BIOECOLOGY AND MANAGEMENT OF *Tuta absoluta* MEYRICK (Lepidoptera: Gelechiidae) ON *Solanum lycopersicum* L. IN SOUTHWESTERN, NIGERIA

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to the memory of Nigerian youths who paid the supreme price for a better Nigeria. May God heal our land and make Nigeria great.

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Praises be to the great Father, who sits enthroned in the heavens reigning as King over all, the Immortal, Invincible, and the One Wise God, to Him be the glory, honour, praises, adoration, and worship now and forevermore.

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ABSTRACT

Tuta absoluta (Ta) is an invasive insect pest causing severe damage and yield loss to tomato in the field and screenhouses. Management of Ta is difficult because its life cycle varies with environment, and it has developed resistance to chemical pesticides. Presently in Southwest Nigeria (SWN), information on the knowledge and life cycle of Ta which is important for its effective management is limited. Therefore, tomato farmers' Knowledge, Perception, and Management Preferences (KPMP) of Ta was surveyed in SWN, while its bioecology and management on tomato were investigated in Ibadan.

A five-stage sampling procedure was used. Three major Tomato-Growing-States (TGS): Ogun, Oyo and Ekiti states were purposively sampled in SWN. Five Agricultural Development Programme Zones (ADPZ) were purposively selected from the TGS. Thereafter, ten Agricultural Development Programme Blocks (ADPB) from ADPZ were purposively selected. Twenty-four Agricultural Development Programme Cells (ADPC) were purposively selected from each ADPB. Finally, 31.3% of tomato farmers from ADPC were randomly selected (n=180). Data were collected on tomato farmers' KPMP of Ta using structured questionnaire. Forty tomato plants were assessed on 15 farms and three screenhouses in each TGS for Ta occurrence. Selected samples of Ta (n=10) were identified at molecular level using specific primers and standard technique. Developmental and behavioural biology of Ta were assessed on sweet-tomato in the laboratory for data on Developmental Period-DP (days), morphometrics (mm), lifetable, parthenogenesis, Days to Adult Emergence (DAE), Adult Longevity (AL, days), sex ratio, and pre-oviposition and oviposition. Twenty tomato accessions were evaluated in the screenhouse (10kg pots) for resistance. Pots were laid out in randomised complete block design with four replicates. Larval-eclosion (%) and yield loss were determined. Data were analysed using descriptive statistics and ANOVA at $\alpha 0.05$.

Tomato farmers (78.8%) identified Ta with pictures and damage characteristics, 69.5% knew severity of Ta infestation on tomato plants. About 52.5% of respondents did not perceive Ta as a major field insect pest of tomato. Management preference for Ta were botanicals (88.5%)>chemical (87.7%)>crop rotation (63.5%). *Tuta absoluta* was not detected on the farms but in screenhouses. *Tuta absoluta* was biphyletic (Brazil and Spain variants). Total DP for eggs, larva and pupa was 3-4, 10-13, and 6-8, respectively. Egg was oval (0.22±0.01); larva comprised four-instars with body-length (1.49±0.02-8.00±0.01), body-width (0.28±0.02-0.67±0.01) and head-capsule-width (0.15±0.01-0.56±0.01). Pupa body-length and body-width were 4.00±0.01 and 0.64±0.01, respectively. Net reproductive rate was 46.01. Deuterotokous parthenogenesis occurred with 33.2% larvae emergence from 94.40±7.21 eggs laid. The DAE was 21 and AL varied with sex (virgin males:7.28±2.13, virgin females:12.87±3.55) and mated females (15.54±3.20). Sex ratio was 1:1.5 (male:female). Pre-oviposition was 1.9 days and oviposition lasted 10.0 days. Accessions were susceptible with larval-eclosion (50-80%) and yield loss (60-100%).

Tuta absoluta is a serious threat to tomato production in the screenhouses in Southwest, Nigeria. It has a short developmental period from egg to adult (20-23 days) that makes it a multivoltine insect. Due to its deuterotokous parthenogenetic nature, *Tuta absoluta* control through the sterile insect technique will not be effective.

Keywords: *Tuta absoluta*, Tomato damage characteristics, Larval-eclosion, Deuterotokous parthenogenicity

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CHAPTER ONE

INTRODUCTION

Tomato, *Solanum lycopersicum* (Linanaues), is a major vegetable cultivated globally with great economic importance to many countries. It belongs to the family *Solanaceae*, and genus *Solanum*, which includes important species such as *S. Cheesemannii, S. glandulosam, S. hirsutm, S. lycopersicum cerasiforme, S. peruvianum, and S. pimpinellifolium* (Bai and Lindhout, 2007). Ancestor of the cultivated tomato is *S. lycopersicum cerasiforme*. The family Solanaceae include crops such as eggplant, peppers, potato, and tobacco which are economically important (Peralta and Spooner, 2005a). The assumption by many researchers is that tomato might have originated from the Andean area in South America, but the place and time of its domestication is still unknown (Naika *et al.*, 2005). Peralta and Spooner (2005b) suggested that distribution of tomato began from Europe moving to Asia (southern and eastern) Asia, Africa, and the Middle East.

The use of tomato as food originated in Mexico and its global spread followed the Spanish colonization of the Americas. It is consumed raw, cooked, steamed, or fried with other vegetables. In Nigeria, it is an important ingredient in many dishes like jollof rice, salads, sauces, and stews. Canned and dried tomatoes are of great economic importance as processed products, and Nigeria spends millions of dollars annually on the importation of tomato paste to augment the local production which is not enough for national demand.

Tomato is a super-food that is rich in micronutrients. It contains an abundance of carotenoids, including beta-carotene; making it a rich source of vitamin A. *Bhowmik et al.* (2012) reported that regular consumption of tomatoes might prevent atherosclerosis, diabetes, asthma, decrease the risk of cancers, osteoporosis and cardiovascular diseases. The report suggested that carotenoids such as alpha- and beta-carotene, lutein, and lycopene have synergistic effects as a group, and when eaten with other plants such as garlic, avocado, olive oil, and broccoli, in prevention of cancers.

In addition, Bhowmik *et al.* (2012) stated other benefits of tomato consumption which include, but not limited to, maintenance of strong bones due to the presence of calcium and vitamin K, helps the body recover from the effects of smoking due to the presence of coumaric acid and chlorogenic acid. Also, the presence of vitamin B and potassium in tomatoes is effective in reducing cholesterol levels and lowering blood pressure in human beings. While the presence of vitamin A in tomatoes is good for the eyes, skin, teeth, and keeps the hair strong and shiny. There are other benefits associated with the consumption of tomatoes hence it is one of the most grown vegetables worldwide.

Tomato production was 180.77 million metric tonnes globally in the year 2019, the highest in the vegetable category for that year, while Africa contributed 0.89% to the global production (FAOSTAT, 2021). Although, tomato production increases yearly yet there is a great demand for more. Pests such as nematodes, viruses, fungi, bacteria and insects, limit tomato production. Insect pests of tomato include stinging and sucking insects, such as whitefly, thrips, and aphids. Tomato leaf miner, *Tuta absoluta,* has more devastating effects as insect pest of tomato, in greenhouse and open field (Cocco *et al.*, 2012; Gharekhani and Salek-Ebrahimi, 2013), causing 80 - 100% damage both on the field and in the screen house (Desneux *et al.*, 2010; Desneux *et al.*, 2011).

The larvae of *Tuta absoluta* cause damage to tomato by mining the leaves, stems, buds, and burrows into fruits causing quality decline of fresh tomato and yield loss that range from 50% to 100% (Cocco *et al.*, 2012; USDA–APHIS, 2011). Its biology and behaviour make control a challenge.

