its variation has a role to play in resistance against pests (Hartman and St Clair, 1998). The source of resistance against *T. absoluta* has been reported (Resende et al., 2006; da Silva et al., 2008; Rodrigues et al., 2011). The role of tomato chemical including acyl sugars in resistance against *T. absoluta* have been documented (Oliveira et al., 2012). Maluf et al. (2010) revealed the importance of tomato resistance against *T. absoluta*. However, these sources have not been exploited yet in sub-saharan Africa, also calling for immediate managerial strategy *T. absoluta*.

Several studies recommend use of Integrated Pest Management (IPM) strategy for effective management of *T. absoluta* (Miranda et al., 2005; Arnó et al., 2009). A combination of physical and biological agents such as parasitoids, predators (Chailleux et al., 2013; Mollá et al., 2014) and traps (Michereff et al., 2000) can create an effective IPM (Mollá et al., 2011). The use of pheromone traps together with entomopathogenic fungi and bacteria is common in IPM programs (Abbes et al., 2012). Parasitoids and predators have been used and are commercially available as part of IPM programs in America and Europe (Abes et al., 2014; Al-Jboory et al., 2012; Cely et al., 2010; Zappala et al., 2013). On other hand, chemical pesticides are common in management of pest, but are not suitable when integrated with other management strategies especially biological ones (Arnó et al., 2009). Chemical pesticides have been reported to cause severe side effects to natural enemies (Zappalá et al., 2012), thus ad. Arnó and Gabarra (2011) reported that conventional pesticides have great effects on natural enemies of parasitoids whereas the use of organic pesticides is also uncertainty (Biondi, 2012). Thus coming up with an effective IPM strategy is challenging but not impossible. Hence development of sustainable and affordable IPM is crucial to rescue tomato production as well as raising income of farmers in Sub-Saharan Africa.

Potential management strategy in Sub Saharan Africa

Tomato is one of the major horticultural crops for income of small holder farmers in Africa. Invasion of tomato borer has led to decline of tomato market. Hence this paper reviews current managerial options against tomato leafminer in tomato growing in sub-Saharan Africa so as to highlight the current situation and future prospects. Pheromone traps are important and best for detection and monitoring of insect population but could not be used as management option. Use of chemical pesticides is

common in Sub-Saharan Africa; however, alternation of classes of compound would reduce the problem of insect resistance to pesticides. On the other hand, entomopathogenic fungi and bacteria such as *Metarhizium anisopliae* and *Bacillus subtilis* are commercially available in the region, validated to be used at early stage of plant development for control of the pest. Conversely, none of parasitoid and resistant tomato variety has been identified for management of *T. absoluta* in Sub-Saharan Africa. This calls for researches to search for best option suitable for the region. However, no single control method or approach has been reported for sub-Saharan Africa, thus this review highlighted potentials strategies that would be adopted immediately to sustainably control *T. absoluta* in sub-Saharan Africa, where cultivation is solely rely on open fields making it more susceptible to pest. The prospective IPM strategy based on native microbial biocontrols, pheromone trap, compounds from plants and some moderate synthetic pesticides would be relevant and environmentally affordable solution for small holder farmers in Sub-Saharan Africa.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

- Abbes K, Biondi A, Zappalá L, Chermi B (2014). Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica* 42:85-92.
- Abbes K, Chermi B (2011). Comparison of two marks of sex pheromone dispensers commercialized in Tunisia for their efficiency to monitor and to control by mass-trapping *Tuta absoluta* under greenhouses. *Plant Prot.* 6:133-146.
- Abbes K, Harbi A, Chermi B (2012). The tomato leafminer *Tuta absoluta* (Meyrick) in Tunisia: current status and management strategies. *EPPO Bull.* 42:226-233.
- Abdel-Raheem A, Ismail A, Abdel-Rahman S, Abdel-Rhman E, Naglaa F (2015). Efficacy of Three Entomopathogenic Fungi on Tomato leaf miner, *Tuta absoluta* in Tomato crop in Egypt. *Swift J. Agric. Res.* 1(2):015-021.
- Al-Jboory J, Katbeh-Bader A, Al-Zaidi S (2012). First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta*(Meyrick)(Lepidoptera: Gelechiidae) in Jordan. *Med. E. J. Sci. Res.* 11:435-438.
- Amizadeh M, Hejazi MJ, Niknam N, Arzanlou M (2015). Compatibility and interaction between *Bacillus thuringiensis* and certain insecticides: perspective in management of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biocon. Sci. Technol.* 25:671-684.
- Arnó J, Gabarra R (2011). Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesitlocoris tenuis* (Hemiptera: Miridae). *J. Pest. Sci.* 84(4):513-520.
- Arnó J, Sorribas R, Prat M, Matas M, Pozo C, Rodríguez, D Perdakis D (2009). *Tuta absoluta*, a new pest in IPM tomatoes in the northeast of Spain. *IOBC/WPRS. Bull.* 49:203-208.
- Ayalew G (2015). Efficacy of selected insecticides against the South American tomato moth, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on tomato in the Central Rift Valley of Ethiopia. *Afr. Entomol.* 23(2):410-417.
- Balzan V, Wäckers L (2013). Flowers to selectively enhance the fitness of a host-feeding parasitoid: adult feeding by *Tuta absoluta* and its parasitoid *Necremnus arynes*. *Biol. Cont.* 67(1):21-31.