Tuta absoluta became a major problem, spreading through Spain in 2006; reaching Senegal in 2012, Kenya in 2014 and Nigeria in 2015 (Cabello *et al.*, 2012; Oke, 2017). In Nigeria, it was first detected in Daura, Katsina State in April 2015, then in Kano State in June 2015 and Abeokuta, Ogun State in September 2015 (Oke *et al.*, 2016, 2017). The invasion led to destruction of tomato farms nationwide. Tomato production was reduced by over 80% loss in the first cycle of the season 2016 in northern parts of the country where the pest has been much restricted (Borisade *et al.*, 2017). In Nigeria, the yield losses attributed to *T. absoluta* infestation and spread was equivalent to 720, 000 metric tonnes (Sanda *et al.*, 2018).

The first management strategy for *T. absoluta,* because of its invasive nature, was the massive use of chemical control, which resulted in the pest developing resistance to chemical pesticides such as spinosad, bifenthrin, cartap hydrochloride, deltamethrin, ermethrin, cypermethrin, indoxacarb, and methamidophos (Lietti *et al.,* 2005). Furthermore, resistance to Diflubenzuron, Triflumuron, Metaflumizone, and Lamdda-cyhalothrin have been documented (Guedes and Picanço, 2012). In Nigeria, there is evidence that *Tuta absoluta* is resistant to Lambda-cyhalothrin (Oke, 2017).

Use of chemical pesticides have harmful effects on the environment by destroying natural enemies of the pest and other beneficial organisms, it also affects human beings and animals adversely hence there is a need for a more environmentally friendly approach to controlling this invasive pest (Luna *et al.,* 2012; Molla *et al.,* 2011). Furthermore, there are an increasing number of resistant strains of this pest (Moreno, 2011; Deliperi and Delrio, 2012). Mass trapping of the male adults and mating disruption methods have not been effective as there are reports of reproduction by parthenogenesis (Caparros *et al.,* 2012). Furthermore, these techniques are more expensive than pesticide applications (Cocco *et al.,* 2012). From the foregoing, it is expedient that we explore wild accession of tomato for new sources of resistance. Thus, there is a need for an effective and ecologically sound management approach.

Fruits and vegetables have essential micronutrients needed for proper growth; however, these are usually unaffordable for many Nigerians due to high cost. The high cost is often a result of losses associated to production. Vegetables also provide farmers with higher income per hectare than cereal, root, and tuber crops. Thus, there is a need to increase production of vegetables such as tomato and reduce loss to vegetable (tomato) production.

There is also a need for government to have a paradigm shift from oil explorations to agricultural production, especially horticultural produce like tomato. Thus, increase in agricultural production can increase the country's gross domestic product (GDP) earnings through exportation of produces like tomato, which has a high demand globally. To achieve these, the limiting factors affecting tomato production need be addressed. Nigeria tomato farmers, in 2015/2016 growing seasons, recorded loss of over 2-billion-naira worth of tomato produce to *Tuta absoluta* infestation, making Tuta absoluta a national concern (Bala *et al.,* 2019). The destruction was rapid and total that it was named “tomato ebola” (Borisade *et al.,* 2017; Sanda *et al.,* 2018).

Although, Nigeria is the highest tomato producer in sub-Saharan Africa there is deficit in her tomato demand, hence there is a need to reduce loss due to insect pest like *T. absoluta.* However, it takes an understanding of the pest’s biology to develop an effective Integrated Pest Management (IPM) for it. Although, there are reports on the biology of the pest, but majority of such reports do not emanate from Nigeria, and the development of *Tuta absoluta* is influenced by environmental factors such as temperature, in addition to that is host plant resistance. Hence, the need to study the pest within the Nigerian context and to discover a low-cost but highly effective control that has minimal impacts on the environment such as use of resistant variety. Furthermore, there is a need to supply baseline information for Pest Risk Assessment (PRA) in Southwest, Nigeria. Also, there is a need to assess stakeholders’ knowledge, perception of the pest, and their management preferences.

The aim of this research is therefore to ascertain the presence and pest status of the insect in Southwest, Nigeria, study its biology and ecology.

The specific research objectives are:

i. Quantitative survey of tomato farmer’s knowledge, perception, and management practices (KPP) employed to curtail activities of *Tuta absoluta* on tomato in selected states in Southwest, Nigeria
ii. Assess occurrence, abundance, and diversity of tomato leaf miners on *Solanum lycopersicum* in selected states in Southwest, Nigeria
iii. Conduct a molecular identification of the pest (*Tuta absoluta*)
iv. Investigate the developmental, behavioural, and reproductive biology of *T. absoluta*
v. Sort to determine the resistance of selected tomato accessions to *T. absoluta* infestation

CHAPTER TWO

LITERATURE REVIEW

2.1. History and cultivation of *Solanum lycopersicum*

First cultivation of tomato, a native to South America, was by the Incas and Aztecs around 700 BC., and was widely distributed from Ecuador to Northern Chile, with two endemic species in the Galapagos Islands (Peralta and Spooner, 2007). Tomato can grow in diverse ecological habitats, from near sea level to over 3,300 m in elevation, in arid coastal lowlands, isolated valleys in the high Andes, and in deserts like the severe Atacama Desert in Northern Chile, Andean topography and different climates. All these have contributed to wild tomato diversity (Darwin *et al.*, 2003). According to Peralta and Spooner (2007), the first description of tomato with the common name "Pomid'oro" (Golden Apples) was by Pietro Andrea Matthioli (1544) in his 'Commentary' on the work of the 1st century Greek botanist Dioscorides of Anazarbos.

The movement of tomato from South America to Europe is credited to the Spanish conquistadors, such as Christopher Columbus as early as 1493 or Hernán Cortés who captured the Aztec city of Tenochtitlan, now Mexico City, in 1521. They carried the plant and its seeds back to Europe, where it gained popularity as a food item in Italy, Spain, and Portugal. The plant then began to spread from there to the Caribbean, Asia, and Africa. Thomas Jefferson, the third president of United States of America, was one of the first Americans to have grown tomatoes in his Virginia home. However, by 1812, tomatoes had become widely accepted by many people and were included in recipes of cooks in Louisiana and Maine (Smith, 1994; Genticore, 2014).

2.2. Taxonomy and botanical description of *Solanum lycopersicum*

Tomato belongs to the family Solanaceae, genus Solanum, subfamily Solanoidaeae and tribe solaneae (Taylor, 1986). The genus includes a small collection of cultivated species like *Solanum lycopersicum* (formerly *Lycopersicon esculentum* Milli.), and wild species like, *S. cheesemannii, S. glandulosam, S. hirsutum, S. peruvianum,* and *S. pimpinellifolium*. It was placed in the genus Solanum by Linnaeus (1753) as *Solanum lycopersicum* L. (derivation, 'lyco', wolf, plus 'persicum', peach, i.e., "wolf-peach"). Philip Miller, who placed it in the genus *Lycopersicon* and then named it as *Lycopersicon esculentum* in 1768, challenged this (Peralta and Spooner, 2005b, Shukla *et al.*, 2013). However, geneticists have correctly placed tomato in the genus *Solanum* (Peralta and Spooner, 2007). Tomato was wrongly believed to be toxic because it belongs to the Solanaceae family, known as the "deadly" Nightshade family; however, the toxicity of tomato is limited to the leaves and not the fruits (Shukla *et al.*, 2013).

Tomato is a perennial plant but usually grown as an annual plant (Plate 2:1). It can reach up to 3 metres in height. It has a weak stem and often requires staking when planted. Branching is monopodial at the base but becomes sympodial at the top. Their leaves sizes range from 10 to 30 cm long and are unevenly imparipinnate with indented/lobed margins. Tomato's inflorescence has small yellow flowers with five pointed lobes on the corolla. The fruits are fleshy berries, which are green when unripe but turn deep red and shiny when ripe. The redness is associated with lycopene (Sharoni and Levi, 2006).

There are different cultivars based on size, shape, and colour (yellow, orange, green and brown accessions of fruits). The shape can vary from small cherry tomatoes, pear-shaped to large irregular-shaped beefy tomatoes (Gerszberg *et al.*, 2015). There are two major types of tomatoes: determinate and indeterminate, under these two types, there are more than 10,000 accessions including Roma, Lemon boy, Jubilee, Celebrity, and Big Beef. These are further divided into different categories such as Plum, Slicing, Heirloom, Beefsteak, Cherry, and Oxheart among many others. The determinate tomatoes have fixed process of growth, flowering, fruiting, and senescence, while indeterminate tomatoes have continuous growth, producing flowers and fruits at the time until the first frost or natural senescence. The harvest from indeterminate accessions often extends over two or three months and the yields are heavier than from determinate types (Gerszberg *et al.*, 2015).

Plate 2.1: An indeterminate tomato cultivar grown under greenhouse conditions

(https://www.agrifarming.in/wp-content/uploads/2015/02/Tomato-Cultivation1.jpg retrieved on 29 November 2021)

2.3. Utilization of *Solanum lycopersicum*

Tomato is economically important; it is one of the most widely grown, eaten, and researched vegetable worldwide. It can be eaten raw or in different processed forms. The processed forms can be in any of the following: tomato preservers such as peeled tomatoes, tomato juice, and tomato paste. It can also be in the form of dried tomato this include tomato powder, tomato flakes and dried tomato fruits, and thirdly it can be processed into tomato-based foods e.g., chili sauces, jollof rice, ketchup, stew, tomato sauces, and tomato soup. Tomato is rich in vitamins (A, B and C), antioxidants (carotenes, Lycopene, and β-carotene), and phenolic compounds, regular consumption has been associated with prevention of several diseases including colon cancer (Wilcox *et al.,* 2003; Sharoni and Levi, 2006; Balasundram *et al.*, 2006; Periago *et al.,* 2009).

Tomato is used for molecular farming, which is the production of recombinant proteins in plants, without altering the plants' genetic make-up, with the express intention to use the protein for therapeutic purposes (Gerszberg *et al.*, 2015). The use is in the production of vaccines or antibodies in a process called "biopharming"; there has been some successful production of recombinant pharmaceutical proteins in tomatoes. For example, there was a successful expression of a malaria antigen (PfCP-2.9) in transgenic tomato plants by Kantor *et al.* (2013).

One of the diseases that scientists are trying to solve using tomato plants is the Alzheimer's Disease (AD). This disease is often associated with the presence of Aβ amyloid, an insoluble protein, deposited in the brain. Inhibiting or lowering the formation of Aβ is thus a promising approach towards the treatment of AD (Vassar, 2004). Hence, many researchers have made different attempts to addressing this issue using tomato plants for the experiments. For example, Youm *et al.* (2008) successfully attempted the production of tomato plants with a satisfactory level of Aβ protein expression used as an oral vaccine assay on mice. In another experiment, Kim *et al.*, (2012) successfully attempted the production of human β -secretase (BACE1) in tomato fruits. BACE1 is a vaccine antigen that promotes immune response in AD patients.

In another experiment, Chen *et al.* (2009) reported the possible production of Thymosine (Tα1), an immune booster, against diseases induced by viral infections (e.g., hepatitis B and C) from tomato plants. This makes tomato plant particularly important to medical research for the advancement of healthy living among human beings.

2.4. Global demand for *Solanum lycopersicum*

There is a great demand for tomato hence the increasing demand in production (fresh and processed) worldwide for the last four decades (Costa and Heuvelink, 2005). A total of 79.9 million metric tonnes was produced worldwide in 2005; by 2012, production had increased to 159.3 metric tonnes, by 2014 production had reached 170.8 million metric tonnes, the latest report being 180.7 million metric tonnes in 2019 (FAOSTAT, 2012; FAOSTAT, 2016; FAOSTAT, 2021). Africa produces 11% of the world production (Figure 2.1). Morocco is the leading producer in Africa while Nigeria is the fourth. In sub-Saharan Africa, Nigeria is the largest producers of tomatoes and ranks 14th in global production, producing about 1.5 million tonnes with an average yield of 5-6 tonnes ha^{-1} (FAOSTAT, 2012; FAOSTAT, 2016).

2.5. *Solanum lycopersicum* production constraints

Unfavourable environmental conditions and pests affect tomato production. Insect pests of tomatoes include the following, cutworms (*Agrotis spp*.), Plusia looper (*Chrysodeixis acuta* Walker), Tobacco caterpillar (*Spodoptera litura* Fabricius) (Lepidoptera: Noctuidae), Gram pod borer: *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and leaf miners such as *Liriomyza trifolii* (Burgess) and *Tuta absoluta* (Meyrick). Tomatoes are subject to attack from a varied array of pests. Severity of these attacks however varies with cultivars, farm management practices, places, and seasons. Apart from the larvae of moths, aphids, thrips, various mites (particularly red spider mite), nematodes, and white fly also cause severe damage to tomato production.

2.5.1. Aphids: These are soft-bodied insects with body length of 1.5 mm to 2.5 mm, having long, frail legs and are slow-moving; some are winged while others are wingless. They have a short life span and can complete twenty or more generations within a year. Increase in population is seasonal, aphid infestations when severe under dry conditions can cause early dieback of plants. Furthermore, the indirect damage, triggered by transmission of disease-causing viruses by the pest, is more severe than the piercing and sucking activities of these sap-sucking insects. Myzus persicae (Sulzer) and Macrosiphum euphorbiae (Thomas) are probably the most important to tomato production.

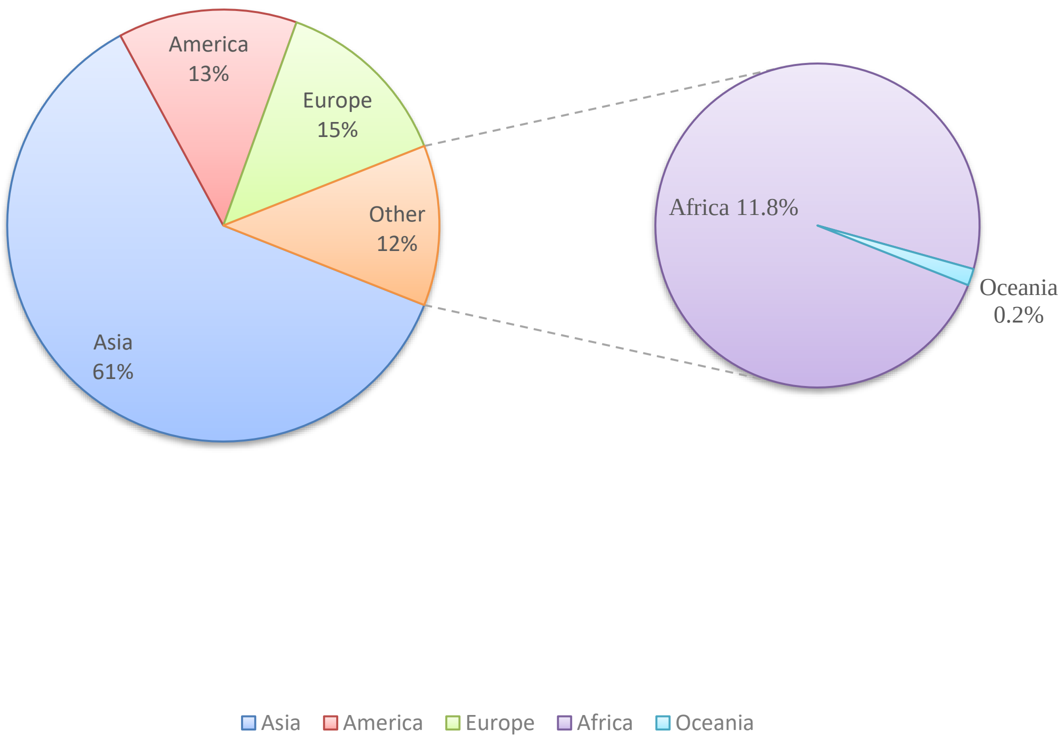

Figure 2.1: Global tomato production in 2017

(http://www.fao.org/faostat/en/#data/QC/visualize) data retrieved 26 Nov, 2019

2.5.2. Mites: Also, tomatoes suffer attack from different mites such as red spider mite (*Tetranychus* spp.) that feeds on the leaves at the lower sides. The leaves become yellowish and bronzed before drying out. They also make fine webbing in severe infestation. Tomato russet mite (*Aculops lycopersici* Tryon) causes a twisting up of the lowermost leaves with shiny sheen on the adaxial surfaces. Leaves of the plants become bronzed, withered, and resulting in death of the plants. The stems also become brownish-purple and the fruits looking rusty brown with coarse surface cracking. Erinose mites (*Eriophyses lycopersici* Tryon) have microscopic size and they cause a dense patch of fine hairs, white in colour, on the fresh leaves and stems of the tomato plants.