- Batalla-Carrera L, Morton A, Garcia-del-Pino F (2010). Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *Biol. Cont.* 55:523-530.
- Bawin T, Dujeu D, De Backer L, Francis F Verheggen J (2015). Ability of *Tuta absoluta* (Lepidoptera: Gelechiidae) to develop on alternative host plant species. *Can. Entomol.* pp. 1-9.
- Biondi A (2012). Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* 87(7):803-812.
- Biondi A, Zappala L, Desneux N, Aparo A, Siscaro G, Rapisarda C, Garzia T (2015). Potential toxicity of α -cypermethrin-treated nets on *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* 100:1191-1197.
- Braham M (2014a). Role of trap colors and exposure time of pheromone on trapping efficacy of males of the tomato leafminer, *Tuta absoluta* (Meyrick, 1917)(Lepidoptera: Gelechiidae). *Afr. J. Agric. Res.* 9:2263-2271.
- Braham M (2014b). Sex Pheromone Traps for Monitoring the Tomato Leafminer, *Tuta absoluta*: Effect of Colored Traps and Field Weathering of Lure on Male Captures. *Res. J. Agric. Environ. Manage.* 3(6):290-296.
- Braham M, Gilda-Gnidez H, Hajji L (2012). Management of the tomato borer, *Tuta absoluta* in Tunisia with novel insecticides and plant extracts. *Bull. OEPP/EPPO Bull.* 42(2):291-296.
- Brevault T, Sylva S, Dialle M, Bernadas G, Diarra K (2014). *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): a new threat to tomato production in sub-Saharan Africa: Short communications. *Afr. Entomol.* 22:441-444.
- Brito F, Baldin L, Silva M, Ribeiro P, Vendramin D (2015). Bioactivity of piper extracts on *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato. *Pesqui. Agropecu. Bras.* 50:196-202.
- Cabello T, Francisco G, Juan F, Francisco G, Garay J (2015). Can interactions between an Omnivorous Hemipteran and an egg Parasitoid limit the level of biological control for the tomato pinworm? *Environ. Entomol.* 44:12-26.
- Calatrava J, Barberá G, Castillo M (2011). Farming practices and policy measures for agricultural soil conservation in semi-arid Mediterranean areas: The case of the Guadalentín basin in southeast Spain. *Land Degr. dev.* 22:55-69.
- Campos R, Rodrigues S, Silva M, Silva M, Silva F, Guedes C, Siqueira A (2014). Spinosad and the tomato borer *Tuta absoluta*: a bioinsecticide, an invasive pest threat, and high insecticide resistance. *PLoS one* 9:e103235.
- Campos MR, Silva TB, Silva WM, Silva JE, Siqueira HA (2015). Spinosyn resistance in the tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *J. Pest Sci.* 86(2):405-412.
- Castillo E, Jiménez J, Delgado A (2010). Secondary metabolites of the Annonaceae, Solanaceae and Meliaceae families used as biological control of insects. *Trop. Subtrop. Agroecosys.* 12:445-462.
- Cely P, Cantor F, Rodríguez D (2010). Determination of levels of damage caused by different densities of *Tuta absoluta* populations (Lepidoptera: Gelechiidae) under greenhouse conditions. *Agron. Colomb.* 26:392-402.
- Cetin B, Vardar A (2005). An economic analysis of energy requirements and input costs for tomato production in Turkey. *Rene En.* 33:426-433.
- Chailleux A, Bearz P, Pizzol J, Amiens-Desneux E, Ramirez-Romero R, Desneux N (2013). Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*. *J. Pest. Sci.* 86:533-541.
- Chailleux A, Desneux N, Seguret J, Khanh T, Malignet P, Tabone E (2012). Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. *PLoS One* 7: 48068.
- Cherif A, Mansour R, Grissa-Lebdi K (2013). Biological aspects of tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) in conditions of Northeastern Tunisia: possible implications for pest management. *Environ. Exp. Biol.* 11:179-184.
- Cifuentes D, Chynoweth R, Bietza P (2011). Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994)(Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest. Manage. Sci.* 67:1155-1162.
- Cocco A, Delliperi S, Delrio G (2012). Potential of mass trapping for *Tuta absoluta* management in greenhouse tomato crops using light and pheromone traps. *IOBC-WPRS Bull.* 50:319-324.
- Cocco A, Delliperi S, Delrio G (2013). Control of *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. *J. Appl. Entomol.* 137:16-25.
- Conzoli L, Farra P, Hassan A (1998). Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *J. Appl. Entomol.* 122:43-47.
- Cork A, Dobson H, Grzywacz D, Hodges R, Orr A, Stevenson P (2009). Review of pre-and post-harvest pest management for pulses with special reference to East and Southern Africa: Natural Resources Institute, University of Greenwich.
- Cuthbertson G, Malher J, Blackburn F, Korycinska A, Luo W, Jacobson J, Northing P (2013). Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under simulated UK glasshouse conditions. *Insects* 4:185-197.
- da Silva H, Abreu B, Callman B, Antonio C, Patel B (2008). Tomatoes: Origin, Cultivation Techniques and Germplasm Resources. In: Tomatoes and tomato products - Nutritional, medicinal and therapeutic properties. Eds: Freedy, V.R. and Watson, R.R., Science Publishers, Enfield, NH, USA, pp. 3-23.
- De Backer L, Megido C, Fauconnier M, Brostaux Y, Francis F, Verheggen F (2015). *Tuta absoluta*-induced plant volatiles: attractiveness towards the generalist predator *Macrolophus pygmaeus*. *Arthr-Plant Int.* 9:465-476.
- Desneux N, Luna G, Guillemaud T, Urbaneja A (2011). The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *J. Pest Sci.* 84:403-406.
- Desneux N, Wajnberg E, Wyckhuys KA, Burgio G, Arpala S, Narvaez-Vasquez CA, González JC, Ruescas DC, Tabone E, Frandon J, Pizzol J (2010). Biological Invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J. Pest Sci.* 83:197-215.
- Do T, Cereja A, Tomateiro T (2009). Sources of resistance to tomato leafminer in cherry tomato. *Sci. Agrar.* 10:327-330.
- Durmusoglu E, Halilpoglu A, Balci H (2011). Efficiency of some plant extracts against *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) under laboratory conditions. *Turk. Entomol. Dergisi-Turkish J. Entomol.* 35:651-663.
- El-Arnaouty A, Pizzol J, Galal H, Kortam N, Afifi I, Beyssat V, Heikal H (2014). Assessment of two *Trichogramma* species for the control of *Tuta absoluta* in North African tomato greenhouses. *Afr. Entomol.* 22:801-809.
- Ferracini C, Ingegno B, Letizia N, Paolo F, Ester M, Marco L, Alma A (2012). Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *J. Econ. Entomol.* 105:1311-1319.
- Gacemi A, Guenaoui Y (2012). Efficacy of emamectin benzoate on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting a protected tomato crop in Algeria. *Acad. J. Entomol.* 5:37-40.
- García-del-Pino F, Alabern X, Morton A (2013). Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. *BioCon* 36:723-731.
- Ghoneim K (2014). Predatory insects and arachnids as potential biological control agents against the invasive tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), in perspective and prospective. *J. Entomol. Zool. Stud.* 2(2):52-71.
- Ghoneim K (2014a). Parasitic insects and mites as potential biocontrol agents for a devastating pest of tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the world: a review. *Int. J.* 2:81-115.
- Goffishu M, Seld A, Dechassa N (2014). Occurrence and population dynamics of tomato leaf miner *Tuta absoluta* (Meyrick), Lepidoptera: Gelechiidae] in Eastern Ethiopia. *East Afr. J. Sci.* 8:59-64.
- Gontijo C, Picanço C, Pereira G, Martins J, Chediak M, Guedes C (2013). Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. *Ann. Appl. Biol.* 162:50-59.