2.5.3. Thrips: The main thrips species that attack tomatoes is *Frankliniella schultzei* Trybom which feeds on the flowers and new fruits; this is done by scraping their surfaces and sucking sap from broken cells in the flowers and fruits. Their attack results in the blossom drop, fruit scarring with some malformation of the leaves. Thrips are important vectors of spotted wilt virus, which is a major disease of tomatoes.

2.5.4. Moths: These are the most common of all the pests of tomato plants. The following constitute pests of economic importance to tomato production. The larvae of American Bollworm (*Helicoverpa armigera*) feed primarily on the flowers causing high rate of dropping and low fruits production, and on the fruits causing severe damage if left uncontrolled. Cutworm (*Agrotis* spp.) cut off the young plants at then stems near the ground level. Plusia looper (*Chrysodeixis acuta*) larvae feed on the abaxial surface of the leaves, skeletonising them. The matured larvae attack the fruits, their feeding causes shallow and often wide holes in the fruits. American leaf miner (*Liriomyza trifolii*) larvae make narrow, twisting tunnels between the abaxial and adaxial surfaces of the leaves. After feeding, they leave trails of black excrement along these mines. In severe infestation, the tunnels merge to form big cavities, which results in low photosynthetic ability of the plant. This damage would affect yield through, increases in incidence of sunburns and poor fruit set. Leaf miner/potato tuber moth (*Phthorimaea operculella*) larvae mine leaves by making uneven cavities in the leaves and in the process leaves brown frass on the leaves. The most aggressive of these moths is *Tuta absoluta*, which can cause 80% - 100% damage in the open field and screen house (Plate 2.2) in a relatively short time hence it is called “Tomato Ebola” in Nigeria. These moths feed on the leaves, fruits, and stems in severe infestation (Plate 2.2).

Plate 2.2: Damage to tomato in the (A) open field and (B) screen house

(www.seedquest.com/news.php?type=news&id_article=94117&id_region=id_category=&id_crop) retrieved 13 December 2021

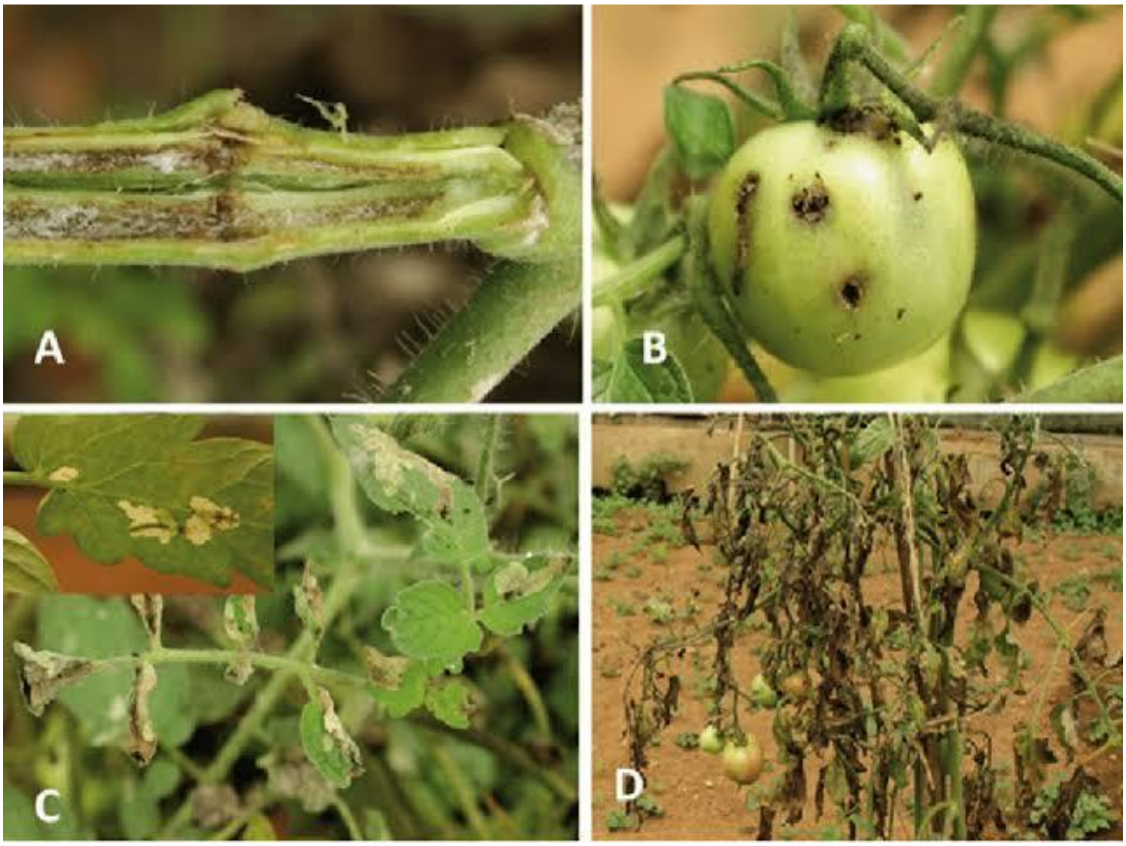

Plate 2.3: Damages caused by *Tuta absoluta* on the A) stem of tomato plant B) fruit of tomato (entrance and exit holes of the larvae) C) leaves of tomato (galleries) D) tomato plant

(Source: Sankarganesh *et al*., 2017)

2.6. Occurrence, distribution, and dispersal of *Tuta absoluta* (Meyrick, 1917; Lepidoptera: Gelechiidae)

Tuta absoluta, also known as tomato leaf miners, tomato borer, South American tomato moth, and South American tomato pinworm, belongs to the Domain: Eukaryota, Kingdom: Metazoa, Phylum: Arthropoda, Subphylum: Uniramia, Class: Insecta, Order: Lepidoptera, Family: Gelechiidae, Genus: *Tuta,* Species: *Tuta absoluta.*

Tomato leaf miner is an invasive moth from South America, which causes severe damage and significant yield loss (80-100%) to tomato in the screen house and open field. It also affects other solanaceous crops in various regions of the earth (Cifuentes *et al.*, 2011; Urbaneja *et al.,* 2013; Zappala *et al.,* 2013; Tonnang *et al.*, 2015; Bawin *et al.,* 2016). This pest was first documented in 1917 and reported as pest of tomato in Peru in 1960 (Seplyarsky *et al.,* 2010; Guedes and Picanço, 2012), from where it migrated into Spain and other part of Europe in 2006 (Harizanova *et al.*, 2009; Roditakis *et al.,* 2010; Desneux *et al.*, 2011).

First reported case of *Tuta absoluta* in Africa was in Morocco in 2007 (Muniappan, 2013), then in 2008 it was reported in Tunisia (Abbes *et al.* 2012), north of the Sahel (Desneux *et al.,* 2010) in 2010 it was reported Egypt (Moussa *et al.*, 2013), and in 2011 by Sudan and South Sudan (Brevault *et al.,* 2014). By 2012, it had invaded Senegal (Pfeiffer *et al.*, 2013), Libya (Harbi *et al.*, 2012) and Ethiopia (Goftishu *et al.*, 2014).

The pest continued its spread like wildfire invading Kenya by 2013 (Mohamed *et al.*, 2015), Tanzania (Biondi *et al.,* 2015) and Senegal (Tonnang *et al.*, 2015) in 2014 and by 2015 Nigeria experienced the great devastation by tomato Ebola (Oke *et al,* 2017).

The report from NAPPO (2013) showed that *T. absoluta* had spread to the following countries: Albania, Argentina, Algeria, Austria, Belgium, Bahrain, Bolivia, Bulgaria, Brazil, Chile, Cayman Islands, Cyprus, Colombia, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Ethiopia, France, Finland, Greece, Germany, Iraq, Hungary, Iran, Ireland, Italy, Israel, Jordan, Kuwait, Kosovo, Latvia, Libya, Lebanon, Luxembourg, Lithuania, Morocco, Malta, Netherlands, Paraguay, Palestinian Authority (West Bank), Panama, Portugal (including the Azores), Peru, Poland, Qatar, Russia, Romania, Spain, Saudi Arabia, Slovakia, Senegal, Slovenia, Sudan, Sweden, Switzerland, Syria, Western Sahara, Tunisia, Turkey, United Kingdom, Venezuela, and Uruguay. Therefore, it can be found in Africa, Europe, the Middle East, and parts of Asia (Soares and Campos, 2021). Yet, it is still spreading locally, and globally. There is a need for increasing quarantine, more information of dispersal dynamics and sustainable management options to contain its spread (Campos *et al.,* 2017).

Retta and Berhe (2015) observed that the swift dispersal of *Tuta absoluta* over these wide range of geographic zones may perhaps be associated to its high fecundity rate, large variety of host plants, which increases its persistence in farmland areas, and hibernating capability. In addition, they corroborated Desneux *et al.* (2010) report, that the absence of co-evolved natural enemies in the newly invaded areas must have aided the increase in pest population when compared to native areas with natural enemies. Furthermore, Desneux *et al*. (2011) suggested that the intra-continental dispersal could be associated to human transportation which was supported by Tropea Garzia *et al.* (2012), while Silva *et al.* (2011) and Gontijo *et al.* (2013) believed that resistance to insecticides also contributed to the wide dispersal. Gontijo *et al.* (2013) reported that the pest is also dispersed by wind, this was corroborated by Sridhar *et al.* (2019).

2.6.1. Life cycle characteristics of *Tuta absoluta*

Tuta absoluta is a holometabolous insect that can produce between 10 to 12 generations in a year; it feeds on cultivated and non-cultivated solanaceous species (Pereira and Sanchez, 2006). The development of Tuta absoluta is adversely affected by several environmental factors, particularly temperature. The pest can survive in a temperature range of 3°C to 35°C (Soares and Campos, 2021). Thus, the life cycle can take from 23.8 days at 27°C to 76.3 days at 14°C (Duarte *et al.,* 2015). The adult insect lays its egg on the leaves (Plate 2.4), buds, stems and/or calyx of unripe fruits. The eggs are oval, 0.4 mm in length and 0.2 mm in diameter. The colour of the egg is creamy white which turns yellow and finally black before hatching (Desneux *et al.,* 2010).

Tuta absoluta has a pre-oviposition period of 2.3 to 4.6 days depending on temperature (Arnó and Gabarra, 2010; NAPPO, 2012; Salama *et al.,* 2014). A gravid female can lay maximum of 260 eggs in a lifetime, more than 70% of which are laid in the first 7 days after mating (Uchoa-Fernandes *et al.,* 1995; Harizanova *et al.,* 2009). Egg hatch between 4-6 days after being laid (Cuthbertson *et al.,* 2013). However, Muniappan, (2015) reported seven days for larva eclosion from eggs. The young larvae then penetrate the leaves, fruits, or stems where they feed and develop through the four larval instars before

Plate 2.4: ***Tuta absoluta*** **laying eggs on tomato leaflet**

(Source: www.tutaabsoluta.org retrieved 21st April 2019) transforming into the pupae. The first two-instar stages mine the leaves by feeding on the mesophyll, while the later stages leave the mines and bore into stalks, apical buds, and fruits (Ferracini *et al.*, 2012). Different researchers have reported different duration for this stage; 8 days (Muniappan, 2015), and 12-15 days (Mutamiswa *et al.*, 2017).

Pupation occurs in the mines, dried leaves or in soil. The fully-grown larvae drop to the ground or dry leaves on a silk thread (Pfeiffer *et al.*, 2013). The duration of transformation to adult varies depending on environmental factor such as temperature. Muniappan, (2013) reported duration of 10 days. Mutamiswa *et al.* (2017) also reported duration of 9 -11 days at benign condition. Pupae are cylindrical in shape, greenish in colour when they are just formed; they become brownish in colour with maturity (NAPPO, 2012).

Tuta absoluta is a microlepidopteran. The adults are mottled grey in colour, about 1 cm long and have a wingspan of about 1 cm (USDA–APHIS, 2011). *Tuta absoluta* can be found in all climatic conditions, Tonnang *et al*. (2015) reported that they have been found in high altitudes, 1000 m above sea level.

2.6.2. The distribution and spread of *Tuta absoluta*

The spread of *Tuta absoluta* has been associated with wind dispersal, porous borders, and poor implementation of quarantine regulations (Desneux *et al.*, 2010). The most obvious pathway for the spread of *T. absoluta* is accidental introduction (Soares and Campos, 2021) through the importation of tomato fruits from infested areas, especially in long distance dissemination of T. absoluta. Examples of this include the introduction of the pest to Argentina from Chile in 1964 (Bajonero *et al.*, 2008) and to Spain from Latin America in 2006 (Urbaneja *et al.*, 2007).

Furthermore, eggs can be dispersed through packing materials, such as box, crate, and pallets among others, used in transporting host plants like eggplant, tomato, potato, peppers, and tobacco from countries with infestation cases (Caparros-Megido, *et al.*, 2013). Agricultural tools originating from infested countries could also be the source of infestation in the new area (Retta and Berhe, 2015). In addition, transporting live plants from areas with infestation increases the probability of the pest's survival during the period of transportation or storage, as the leaves provide a haven for the eggs and larvae during transport (Karadjova *et al.*, 2013). Also, fresh tomatoes offer refuge for the eggs and larvae during transport and are thus high-risk source of transferring *Tuta absoluta* from one source to another. Hence to avoid this risk, fruits must be properly quarantined before being imported or exported to other countries.

Other sources of spread might be through repackaged tomato consignments (Karadjova *et al.,* 2013). In many production facilities, tomatoes are packed, repacked, and distributed for sales and export, some of these consignments might harbour eggs, early or late larval stage or pupa that are concealed. These can develop into moths at the packing station where the consignments are received. With suitable environmental conditions, these would grow to become fully-grown moths, thus causing a great infestation in the new area.

Another possibility of spread of the tomato miner is through farm visitors, as the eggs and larvae easily attach to surfaces. Consequently, to avoid infestation in non-infested areas there must be proper sterilization of all materials brought into the farm, such as crates, packing cartons, farm equipment, among others. Furthermore, seedlings must be thoroughly checked and disinfected, while visitors and other farm workers must be properly sanitized before having contact with the farm.

2.6.3. Economic impact of *Tuta absoluta* infestation

This pest is a threat to solanaceous crops like potato, but it poses a main peril to tomato production globally (Biondi *et al.,* 2018). The economic impacts include costs of pest management, decrease in marketability of the produce, and potential loss of trading partners through import restrictions from non-infested countries (Soares and Campos, 2021).