- González-Cabrera J, Mollá O, Montón H, Urbaneja A (2011). Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioCont* 56:71-80.
- Guedes C, Picanço C (2012). The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. *EPPPO. Bull.* 42:211-216.
- Guedes C, Siqueira A (2013). The tomato borer *Tuta absoluta*: Insecticide resistance and control failure. *Plant Sci. Rev.* 7(055):1-7
- Haddi K (2012). Studies on insecticide resistance in *Tuta absoluta* (Meyrick), with special emphasis on characterisation of two target site mechanisms. <http://archiv.unicl.it/handle/10761/1226>
- Harbi A, Abbes K, Chemill B (2012). Evaluation of two methods for the protection of tomato crops against the tomato leafminer *Tuta absoluta* (Meyrick) under greenhouses in Tunisia. *EPPPO. Bull.* 42:317-321.
- Harizanova V, Stoeva A, Mohamedova M (2009). Tomato leaf miner, *Tuta absoluta* (Povolny)(Lepidoptera: Gelechiidae)—first record in Bulgaria. *Agric. Sci. Technol.* 1:95-96.
- Hartman B, St Clair A (1995). Variation for insect resistance and horticultural traits in tomato inbred backcross populations derived from *Lycopersicon pennellii*. *Crop Sci.* 35:1501-1506.
- Inanli C, Yoldas Z, Birgücü K (2012). Effects of entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) on larvae and egg stages of *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Ege Üni Zir Fak Der* 49:239-242.
- Isman MB (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Ann. Rev. Entomol.* 51:45-66.
- Jervis MA, Eilers J, Harvey JA (2005). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Ann. Rev. Entomol.* 53:361-385.
- Kaoud A (2014). Alternative methods for the control of *Tuta absoluta*. *GJMAS. J.* 2:41-46.
- Kiliç T (2010). First record of *Tuta absoluta* in Turkey. *Phytoparas* 35:243-244.
- Lietli M, Botto E, Alzogaray A (2005). Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop. Entomol.* 34:113-119.
- Lobos E, Occhionero M, Werenitzky D, Fernandez J, Gonzalez M, Rodriguez C, Oehlschlager C (2013). Optimization of a trap for *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and trials to determine the effectiveness of mass trapping. *Neotrop. Entomol.* 42:445-457.
- Luna G, Pereyra C, Coviella E, Nieves E, Savino G, Nadia S, Sánchez E (2015). Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina. *Florida Entomol.* 98:489-494.
- Matra-Neto A, De Lame FM, Fettig CJ, Munson AS, Perring TM, Stelinski LL, Vargas RI (2013). Manipulation of insect behavior with specialized pheromone and lure application technology (SPLAT®). *Pest Manage. Nat. Prod.* 1141:31-58.
- Maluf R, de Fátima Silva V, das Graças C, Maria G, Luiz Antonio A, Neto C, Gonçalves M, Nizio C (2010). Resistance to the South American tomato pinworm, *Tuta absoluta* in high acylsugar and/or high zingiberene tomato genotypes. *Euphytica* 176:113-123.
- Materu CL, Shao EA, Losujaki E, Chidege M, Mwambela N (2016). Farmer's perception knowledge and practices on management of *Tuta Absoluta* Meyerick (Lepidoptera Gelechiidae) in tomato growing areas in Tanzania. *Int. J. Res. Agric. For.* 3(2):1-5.
- Megido C, De Backer L, Eltalib R, Brostaux Y, Fauconni M-L, Delaplace P, Francis F (2014). Role of larval host plant experience and solanaceous plant volatile emissions in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. *Arth-Plant Int.* 5:293-304.
- Megido C, Haubruge E, Verheggen J (2013). Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review *Synthese bibliographique: les stratégies de lutte phéromonale utilisées pour contrôler la mineuse de la tomate, Tuta absoluta* (Lepidoptera: Gelechiidae). *Biotechnol. Agron. Soc. Environ.* 17:475.
- Melo PC, Melo AM, Bolteux LS (2006). Overview and perspectives of tomato breeding for fresh market adapted to mild tropical climates of Brazil. Paper presented at the International Symposium on Tomato in the Tropics 821p.
- Michel-Salzat A, Whitfield B (2004). Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgasterinae): combined analysis of four genes. *Syst. Entomol* 29:371-382.
- Michereff FM, Vilela F, Attygalle B, Meinwald J, Svatoš A, Jham N (2000). Field trapping of tomato moth, *Tuta absoluta* with pheromone traps. *J. Chem. Ecol.* 26:875-881.
- Miranda M, Picanço C, Zanuncio C, Bacchi L, Silva D (2005) Impact of integrated pest management on the population of leafminers, fruit borers, and natural enemies in tomato. *Clénc. Rural* 35:204-205.
- Miranda M, Picanço M, Zanuncio C, Guedes C (1998) Ecological life table of *tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). *Biocont. Sci. Technol.* 5:597-606.
- Mohamed I, Mahmoud E, Elhaj M, Mohamed A, Ekesi S (2015). Host plants record for tomato leaf miner *Tuta absoluta* (Meyrick) in Sudan. *EPPPO. Bull.* 45:106-111.
- Mollá O, Biondi A, Alonso-Valiente M Urbaneja A (2014). A comparative life history study of two mirid bugs preying on *Tuta absoluta* and *Ephesia kuehniella* eggs on tomato crops: implications for biological control. *BioCont* 59:175-183.
- Mollár O, González-Cabrera J, Urbaneja A (2011). The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer, *Tuta absoluta*. *BioCont* 56:583-591.
- Moreno, Shalene C, Carvalho, Geraldo A, Picanço, Marcelo C, Morais, Eilsangela GF, Pereira RM (2012). Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and selectivity to two non-target species. *Pest Manage. Sci.* 68:386-393.
- Moussa A, Balomy F, El-Adl E (2013). The status of tomato leafminer; *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Egypt and potential effective Pesticides. *Acad. J. Entomol.* 6:110-115.
- Muniappan R, Heinrichs A (2015). Feed the Future IPM Innovation Lab: A Critical Role in Global Food Security. *Outl. Pest Manage.* 26:148-151.
- Mwangi F (2015). Evaluation of botanical pesticides and coloured sticky insect traps For Management of insect pests (Thrips, Whiteflies And Aphids) in French Beans (*Phaseolus Vulgaris* L.). University of Nairobi.
- Oerke C, Dehne W, Schönbeck F, Weber A (2012). Crop production and crop protection: estimated losses in major food and cash crops: Elsevier.
- Oliveira A, da Silva H, Leite, D, Jham G, Picanço M (2009). Resistance of 57 greenhouse-grown accessions of *Lycopersicon esculentum* and three cultivars to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Sci. Hortic.* 119:162-167.
- Parra JR (2009). Egg parasitoids commercialization in the new world. In *Egg parasitoids in agroecosystems with emphasis on Trichogramma*. Springer, Netherlands pp. 373-386.
- Pfleiffer G, Muniappan R, Sall D, Diatta P, Diongue A, Dieng O (2013). First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Senegal. *Flo. Entomol.* 96:661-662.
- Pires M, Marques J, Wanderley-Teixeira T, Álvaro C, Alves C, Alves B (2009). Ultrastructure of *Tuta absoluta* parasitized eggs and the reproductive potential of females after parasitism by *Metarhizium anisopliae*. *Micron* 40:255-261.
- Ponti L, Gilloli G, Biondi A, Desneux N, Gutierrez P (2015). Physiologically based demographic models streamline identification and collection of data in evidence-based pest risk assessment. *EPPPO. Bull.* 45:317-322.
- Proffitt M, Birgersson G, Bengtsson M, Reis Jr R, Witzgall P, Lima E (2011). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. *J. Chem. Ecol.* 37:565-574.
- Reddy P, Guerrero A (2010). Chapter twenty-new pheromones and insect control strategies. *VII. Hortic.* 83:493-519.
- Regnault-Roger C (2012). Trends for commercialization of biocontrol agent (biopesticide) products. In *Plant Defence: Biol. Cont. Spri* Netherlands pp. 139-160.
- Resende V, Maluf R, Faria V, Pfann, Alessan Z, Nascimento R (2006). Acylsugars in tomato leaflets confer resistance to the South American tomato pinworm, *Tuta absoluta* Meyr. *Sci. Agric.* 63:20-25.
- Rodilakis E, Papachristos D, Rodilakis E (2010). Current status of the tomato leafminer *Tuta absoluta* in Greece. *EPPPO. Bull.* 40:163-166.

- Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouli M, Bassi A (2015). First report of *Tuta absoluta* resistance to diamide insecticides. *J. Pest Sci.* 88:9-16.
- Rodrigues B, Elsayed Y, da Silva H (2011). Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* MILL.). *Rev. Bras. Ciênc. Agrár.* 6:243-252.
- Sabbour M (2014). Biocontrol of the tomato pinworm *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Egypt. *Mid East J Agric Res* 3: 499-503.
- Sabbour M, Nayera S (2014). Evaluations of three *Bacillus thuringiensis* against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *Int. J. Sci. Res.* 3(5):2319-7004.
- Sánchez NE, Pereyra, Patricia CL, María G (2009). Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environ. Entomol.* 38(2):365-374.
- Serifli-Nathan S (2013). Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. *Front Physiol.* 4:359.
- Seplyarsky V, Weiss M, Haberman A (2010). *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. *Phytoparasitica* 38:445-446.
- Sharma N, Bhandari S (2014). Management of pathogens of stored cereal grains. *Sustainable Agric Rev* (pp. 67-107): Springer.
- Silva A, Picanço C, Bacchi L, Crespo B, Rosado F, Guedes C (2011). Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Manage. Sci.* 67:913-920.
- Siqueira A, Guedes C, Picanço C (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agric. For. Entomol.* 2:147-153.
- Soliman A, Hegazi M, Attala M, Imam I (2013). Evaluating the role of sex pheromone in monitoring and controlling tomato leaf miner, *Tuta absoluta* (Meyrick), under the Egyptian north western coast conditions. *Egypt. J. Biol. Pest Cont.* 23:169.
- Speranza S, Sannino L (2012). The current status of *Tuta absoluta* in Italy. *EPPO. Bull.* 42:326-332.
- Sridhar V, Chakravarthy K, Asokan R (2014). New record of the invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India. *Pest Manage. Hortic. Ecosys.* 20:140-154.
- Steiner M, Goodwin S (2015). *Tuta absoluta*: Coming soon to a tomato crop near you. *Pract. Hydroponics Greenhouses* 106:44-49.
- Taha M, Homam H, Afsah E, EL-Sharkawy M (2012). Effect of trap color on captures of *Tuta absoluta* moths (Lepidoptera: Gelechiidae). *Int. J. Environ. Sci. Eng.* 3:43-46.
- Tonnang Z, Mohamed F, Khamis F, Ekesi S (2015). Identification and risk assessment for worldwide invasion and spread of *Tuta absoluta* with a focus on sub-saharan Africa: Impl. *Phyto Meas Manage. PloS one* 10:e0135263.
- Trotin-Caudal Y, Baffert V, Leyre M, Hulas N (2012). Experimental studies on *Tuta absoluta* (Meyrick) in protected tomato crops in France: biological control and integrated crop protection. *EPPO. Bull.* 42:234-240.
- Urbaneja A, Desneux N, Gabarra R, Arno J, González-Cabrera J, Mañá Nelo A, Peña JE (2013). Biology, ecology and management of the South American tomato pinworm, *Tuta absoluta*. *Potent. Invas. Pests Agric. Crops* pp. 96-125.
- Vacas S, Alfaro C, Primo J, Navarro-Llopis V (2011). Studies on the development of a mating disruption system to control the tomato leafminer, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae). *Pest Manage. Sci.* 67:1473-1480.
- Van Damme V, Berckvens N, Moerkens R, Berckmoes E, Willems L, De Vis R, De Clercq P (2015) Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. *J. Pest Sci.* 88:533-541.
- Van Lenteren C, Bueno P (2003). Augmentative biological control of arthropods in Latin America. *BioCont* 40:123-139.
- VanDoorn A, de Vos M (2013). Resistance to sap-sucking insects in modern-day agriculture. *Front. Plant Sci.* 4:222.
- Witzgall P, Kirsch P, Cork A (2010). Sex pheromones and their impact on pest management. *J. Chem. Ecol.* 36:80-100.
- Yankova V, Valchev N, Markova D (2014). Effectiveness of phytopesticide Ne m Azal T/S® against tomato leaf miner *Tuta absoluta* Meyrick in greenhouse tomato. *Bul. J. Agric. Sci.* 20 (5):1116-1118.
- Zappala L, Bernardo U, Biondi A, Cocco A, Deliperi S, Delrio G, Garzia T (2012). Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bull. Insectol.* 65:51-61.
- Zappala L, Biondi A, Alma A, Al-Jboory J, Arno J, Bayram A, Guenaoui Y (2013). Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *J. Pest Sci.* 86:635-647.
- Zekeya NM, Shahada F, Chacha M (2014). Bioefficacy of *Bersama abyssinica* extracts against cowpea beetle; *Callosobruchus maculatus* in Storage. *Int. J. Innov. Res. Dev.* 3:5.
- Zouba A, Chermili B, Kadri K, Fattouch S (2013). Molecular characterization of *Trichogramma bourarachae* strains (Hymenoptera: Trichogrammatidae) from open field tomato crops in the South West of Tunisia. *Biomirror* 4:05-11.
- Zucchi A, Querino B, Monteiro C (2009). Diversity and hosts of *Trichogramma* in the new world, with emphasis in South America. In *Egg parasitoids in agroecosystems with emphasis on Trichogramma*. Springer Netherlands, pp. 219-236.



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First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania

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ABSTRACT

Tuta absoluta (Meyrick) is a destructive pest causing adverse environmental and economic effects globally. Management of *T. absoluta* by synthetic chemicals is of diminutive achievement due to resistance trick of the pest. Harnessing of biocontrols is currently fascinating and efficient for management of the tremendous pests. This study isolated an entomopathogenic fungus from dead larvae of *T. absoluta* on tomato leaves from farmer's field Tanzania. Two *Aspergillus* fungal isolates were isolated and tested against larvae and adults of *T. absoluta* under conditions of 19.5°C and 50%RH, 30.4°C and 70% RH, respectively, in the laboratory. Sequence analysis of *Aspergillus* isolates that we referred as A-Tz1 and A-Tz2 were analysed with the BLAST program available at the National Center for Biological Information (NCBI). The two isolates (A-Tz1 and A-Tz2) showed 100% similarity with *Aspergillus oryzae* of GeneBank accession numbers EF121337.1 and MG519722.1. Such similarity implied that both A-Tz1 and A-Tz2 isolates were *A. oryzae*. To confirm pathogenicity, bioassays were conducted against the larvae, pupa and adults of *T. absoluta*. Results showed that at 1.0×10^8 conidia/mL, fungal isolates caused up to 70% larval mortality 3 days post inoculation and consequently inhibited pupation by 84.5% and adult emergence by 74.4%. The identified *A. oryzae* reduced the life span of adult *T. absoluta* up to 5 days post inoculation at 1.0×10^8 conidia/mL whereas in control *T. absoluta* survived up to 25 days. This study found that isolates of *Aspergillus* species have the potential to be used as active ingredient for the formulation of a new bioinjections for application by farmers to control the pest in fields.

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1. Introduction

Tuta absoluta (Meyrick 1917) (Lepidoptera: Gelechiidae) is a serious pest damaging several Solanaceae's crops (Biondi, Guedes, Wan, & Desneux, 2018). Recently, *T. absoluta* invaded Africa disrupting tomato and several Solanaceous crops including potato, night

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shade and peppers (Retta, Berhe, & Management, 2015; Zekeya et al., 2016). The pest is spreading quickly across continents threatening cultivation of tomato and related crops in Africa (Brévault, Sylla, Diatte, Bernadas, & Diarra, 2014).

Management of *T. absoluta* with synthetic chemicals are prevalent in Africa, however they are unaffordable and reported to pose hazardous health effects in other regions (Guedes & Picanço, 2012; Silva et al., 2011; Siqueira, Guedes, Picanço, & Entomology, 2000).

Feeding behaviour of larvae by mining between plant tissues has also been thought to attribute to the failure of most chemical pesticides with contact toxicity, leading to search for systemic pest control options (Desneux et al., 2010).

Approaches such as predators, parasitoids and microbial control including fungi and bacteria are sought to be efficient for management of *T. absoluta* (Contreras et al., 2014; Pires et al., 2009; Polaszek et al., 2012; Sabbour, 2014). Some fungi including *Beauveria bassiana* and *Metarhizium anisopliae* have been reported as effective biocontrol of pests (Inanlı, Yoldaş, & Birgücü, 2012). *M. anisopliae* has been reported as effective control of several crop pests (Santi et al., 2011) and has been reported to parasitize eggs of *T. absoluta* (Pires et al., 2009). However, the genus, *Aspergillus* has been reported to control several pests including *T. absoluta* in the laboratory (Dahliz et al., 2013; Lakhdari et al., 2016) whereas *Aspergillus oryzae* was reported to control Locust (Zhang et al., 2015). Despite the entomopathogenic activity of some *Aspergillus* species, there is scarce information regarding detailed entomopathogenic activity on the tomato leafminer. This study characterised the entomopathogenic activity of local isolates of *A. oryzae* against *T. absoluta* in the laboratory. However, this study recommends future study on mass production, evaluation in field conditions and commercialisation of the new biocontrol for application by farmers in Tanzania.

2. Materials and methods

2.1. Tomato plants

Twenty-one days old tomato seedlings (cultivar Tanya) were transplanted in plastic pots (diameter 14 cm and height 20 cm) containing sterile sand: compost soil (1:3 ratio) filled to a depth of about 12 cm. Then plants were allowed to grow under screen-house conditions for 14 more days at temperatures of about $23 \pm 2^\circ\text{C}$ and 80% relative humidity (RH).

2.2. Isolation of *Aspergillus* species

Fungi were isolated from dead *T. absoluta* larvae and adults from tomato leaves collected in farmers' field (3.14°S, 36.88°E) in Arusha. Isolation of fungi was achieved by sterile needle under stereomicroscope and cultured on potato dextrose agar in glass petri dishes. Out of 25 isolates identified, only two isolates (A-Tz1 and A-Tz2) with high entomopathogenic activity were selected for this study.

2.3. Morphological identification of isolates

Identification of fungal isolates was done using morphological features and colour as described by Diba, Kordbacheh, Mirhendi, Rezaie, and Mahmoudi (2007). Colour,

structure, appearance of mycelia and spore on PDA media were used to distinguish the fungus from another *Aspergillus* sp. (Zhang et al., 2015). A series of microscopic observation to verify morphology and colour pattern was used to distinguish the A-Tz1 and A-Tz2 isolates from *Aspergillus flavus* that is quite similar (Zhang et al., 2015).

2.4. Molecular identification

2.4.1. DNA extraction

In order to confirm whether these fungal isolates belong to *A. flavus* with aflatoxin compounds, molecular characterisation of all isolates was conducted. DNA from fungal isolates was extracted according to Mahuku (2004) with minor modifications. Two hundred milligrams of fresh fungal mycelia were transferred to a sterilised 1.5 mL Eppendorf tube containing 300 μ L of TES, extraction buffer (0.2 M Tris-HCl (Bezemer, Jones, & Knight), 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) and 800 mg of acid washed sterilised sea sand. Micro-centrifuge tube 1.5 mL containing mycelium were fitted into homogeniser (Retsch) and macerated for 4 min. Samples were then vortexed for 30 s followed by the addition of 200 μ L of TES extraction buffer containing proteinase K. The mixture was vortexed to thoroughly mix, and tubes were placed on water bath at 65°C for 30 min. Two hundred and fifty microlitres of 7.5 M ammonium acetate was added, mixed thoroughly and incubated at -5°C for 10 min. The mixture was centrifuged for 15 min at 12,000g to pellet DNA and the supernatant was transferred to a new tube and 500 μ L of ice-cold isopropanol was added.

Tubes were then incubated at -5°C for overnight. The mixture was centrifuged for 10 min at 12,000g to pellet DNA. The supernatant was decanted, and DNA pellet was washed with 800 μ L of cold 70% ethanol. Tubes were kept upside-down on a clean sterile paper towel for 10 min to air dry. DNA was eluted from the pellet with twice repeated extraction with 100 μ L of 1 \times TE buffer (10 Mm Tris-HCl [pH 8], 1 Mm EDTA) each time centrifuged for 2 min to avoid collecting polysaccharides. DNA solution was transferred to micro-centrifuge tube, followed by the addition of 5 μ L of RNase (20 mg/mL) and incubated at 37°C for 60 min. Finally, 1 drop (1 μ L) DNA solution was dropped into Nano drop to determine concentration and purity of DNA.