There could be total yield loss of tomato crops in the absence of control measures (Guedes *et al.,* 2019), especially in sub-Saharan Africa where the pest is new (Zekeya *et al.,* 2017), and knowledge of management is scanty. In many parts of sub-Saharan Africa, smallholder farmers rely on horticultural crops like tomato for income (Oerke *et al.*, 2012) this is because of their high nutritive value (Cetin and Vardar, 2008). Hence, the effect of the damage could be so devastating, to the extent that some farmers have stopped tomato production due to losses incurred from *Tuta absoluta* infestation (Muniappan and Heinrichs, 2015). In Nigeria, tomato is a major horticultural crop, with an estimated production of over 17 million tonnes/ha per year (Materu *et al.*, 2016).

Unfortunately, the country experienced invasion of the pest in the year 2016, which caused a great devastation to production in Northern Nigeria.

2.7. Management strategies for *Tuta absoluta*

There are different methods used in managing *Tuta absoluta* infestation. These include but not limited to semiochemicals (Cocco *et al.*, 2013), cultural methods (Bawin *et al.*, 2016), chemical methods (Guedes *et al.*, 2019), sterile males technique (Cagnotti *et al.*, 2012) and physical methods, botanicals, and Biopesticides (González-Cabrera *et al.*, 2011; El-Arnaouty *et al.*, 2014) and use of resistant accessions (Guedes and Picanço, 2012).

2.7.1. Use of pheromone traps for *Tuta absoluta*

Pheromones are chemicals substances secreted in bodily fluids and released into the environment by an animal (mammals, insects), which influence the behaviour and/or physiology of others of its specie such as triggering sexual interest, aggregation, trail, excitement etc. Such include sex pheromones which are released by an organism (male or female) for the purpose of attracting opposite individuals of the same species for mating (Subramoniam, 2017). Prasad and Prabhakar (2012) reported that sex pheromone has an extensive use in monitoring, forecasting, or controlling populations of insect pests, particularly moths. Witzgall *et al.* (2010) believed that the most widespread and successful applications of sex pheromones concern their use in detection and population monitoring. This was similar to Refki *et al.* (2016) report, who that stated the presence of *T. absoluta* in a field can be detected through the use of pheromone traps. Witzgall *et al.* (2010) further reported that sex pheromones are also used to control insect populations through mass annihilation and mating disruption, this was corroborated by El-aassar *et al.* (2015).

The Tuta pheromone traps have *T. absoluta* natural sexual attractants, which attract adult male moths only. The trapping of males is expected to control the population of tomato leaf miners through mating interference and mass extermination. There are different types of pheromones trapping techniques namely:

2.7.1.1. Water trap: this trap consists of a container (plastic, stainless steel etc) with water and pheromone bait (Plate 2.5). The lure is safely placed higher than the water on

Plate 2.5: Two types of pheromone trap: Delta trap with removable liner (A) and Water trap (B)

Source: https://www.tutaabsoluta.com/tuta-absoluta (retrieved 12 April 2019) a firm object like stone, or it could be dangled on the water with attached wire/rope at both ends of the container. Thereafter, droplets of vegetable oil or pinch of detergent is added to the water, which will help in reducing the surface tension, and as a result it minimizes the trapped insect's ability to escape from the setup. This also limits the rate at which the water evaporates therefore reducing the number of times of refilling with water (USDA APHIS, 2011; Chermiti and Abbes, 2012). This is the most common pheromone trap that is used for mass trapping of *T. absoluta.* This trap can be easily maintained, and unlike Delta or light traps, it is not sensitive to dust. Moreover, it can trap more insects than Delta traps (Salas, 2004; USDA APHIS, 2011).

Regrettably, using only this method has not been successful in controlling damage by *Tuta absoluta* on tomato plants (Cocco *et al.*, 2012). This might be associated with the fact that trap cannot to trap all the males in the infested area, and the ability of the females to reproduce parthenogenetically (Caparros-Megido *et al.* 2012). Even then, some of the males might have mated before being trapped.

2.7.1.2. Delta trap: This is a triangular trap that is made from plastic or waterproof card, the surface is coated with non-drying gum and pheromone of the target insects (Plate 2.4). When the targeted insect is attracted by the pheromone, it flies into the trap and gets stuck on the coated surface. The trap can be made of cardboard, triangular, lined with sticky surface or with a removable liner. Any of the two can be used, although the traps with non-drying adhesive liners are preferred by many.

2.7.1.3. Sticky rolls: these rolls contain sticky glue, with incorporated *T. absoluta* pheromone which is released intermittently from the roll (Hassan and Al-Zaidi, 2010). There are two types of this sticky roll namely the clear sticky film, and yellow sticky film. Clear sticky film contains beneficial insects as biocontrol agent, while the yellow sticky film rolls are just for trapping *T. absoluta* alone. The yellow films also trap other insect like whiteflies, aphids, due to their bright colour. However, it is recommended that the sticky film (yellow) should not be used in greenhouses so that non-targeted insects would not be trapped with the pests (Hassan and Al-Zaidi, 2010).

2.7.2. Mating disruption: The aim of this technique is to create sexual confusion in the males. Female pheromone (synthetic) is released into the atmosphere, which prevents the pest from mating, consequently, reducing the pest's population (Cardé 2007; Cocco *et al.*, 2013). Illakwahhi and Srivastava, (2017) reported that there has been successful application of pheromone traps in controlling leaf miners both in screen houses and open field in Asia, Europe, South America, and North Africa. In Egypt, El-aassar *et al.* (2015) reported that sex-pheromone when combined with synthetic pesticides showed promising results in managing the leaf miners.

2.7.3. Physical methods: Insect-proof nets and plastic tunnels are some of the methods of physical control (Holt *et al.,* 2008). Nevertheless, protected tomato crops are often infested by *Tuta absoluta* (Desneux *et al.,* 2010, Zappala *et al.,* 2012) in screen houses, higher infestations are seen on plants closer to the screen house openings (Cocco *et al.,* 2013). Bearing in mind that *T. absoluta* female must have actual contact with the tomato canopy to lay eggs (Proffit *et al.,* 2011), there is all possibility that adults immigrating from the exterior can walk on the protective materials and find a way to lay eggs on the protected plants (Biondi *et al.,* 2015). Thus, screen houses should be fitted with aphid-proof insect nets. The plants should be some centimetres away from the net, and the doors should close tightly. There should be no gaps in the construction, while persons coming from infested areas should be avoided, and growers should be thoroughly disinfected before they enter the screen houses.

Although, through this method adult *T. absoluta* might be screened out, yet 100% prevention against *T. absoluta* is not guaranteed. This being that screening will limit the movement of parasitoids and predators, thus biological control hindered. Nets also reduce ventilation in screen houses, making the screen houses warmer than the surrounding, thus encouraging the growth of the strayed leaf miner in the kit.

2.7.4. Cultural control: This method involves planting clean seedlings that are pest free, destroying crop residue in the field, and crop rotation preferably with non-host crops such as cabbage (cruciferous vegetables) (Illakwahhi and Srivastava, 2017). In addition, soil solarisation and adequate irrigation and fertilization, these are some of the cultural control methods that can be used. Furthermore, ploughing, uprooting infested plants and total removal of plant debris and fruits after harvest are good farm practices that can reduce leaf miner infestation. For example, after the harvest of solanaceous crops like eggplants, peppers, potatoes, or tomato, the crop residues should at once be destroyed completely. This can be done through burning, burying, or covering with transparent plastic film to ferment them. Pupae left in the soil can be killed by soil solarisation, after which the land should be left fallow for a minimum of six weeks before planting susceptible crops (Illakwahhi and Srivastava, 2017). In addition, wild solanaceous host plants around the farm/screen house must be removed to avoid re-infestation. Through these good agricultural practices, *T. absoluta* can be controlled. However, cultural method has not been successful in controlling *T. absoluta* infestation Muniappan, (2013).