2.4.2. Amplification, sequencing and phylogenetic analysis of the ITS rDNA region

Fragments containing the region encoding ITS-1, 5.8S rDNA and ITS-2 were amplified using universal primer, ITS-1(F 5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') as described by Zhang et al. (2015). Polymerase chain reaction (PCR) was conducted with TaqTM mix polymerase in a thermocycler amplification of total of 20 μ L DNA mixture. The initial denaturation temperature was 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 30 s, extension at 72°C for 1 min and incubation at 72°C for 10 min. Electrophoresis of all PCR products were performed on the 1.5% agarose gel and was run against the 1 kb ladder (Promega). DNA was stained using Gel red stain for easy visualisation and TAE buffer was used. Products were visualised through gel documentation system. The 5.8s gene of fungal isolates were aligned with the sequences in the NCBI GenBank. A phylogenetic analysis was conducted using neighbour joining (NJ) method with MEGA5

program. Bootstrap tests were conducted with 1000 replications in the NJ analysis and *Davidiella* sp. was used as outgroup.

2.4.3. Aflatoxin detection test

In order to discriminate Isolate A-Tz1 and A-Tz2 from aflatoxin producing *Aspergillus* species, a RIDASCREEN Aflatoxin total kit containing (100%-aflatoxin B₁, 48%-B₂, 78%-G₁, 18%-G₂) was used to characterise isolates according to manufactures instructions. Tests were conducted using dried spores of A-Tz1 and A-Tz2, aflatoxin producing *A. flavus* (*A. flavus* B₁+), new *A. flavus* isolate grown for 12 days on PDA (30°C) and PDA powder contaminated with aflatoxin B₁(PDA B₁+). Six standards (STD1, STD2, STD3, STD4, STD5 and STD6) with known concentration of aflatoxins: 0.00, 0.05, 0.15, 0.45, 1.35 and 4.05 µg/L, respectively, were used together to compare aflatoxin content with tested isolates. Samples were prepared as per manufacturer's instructions and extracted in 70% methanol and centrifuged at 3500g at room temperature (23°C) and the filtrate was diluted six times for the test. The optical densities (OD) were measured at 450 nm using SYNERGY HTX Multi-mode reader (BioTek Digital, USA). Samples and standards were analysed together in duplicate wells on plate and the experiment was conducted twice.

2.5. Preparation of *Aspergillus* concentrations

After 10 days of isolates A-Tz1 and A-Tz2 of conidial maturation on PDA in Petri dishes, spores were gently scrapped from the media by suspending into 10 mL sterile distilled water with 0.1% Triton X-100 per Petri dish to make a stock suspension. From each stock suspension of A-Tz1 and A-Tz2, concentration was assessed by counting spores using hemocytometer Neubauer (Manfield, German). After mixing suspension in one beaker the stock solution concentration was revealed to be 1.1×10^{10} conidia/mL in which working concentrations were prepared by the addition of water followed by counting spore using hemocytometer Neubauer at temperature range of 25.5°C under stereo microscope to obtain 1.0×10^8 , 1.0×10^7 and 1.0×10^6 conidia/mL for pathogenic test against stages of *T. absoluta*.

2.6. Pathogenicity of isolate A-Tz1 and A-Tz2 against *T. absoluta* larvae

Pathogenic effect of A-Tz1 and A-Tz2 against second instar larvae was conducted in the laboratory at 19.5°C and 50% RH, 30.4°C and 70% RH, (the lowest and optimal, respectively) temperature for cultivation of *A. oryzae* (Narahara et al., 1982). Tomato leaflets (5 × 8 cm area) were used as experimental units that were dipped into (1.0×10^6 , 1.0×10^7 and 1.0×10^8 conidia/mL) of isolates A-Tz1 and A-Tz2 and control (water containing 0.1% Triton X-100). Treated leaflets were dried at room temperature for elimination of excess water then were placed in Petri dishes (21 cm diameter) lined with wet paper towel and cottons covering the petiole to prevent leaf dehydration. Then, ten (10) *T. absoluta* larvae were inoculated separately into each of three treated leaflets and control. Each concentration was replicated three times with 10 larvae/replication. Treated leaflets were used only once at the beginning of the bioassay. After 24 h, when treated leaflets were depleted, treated larvae were fed on fresh untreated leaves. Dead insects were placed on another Petri dish lined with moist paper towel for re-isolation of fungus. Data were

recorded at interval of 24 h and trials were monitored up to the completion of *T. absoluta* life cycle and lifespan.

2.7. Data collection and analysis

Larval survival duration was recorded at interval of 24 h until death. Percentage of larvae mortality was recorded at interval of 24 h in which number of dead larvae was deducted from initial total number of larvae times 100 over total number of larvae. Active larvae (treated but persisted) were monitored for pupation rate by subtracting number of pupae from treated larvae times 100 over total number of larvae. Successful pupae (treated) were also monitored for percentage of adult emergence by subtracting number of adults emerged from total treated pupae times 100 over total pupae. Pupation, adult emergence and lifespan were also recorded by counting days taken for all pupae and adult to emerge over total insect set at the beginning of experiment. All data were analysed with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA) and tested for normality and homogeneity of variance. Mortality rate and adult life span were \log_{10} -transformed to obtain normally distributed data sets with equal variance. Larval duration, pupation duration and adult emergence duration were subjected to analysis of variance (ANOVA) whereas larval mortality, pupation rate and adult life span were subjected to Kruskal Wallis. Bonferroni was used to separate mean difference of larval mortality, pupation rate and adult life span whereas Tukey's honest significant difference (HSD) was used to separate mean differences of larvae duration, pupal duration and adult emergence duration at 5% level of significance.

3. Results

3.1. Morphological and molecular characterisation of *Aspergillus* sp. A-Tz1 and A-Tz2

Morphological comparison of *Aspergillus* sp. A-Tz1 and A-Tz2 with *A. flavus* using culture on PDA showed that both isolates had dark color-olive/brown colour, covered with wooly tufts and smooth surface, whereas *A. flavus* showed brighter bright-yellow/green colour, covered with thick short pile like cotton on side and rough surface (Figure 1).

The sequence similarity search using ITS sequences of *Aspergillus* sp. A-Tz1 and A-Tz2 in BLASTN program revealed that both *Aspergillus* sp. A-Tz1 and A-Tz2 belong to *A. oryzae* with 100% sequence similarity to other *A. oryzae* isolates from China and India. A-Tz1 and A-Tz2 showed 100% similarity with *A. oryzae* GeneBank accession numbers EF121337.1 and MG519722.1, respectively, and *A. oryzae*/India/2016 and *A. oryzae*/China/2017 (Figure 2). Such similarity implied that both A-Tz1 and A-Tz2 isolates belong to *A. oryzae* due to close relation with other isolates depicted in phylogenetic tree (Figure 2).

Based on aflatoxin test, result showed a negative correlation between optical density values and aflatoxin concentration (Figure 3). The presence of aflatoxins is indicated by high level of the optical density (OD) values in aflatoxin containing standards. Isolates A-Tz 1 and A-Tz 2 exhibited OD of 3.01 and 3.19 nm, respectively, with 0.00 μ g of

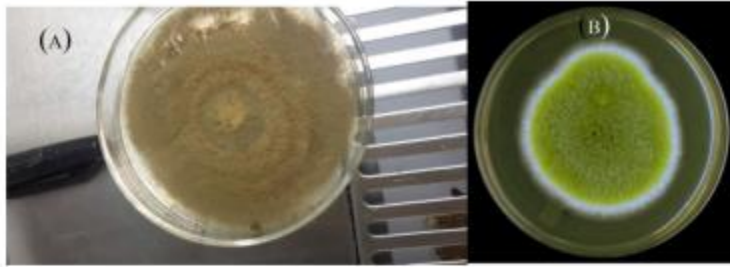


Figure 1. Morphological difference between *A. oryzae* (A-Tz1 and A-Tz2) (A) and *A. flavus* (B) on PDA incubated at 28°C for 12 days.

aflatoxin metabolites that was similar to control (STD 1) with aflatoxin level of 0.00 µg. In contrast, standard 2 up to 6 had aflatoxin concentration of 0.05, 0.15, 0.45, 1.35 and 4.05 µg, respectively, with inverse OD ranging from 3.4 to 0.5 nm (Figure 3).

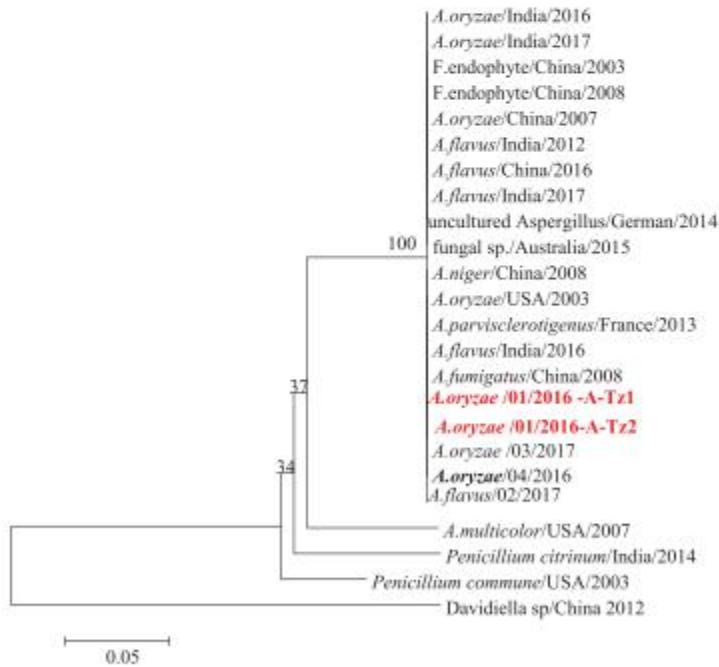


Figure 2. Neighbour joining tree based on ITS sequence data of isolate A-Tz1 and A-Tz2 and accepted species of section *flavi*.

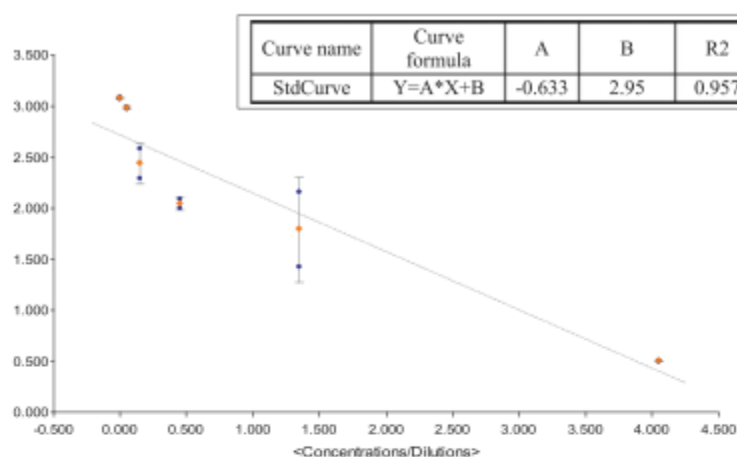


Figure 3. A standard curve presenting a relationship between optical density (450) on Y-axis, and aflatoxin concentration level on X-axis.

3.2. Pathogenicity of A-Tz1 and A-Tz2 on *Tuta absoluta* at different conditions

There was significant pathogenic effect of *Aspergillus* isolates A-Tz1 and A-Tz2 on of *T. absoluta* larvae elevated at 30.4°C and 70%RH and 19.5°C and 50%RH compared to control. The effect of temperature and RH on larval duration, pupation duration and adult emergence duration varied significantly between treatments (Tables 1 and 2). The pathogenic activity was concentration dependent in which higher concentration exhibited higher activity at both conditions.

At 30.4°C and 70%RH larval, pupal and adult duration was shorter compared to 19.5°C and 50%RH in both isolate and control (Tables 1 and 2). The effect of treatment increased with increase in concentration where at 1.0×10^8 conidia/mL larvae had short duration averaging 3.5 ± 0.3 days and 6.8 ± 0.5 days in A-Tz1 and A-Tz2, treated larvae, respectively, whereas in control larvae survived longer averaging 12.0 ± 0.8 days (Table 1). The effect of treatment on pupation was significant in which control exhibited shorter pupation period averaging 9.6 ± 0.3 days compared to sick larvae (A-Tz1) that spent 15.0 ± 0.3 days

Table 1. Effect of isolate A-Tz1 and A-Tz2 on *Tuta absoluta* at 30.4°C and 70%RH.

Treatment	Concentration (conidia/mL)	Larval duration (days)	Pupation duration (days)	Adult emergence duration (days)
Control	0	$11.6 \pm 0.6a$	$9.6 \pm 0.3c$	$16.6 \pm 0.5a$
A-Tz 2	1.0×10^8	$11.0 \pm 0.4a$	$13.07 \pm 0.4b$	$11.6 \pm 0.3b$
A-Tz 1	1.0×10^8	$9.4 \pm 0.4ab$	$13.6 \pm 0.3b$	$11.1 \pm 0.3b$
A-Tz 2	1.0×10^7	$8.6 \pm 0.5bc$	$13.0 \pm 0.3b$	$10.0 \pm 0.6b$
A-Tz 1	1.0×10^7	$7.3 \pm 0.5ab$	$14.6 \pm 0.4ab$	$7.2 \pm 0.9c$
A-Tz 2	1.0×10^6	$5.8 \pm 0.6ab$	$15.1 \pm 0.4a$	$2.3 \pm 0.4d$
A-Tz 1	1.0×10^6	$3.5 \pm 0.5c$	$15.0 \pm 0.3a$	$2.2 \pm 0.5d$
P value		<.0001	<.0001	<.0001

Table 2. Effect of isolate A-Tz 1 and A-Tz 2 on *Tuta absoluta* at 19.5°C and 50%RH.

Treatments	Concentration (conidia/mL)	Larval survival duration (days)	Pupation duration (days)	Adult emergence duration (days_
Control	0	12.0 ± 0.8a	10.6 ± 0.2c	19.9 ± 0.3a
A-Tz 2	1.0 × 10 ⁶	10.9 ± 0.6a	10.8 ± 0.2c	8.7 ± 0.9b
A-Tz 1	1.0 × 10 ⁶	10.6 ± 0.4a	11.7 ± 0.6c	10.2 ± 1.0b
A-Tz 2	1.0 × 10 ⁷	7.6 ± 0.4b	11.7 ± 0.6c	4.5 ± 1.0cd
A-Tz 1	1.0 × 10 ⁷	6.1 ± 0.4b	11.7 ± 0.6c	7.0 ± 0.7bc
A-Tz 2	1.0 × 10 ⁸	6.8 ± 0.5b	14.2 ± 0.3ab	2.9 ± 0.4d
A-Tz 1	1.0 × 10 ⁸	4.8 ± 0.8c	15.5 ± 0.3a	2.8 ± 0.2d
P value		<.0001	<.0001	<.0001

for pupation (Table 1). The effect of *A. oryzae* isolates on emergence of adult *T. absoluta* was also high at concentration of 1.0 × 10⁸ conidia/mL of both A-Tz1 and A-Tz2 that exhibited 2.2 ± 0.5 days and 2.3 ± 0.4 days, respectively, to undergoes mortality compared to control that exhibited longer emergence time averaging 16.6 ± 0.5 days (Table 1).

At 19.5°C and 50%RH, isolate A-Tz1 exhibited longer larvae duration averaging 4.8 ± 0.8 days compared to A-Tz2 (6.8 0.5 days) and control treated larvae that caused mortality in average of 12.0 ± 0.8 days post inoculation (Table 2). The effect of treatments on pupation of *T. absoluta* differed between treatment in which A-Tz2 and A-Tz1 treated insects (sick pupae) exhibited the longer pupation time averaging 15.8 ± 0.7 days at 1 × 10⁶ conidia/mL compared to 1 × 10⁶ conidia/mL treated insect and control that completed pupation in 10.6 ± 0.2 days post inoculation (Table 2). The duration for adult emergence also varied between treatments in which isolate A-Tz1 and A-Tz2 treated insects emerged after an average of 2.8 ± 0.2 and 2.9 ± 0.4 days, respectively, in contrast to control where longer adult emergence duration of up to 19.9 ± 0.3 days was observed due to high number of live insects that survived for longer period (Table 2).

Mortality rate of larvae, pupation rate and adult emergence rate varied significantly between treatments at 30.4°C, 70%RH and 19.5°C, 50%RH (Tables 3 and 4). Isolate A-Tz1 exhibited the highest mortality compared to isolate A-Tz2 and control. At higher concentrations of 1.0 × 10⁸ conidia/mL, both isolate A-Tz1 and A-Tz2 depicted higher mortality rate of 76.66% and 70%, respectively, compared to control that only 13.33% larval mortality was observed (Table 3). The rate of pupation was 23.3% in A-Tz1 at high concentration of 1.0 × 10⁸ conidia/mL compared to low concentration of A-Tz2 and compared control that was 86.6% of pupation. The percentage of adult emerged from treated (sick pupae) was lower ranging from 18.6% to 33.3% in higher and moderate concentration, respectively, compared to control that was successful by

Table 3. Effect of isolate A-Tz 1 and A-Tz mortality of *Tuta absoluta* at 30.4°C and 70%RH.