2.7.5. Chemical control: This is the use inorganic insecticides as the primary method to pest management. The severe nature of *T. absoluta* infestations requires immediate and effective measure hence the widespread use of insecticide by tomato growers (Desneux *et al.,* 2011; Gontijo *et al.,* 2013). Different chemical pesticides have been used such as pyrethroids, bifenthrin, deltamethrin, permethrin, cypermethrin, indoxacarb, and methamidophos, diflubenzuron, triflumuron, metaflumizone, and lambda-cyhalothrin (Lietti, *et al.,* 2005; Guedes and Picanço, 2012; Muniappan, 2013).

The major drawback of this method is the endophytic nature of *T. absoluta*; they feed inside the leaves, fruits and stems of the tomato plant making it difficult to easily access. Additionally, the high cost of insecticide reduces profits; there is destruction of natural enemy populations (Campbell *et al.,* 1991), and there is the building-up of these insecticide residues in tomato fruits (Walgenbach *et al.,* 1991) which will result in bioaccumulation of toxic substance in the food web. Moreover, the indiscriminate use of insecticide has resulted in the development of insecticide resistant strains (Silva *et al.,* 2010; Haddi *et al.,* 2012; Campos *et al.,* 2014). The high reproductive ability and noticeably short generations of the pest could have also contributed to the development of the resistant strain. In addition, the use of synthetic insecticides causes multiple detrimental side effects on the agro ecosystem (Biondi, 2013).

Different studies have showed the resistance of *T. absoluta* to many chemical pesticides. Fe *et al.* (2005) reported resistance to deltamethrin, abamectin, methamidophos in Argentina. Roditakis *et al.* (2015) had reported the first case of *Tuta absoluta* resistance to diamide, which include chlorantraniliprole and flubendiamide. In Brazil, resistance to certain insecticides have been reported such as Abamectin, and permethrin by Siqueira, *et al.* (2000), and spinosad by Campos *et al.* (2014). Abbes *et al.* (2012) reported resistance to more than 18 chemicals in Tunisia during 2009-2011 invasions. Thus, chemical pesticide, though widely used, is ineffective and ecologically unsound in the management of *Tuta absoluta* (Kaoud, 2014).

2.7.6. Biological control: This is the use of biological agents such as parasitoids, natural enemies, and entomopathogens to control insect pests. The control strategies include classical biological control, augmentative biological control, and conservation biological control.

Classical Biological Control (CBC) is the introduction of a natural enemy of exotic origin to control a pest, usually also exotic, aiming at permanent control of the pest (Hajek, 2005; Van Driesche *et al.*, 2008). It involves the use of parasitoids, predators, and pathogens.

Augmentative Biological Control (ABC) is used against native and invasive pests. Many predators and parasitoids are already identified as natural enemies of *Tuta absoluta*, such as *Trichogramma* species (*T. exiguum* Pinto and Platner, *T. nerudai* Pintureau and Gerding, *T. pretiosum* Riley and *T. achaeae* Nagaraja and Nagarkatti) and have been reported as efficient in controlling the menace of this pest (Parra *et al.*, 2004; Chailleux *et al.*, 2012).

2.7.7. Host plant resistance: This is the planting of plant variety/species/accession or cultivar/landrace that is resistant to an insect pest. In tomato cultivation, resistance to insect pests has been associated with the presence of glandular trichomes and the secondary metabolites they possess such as acyl sugars, methyl ketones, jasmonic acid, salicylic acid, and total phenols (Oliveira *et al.*, 2009; Bitew, 2018; Sridhar, *et al.*, 2019; Chen *et al.*, 2021).

Some tomato species have been documented as being resistant to *Tuta absoluta*, such as *S. hirsutum* Dunal, *S. hirsutum* f. *glabratum* Dunal, *S. Chilense*, *S. Arcanum*, *S. corneliomulleri*, *S. habrochaites* and *S. pennellii* Correll (Bitew, 2018; Sridhar, *et al*. 2019). *S. habrochaites* and *S. pennellii* were reported as the most resistant with low oviposition rate and high larvae mortality by Bitew (2018); this was associated with the presence of trichome Type I and IV. Also, the accessions have shown resistance to other insect pests such as spider mites *Tetranychus urticae* and Silverleaf whitefly *Bemisia tabaci*. A lot still need to be done in tomato resistant research, especially in sub-Saharan Africa (Guedes and Picanço, 2012; Zeleya *et al.*, 2017).

2.7.8. Biopesticides: This is a contraction of two words “biological pesticides”. It involves the use of natural organisms (animals, bacteria, certain minerals, or plants), or substances derived from them which includes their genes or metabolites to control agricultural pests (Sporleder and Lacey, 2013). The use of biopesticides in management of *Tuta absoluta* is still in the developing stage (Inanli *et al.*, 2012). However, there are few available biopesticides for use such as *Bacillus thuringiensis* formulation, and *Bacillus subtilis* which have been effectively used to control *T. absoluta* in America and Europe. Other biopesticide which have been used against *T. absoluta* infestation include *Beauveria bassiana* applications, Nucleopolyhedrosis virus, Nucleogranulosis virus (Oke, 2017).

2.7.9. Botanicals: This is the use of plants' extracts either in aqueous, ash, or other forms to control insect pests. Botanicals have played a key role in pests management over the years (Isman, 2006; Zekeya *et al.*, 2017). Several studies have shown that many insect pests, including *T. absoluta,* can be controlled using plant compounds (Castillo *et al.*, 2010). For instance, Durmusoglu *et al*. (2011), in laboratory experiment, reported the efficacy of Neem extracts in the management of *T. absoluta*. Yankova *et al,* (2014) substantiated this, that Neem plant holds several active secondary metabolites such as alkaloids that are effective in insect pest control. Furthermore, Moreno *et al.* (2012) reported that secondary compounds from the plant *Acmella oleracea* L. (toothache plant) have strong activity against *Tuta absoluta*. There are other promising plants for management of tomato leaf miner, which include Piper spp. (Brito *et al.*, 2015).

Although, many botanicals have been reported as having great potential for pest control, yet the application of this novel idea in the sub-Saharan Africa is still limited. It is therefore encouraged that more research should be conducted to validate the efficacy of these natural resources' potential to protect crop from damage and loss. It is a known fact that plant-based pesticides are better than inorganic chemical pesticides because they are available, biodegradable, and environmentally friendly to non-targeted organisms. However, the effectiveness of these methods against *Tuta absoluta* is poorly documented in sub-Saharan Africa (Zekeya *et al.*, 2017).

2.7.10. Integrated Pest Management: According to Mansour *et al.* (2018) the strategy used in Africa today in managing *T. absoluta* is by applying synthetic insecticides. Also, included is the implementation and incorporation of other approaches, such as prophylactic and cultural practices, use of pheromone-based trapping systems either for early detection, monitoring and/or mass trapping. Other methods include biological control using generalist predatory bugs, egg parasitoids, and application of microbial-based and plant extract-based insecticides. In conclusion, potential IPM strategy that is based on the use of native microbial biocontrol, pheromone trap, botanicals, and some moderate inorganic pesticides would be applicable and environmentally reasonable solution for stakeholders in Sub-Saharan Africa.

CHAPTER THREE

MATERIALS AND METHODS

3.1. The Study Site

This research was carried out at the Department of Crop Protection and Environmental Biology, University of Ibadan, Oyo State, Nigeria. The experiments, in the Entomological laboratories and screen houses, were conducted under ambient conditions of 27.5 ± 3 °C temperature and 75.5 ± 3% relative humidity and 29.2 ± 3 °C temperature and 83.3 ± 4% relative humidity respectively. The field work (7.4052º N and 3.8500º E) was set up at National Horticultural Research Institute, (NIHORT), Ibadan, Oyo State; also, the genetic characterization of *T. absoluta* was carried out at the Genetic Laboratory of the Institute.

Field and behavioural surveys were purposively conducted in three states in Southwest, Nigeria namely Ogun, Oyo, and Ekiti States (Figure 3.1).