Treatments	Concentration (conidia/mL)	% of Larval mortality	% of Pupation	%Adult emerged	Adult lifespan (days)
Control	0	13.3	86.7	70.7	16.6 ± 0.5a
A-Tz 2	1.0 × 10 ⁶	43.3	66.3	50.0	10.2 ± 1.0b
A-Tz 1	1.0 × 10 ⁶	40.0	40.7	43.6	8.7 ± 0.9b
A-Tz 2	1.0 × 10 ⁷	60.0	33.3	33.3	7.0 ± 0.7bc
A-Tz 1	1.0 × 10 ⁷	63.3	33.3	23.3	4.5 ± 1.0cd
A-Tz2	1.0 × 10 ⁸	70.0	26.6	23.3	1.3 ± 0.4d
A-Tz1	1.0 × 10 ⁸	76.7	23.7	18.6	0.8 ± 0.2d
P value		<.0001	<.0001	<.0001	<.0001

Table 4. Effect of A-Tz1 and A-Tz2 on mortality of *Tuta absoluta* at 19.5°C and 50%RH.

Treatments	Concentration (conidia/mL)	% Larval mortality	% of Pupation	%Adult emerged	Adult life span
Control	0	14.00	80.00	76.00	19.9 ± 0.3a
A-Tz 2	1.0 × 10 ⁶	50.00	50.00	50.00	11.6 ± 0.3b
A-Tz 1	1.0 × 10 ⁶	50.00	46.00	46.00	11.1 ± 0.3b
A-Tz 2	1.0 × 10 ⁷	53.33	33.33	33.33	10.0 ± 0.6b
A-Tz 1	1.0 × 10 ⁷	66.00	33.33	33.33	7.2 ± 0.9c
A-Tz 2	1.0 × 10 ⁸	66.00	33.33	33.33	5.0 ± 0.7c
A-Tz 1	1.0 × 10 ⁸	70.00	26.66	26.66	2.7 ± 0.4d
P value		<.0001	<.0001	<.0001	<.0001

76.7% (Tables 3 and 4). However, effect of both isolate A-Tz1 and A-Tz2 on life span of *T. absoluta* was significantly across both conditions 19.5°C and 50%RH and 30.4°C and 70%RH but with moderately higher activity in the later conditions (Tables 3 and 4).

4. Discussion

Based on morphological and molecular identification, we confirmed that isolates *Aspergillus* sp. A-Tz1 and A-Tz2 belong to *A. oryzae*. Morphological features including olive colour, fluffy mycelia and revealed that isolates were *A. oryzae* (Zhang et al., 2015). Although morphology and physiology are traditionally used for the identification of fungi, such characteristics were not quite sufficient (Geiser et al., 2007), thus it was complemented with the molecular identification by the use of ITS1-5.8S rDNA-ITS2 as appropriate locus for the identification of *Aspergillus* species (Zhang et al., 2015). However, a combination of morphological and molecular characterisation has been documented for identification of *A. oryzae* infecting locust in China (Zhang et al., 2015). Thus, we were also able to use both morphological and molecular identification approaches to confirm the identity of *Aspergillus* sp. A-Tz1 and A-Tz2 as *A. oryzae* in the current study.

Additionally, aflatoxin test revealed that both isolates *Aspergillus* sp. A-Tz1 and A-Tz2 are non-aflatoxin producer thus could not be associated with aflatoxin producing *Aspergillus* species especially *A. flavus* that is similar to in most morphological features (Zhang et al., 2015).

An entomopathogenic activity of the newly identified isolates was conducted as it has been reported from several fungi infecting *T. absoluta* (El-Hindi, 2016). The Efficacy of *Aspergillus* sp. against *T. absoluta* has also been reported in some African countries (Lakh-dari et al., 2016). Specifically, Zhang et al. (2015) revealed the entomopathogenic activity of *A. oryzae* against locusts in China. This study revealed the efficacy of *A. oryzae* against *T. absoluta* which concur the study by Zhang et al. (2015) that established an entomopathogenic activity of the same fungus on different pest. In this study, the pathogenic activity of *A. oryzae* against *T. absoluta* increased with rise in concentration, in which the higher concentrations caused higher mortality within a short time. In addition, *A. oryzae* was also effective than control at lower concentrations although its activity on larvae, pupae and adult mortality was sluggish compared to higher concentration. *A. oryzae* infected *T. absoluta* in all stages in which treated insects were weak leading to delayed pupation than healthy insects in control. It was also observed that few insects pupated and emerged into adult in which the period for adult emergence and survival duration was very short in *A. oryzae* treated insect regardless of concentration high compared

to control. This could be due to low number of insects in *A. oryzae* treated plates due to high mortality exerted by *A. oryzae* in short time leading to low average duration at the end of the experiment compared to control.

Despite concentrations of *A. oryzae* in treated dead insects, the fungal mycelia and spore were finally, observed propagating on insects' cuticles (Pedrini, Ortiz-Urquiza, Zhang, & Keyhani, 2013; Petlamul & Prasertsan, 2012) and eventually the entire insect body was found degraded due to fungal activity (Charnley & Leger, 1991). The destruction on insect body could be due to enzymatic activity secreted by fungus in insects' cuticles and exoskeletons as a result of hydrophobic interaction between fungus and insect attachment sites (Charnley & Leger, 1991; Zhang et al., 2012).

Temperature and RH have been reported to affect entomopathogenic activity of fungi (Li et al., 2014). This study revealed that at moderate conditions of 28–32°C and 60–80% RH temperature and RH, respectively, the activity of *A. oryzae* was higher than that below 20°C and 50% RH. This could be associated with high fungal metabolic rates in warm temperature, compared to cool temperature. These findings are in consistent with previous report that revealed the entomopathogenic activity of fungal species including *Metarhizium* and *Beauveria spp.* at 25–30°C (Shi & Feng, 2004). Thus, the higher infectivity rate in warmer temperature suggests the shorted duration for entomopathogenic fungi activity for control of *T. absoluta* with short life cycle.

5. Conclusion

This study discovered that *A. oryzae* is efficacy against life stages of *T. absoluta*, a devastating pest of Solanaceous crops and weeds. Hence, these findings suggest for field evaluation and formulation of biopesticide from *A. oryzae* isolates that could save as biocontrol of *T. absoluta* to minimise use synthetic chemicals for protection of crops and biodiversity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Biondi, A., Guedes, R. N. C., Wan, F.-H., & Desneux, N. (2018). Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: Past, present, and future. *Annual Review of Entomology*, 63, 239–258.
- Brévault, T., Sylla, S., Diatte, M., Bernadas, G., & Diarra, K. (2014). *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae): A new threat to tomato production in Sub-Saharan Africa. *African Entomology*, 22(2), 441–444.

- Charnley, A., & Leger, R. S. (1991). The role of cuticle-degrading enzymes in fungal pathogenesis in insects. In *The fungal spore and disease initiation in plants and animals* (pp. 267–286). Boston, MA: Springer.
- Contreras, J., Mendoza, J., Martínez-Aguirre, M., García-Vidal, L., Izquierdo, J., & Bielza, P. J. (2014). Efficacy of entomopathogenic fungus *Metarhizium anisopliae* against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology*, 107(1), 121–124.
- Dahliz, A., Lakhdari, W., Soud, A., Hammi, H., Boučekima, H., & Belaidi, M. (2013). Complex of natural enemies and control methods of the exotic invasive pest *Tuta absoluta*. Lepidoptera: Gelechiidae in Southern Algeria. 48.
- Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., ... Frandon, J. J. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *Journal of Pest Science*, 83(3), 197–215.
- Diba, K., Kordbacheh, P., Mirhendi, S., Rezaie, S., & Mahmoudi, M. J. P. (2007). Identification of *Aspergillus* species using morphological characteristics. *Pakistan Journal of Medical Sciences*, 23(6), 867.
- El-Hindi, M. J. I. (2016). Safe approach to the Biological control of the tomato leafminer *Tuta absoluta* by entomopathogenic fungi *Beauveria bassiana* isolates from Gaza Strip. *International Journal of Applied Research*, 2(4), 351–355.
- Geiser, D., Klich, M., Frisvad, J. C., Peterson, S., Varga, J., & Samson, R. A. (2007). The current status of species recognition and identification in *Aspergillus*. *Studies in Mycology*, 59, 1–10.
- Guedes, R., & Picanço, M. (2012). The tomato borer *Tuta absoluta* in South America: Pest status, management and insecticide resistance. *EPPO Bulletin*, 42(2), 211–216.
- Inanlı, C., Yoldaş, Z., & Birgücü, A. K. (2012). Effects of entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) on larvae and egg stages of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 49(3), 239–242.
- Lakhdari, W., Dehliz, A., Acheuk, F., Mlik, R., Hammil, H., Matallah, S., & Doumandji-Mitiche, B. J. (2016). Biocontrol test against the leaf miner of tomato *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) by using entomopathogenic fungi in the Algerian Sahara. *Algerian Regional Journal*, 13, 85–89.
- Li, M., Liu, X., Wang, Y., Wang, X., Zhou, L., & Si, S. J. A. E. (2014). Effect of relative humidity on entomopathogens infection and antioxidant responses of the beet armyworm. *Spodoptera exigua* (Hübner), 22(3), 651–659.
- Mahuku, G. S. (2004). A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. *Plant Molecular Biology Reporter*, 22(1), 71–81.
- Narahara, H., Koyama, Y., Yoshida, T., Pichanigkura, S., Ueda, R., & Taguchi, H. J. (1982). Growth and enzyme production in a solid-state culture of *Aspergillus oryzae*. *Fermentation Technology*, 60(4), 311–319.
- Pedrini, N., Ortiz-Urquiza, A., Zhang, S., & Keyhani, N. O. (2013). Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: Hydrocarbon oxidation within the context of a host-pathogen interaction. *Frontiers in Microbiology*, 4(24), 24. Abstract.
- Petlamul, W., & Prasertsan, P. J. M. (2012). Evaluation of strains of *Metarhizium anisopliae* and *Beauveria bassiana* against *Spodoptera litura* on the basis of their virulence, germination rate, conidia production, radial growth and enzyme activity. *Mycobiology*, 40(2), 111–116.
- Pires, L. M., Marques, E. J., Wanderley-Teixeira, V., Teixeira, A. A., Alves, L. C., & Alves, E. S. B. (2009). Ultrastructure of *Tuta absoluta* parasitized eggs and the reproductive potential of females after parasitism by *Metarhizium anisopliae*. *Micron*, 40(2), 255–261.
- Polaszek, A., Rugman-Jones, P. F., Stouthamer, R., Hernandez-Suarez, E., Cabello, T., & del Pino Pérez, M. J. B. (2012). Molecular and morphological diagnoses of five species of Trichogramma: Biological control agents of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae) in the Canary Islands. *BioControl*, 57(1), 21–35.

- Retta, A. N., Berhe, D. H. J., & Management, E. (2015). Tomato leaf miner–*Tuta absoluta* (Meyrick), a devastating pest of tomatoes in the highlands of Northern Ethiopia: A call for attention and action. *Research Journal of Agriculture and Environmental Management*, 4(6), 264–269.
- Sabbour, M. J. (2014). Biocontrol of the tomato pinworm *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) in Egypt. *Middle East Journal of Agriculture Research*, 3(3), 499–503.
- Santi, L., e Silva, L. A. D., da Silva, W. O. B., Corrêa, A. P. F., Rangel, D. E. N., Carlini, C. R. ... Biotechnology (2011). Virulence of the entomopathogenic fungus *Metarhizium anisopliae* using soybean oil formulation for control of the cotton stainer bug, *Dysdercus Peruvianus*, 27 (10), 2297–2303.
- Shi, W.-B., & Feng, M.-G. (2004). Lethal effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with a description of a mite egg bioassay system. *Biological Control* 30(2), 165–173.
- Silva, G. A., Picanço, M. C., Bacci, L., Crespo, A. L. B., Rosado, J. F., & Guedes, R. N. C. (2011). Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm. *Tuta absoluta*, 67(8), 913–920.
- Siqueira, H. A., Guedes, R. N. C., Picanço, M. C. J. A., & Entomology, F. (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology*, 2(2), 147–153.
- Zekeya, N., Chacha, M., Ndakidemi, P. A., Materu, C., Chidege, M., & Mbega, E. R. J., & International, (2016). Tomato leafminer (*Tuta absoluta* Meyrick 1917): A threat to tomato production in Africa. *Journal of Agriculture and Ecology Research International*, 10(1), 1–10.
- Zhang, S., Widemann, E., Bernard, G., Lesot, A., Pinot, F., Pedrini, N., & Keyhani, N. O. J. (2012). CYP52X1, representing a new cytochrome P450 subfamily, displays fatty acid hydroxylase activity and contributes to virulence and growth on insect cuticular substrates in the entomopathogenic fungus *Beauveria bassiana*. *Journal of Biological Chemistry*, M111, 338947.
- Zhang, P., You, Y., Song, Y., Wang, Y., & Zhang, L. J. B. s. & technology (2015). First record of *Aspergillus oryzae* (Eurotiales: Trichocomaceae) as an entomopathogenic fungus of the locust. *Locusta Migratoria* (Orthoptera: Acrididae), 25(11), 1285–1298.



Occurrence and distribution of *Tuta absoluta* (Gelechiidae) in agro-ecological zones of Tanzania

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INTRODUCTION

Tomato leafminer (*Tuta absoluta*) is a destructive pest of tomato and other solanaceous crops worldwide. The pest adapts quickly in the new environment and its high reproduction rate leads to high damage in all aerial plant parts including leaves, stem, shoot and fruits in tomato leading to yield loss.

OBJECTIVE

The aim of this study was to evaluate the occurrence and distribution of *T. absoluta* in the major tomato growing zones of Tanzania during dry and wet season for document the status of the pest and its threat to tomato growers.

MATERIAL AND METHODS

Thirteen regions were surveyed for the study on presence and abundance of *T. absoluta* in Tanzania. The survey was conducted in 2015, 2016 and 2017 to measure its occurrence across selected agro-ecological regions of Tanzania. Monitoring of the pest was conducted using pheromone traps in randomly selected tomato fields. Adult *T. absoluta* trapped were counted once per week for four months in each season.

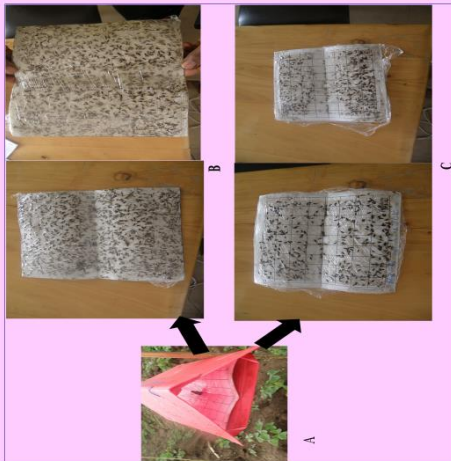


Figure 2: Pheromone trap (A) for capturing adult *Tuta absoluta* in dry (B) and wet (C) season



Figure 1: Severe damage on a tomato leaf by tomato leafminer by *Tuta absoluta*

RESULTS

This study found that *T. absoluta* was present in all thirteen regions surveyed and populations of density varied significantly between regions. Regions with the highest populations were Iringa, Arusha and Morogoro, with trap catches of 503.8 ± 17 , 292.9 ± 28.9 and 239.4 ± 24.2 adults/trap/week respectively. Regions with the lowest populations were Dodoma and Mwanza, with trap catches of 16.2 and 13.3 adults/trap/week respectively. The highest densities of *T. absoluta* and tomato damage are occurring in Tanzania's most tomato producing regions (Iringa, Arusha and Morogoro), which reflects the close relationship between this pest and its preferred host plant, tomato.

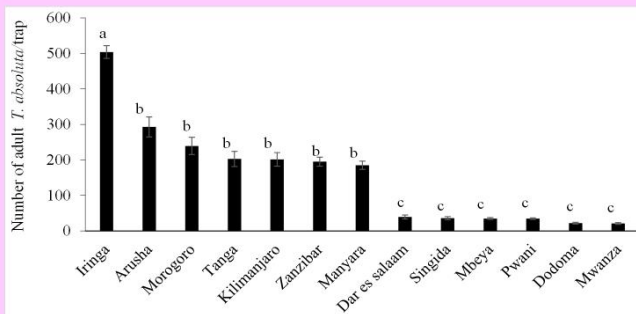


Figure 3: Number of adult *Tuta absoluta* per trap in different regions of Tanzania

CONCLUSION

These findings inform farmers and other stakeholders about the highest density and distribution of the pest across Tanzania. It also indicates the status of the pest and its threat on tomato and other solanaceous crops hence calls for the development of effective and sustainable management strategies for control of *T. absoluta* in Tanzania.

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