3.2. Experimental materials

a) Screen cage: Rearing cage (50 × 60 × 80) cm covered with aphid-proof polyester net for mating and oviposition (Plate 3.1); Petri dishes; Plastic vials (9 cm diameter by 4 cm high and 15 cm diameter by 5 cm high dimension) for larvae culture, and Kliner jars for rearing pupae to adult (Plate 3.2)
b) Experimental units and Instrument: Petri dishes, handheld lens, Olympus compound microscope, and Digital Microscope (CMOS Sensor, High Performance USB 2.0 UVC compliant controller, SNAP, and Digital Zoom) and Olympus Microscope
c) Source of tomato seed: Twenty (20) tomato accessions were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan.

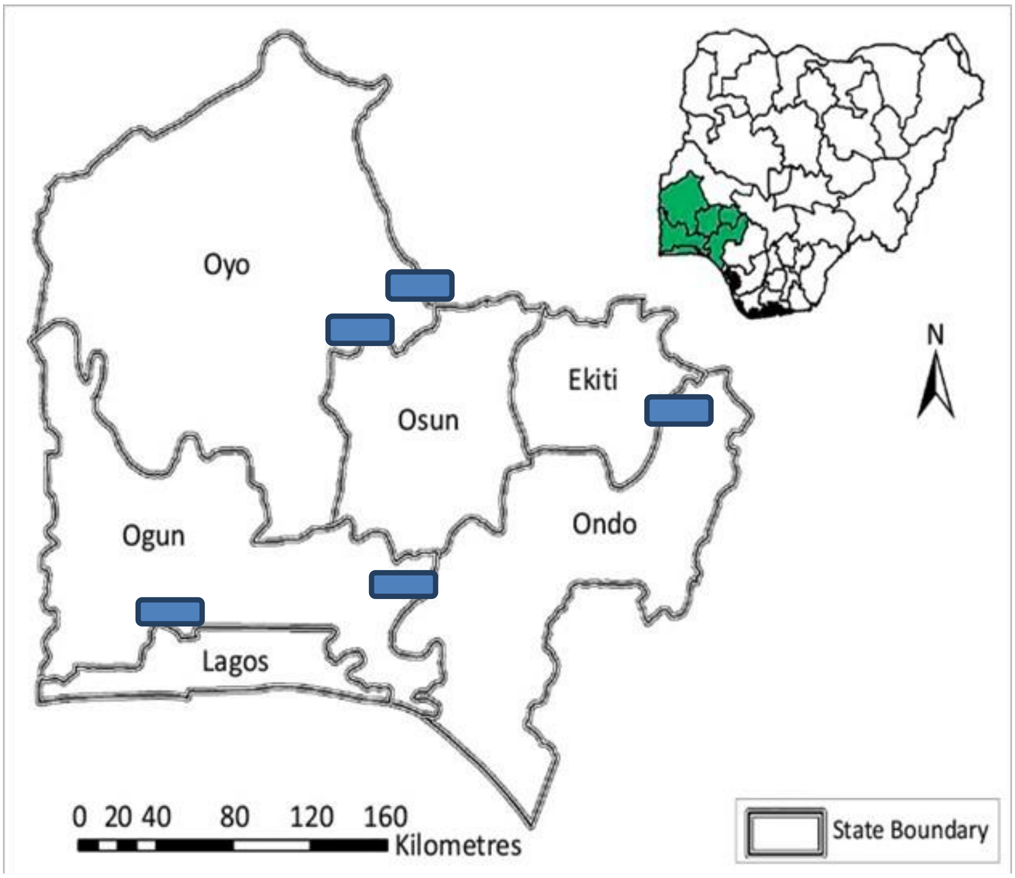

Figure 3.1: States surveyed for *Tuta absoluta* infestation

Legend:

: zone surveyed

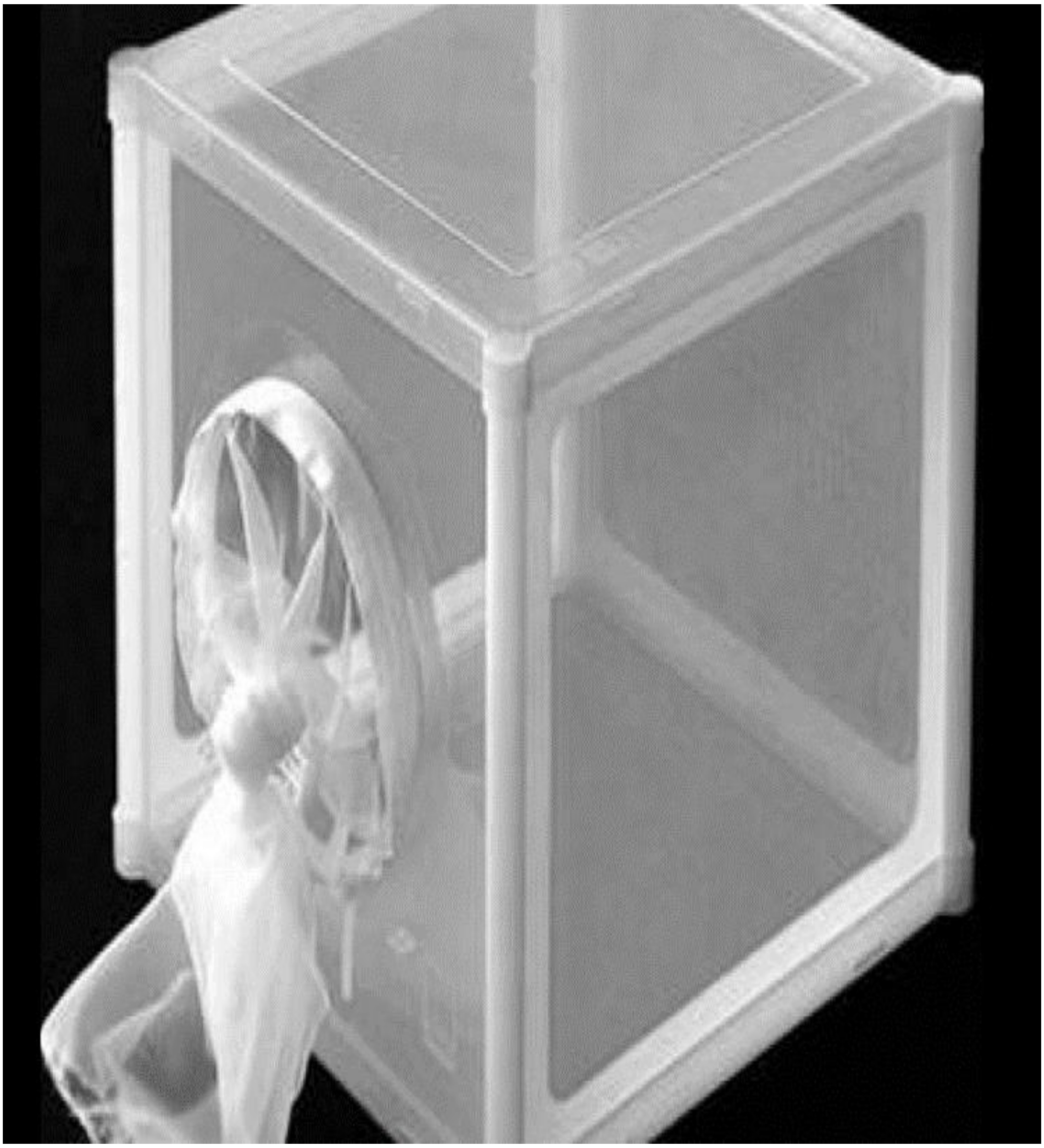

Plate 3.1: A sleeved cage for rearing adult *Tuta absoluta*

Plate 3.2: Plastic cage and Kliner jar used for larvae and pupae rearing in the laboratory

d) DNA extraction kits, reagents such as Protease K (20 mg/ml), reaction buffer (0.5 M EDTA, 5 M NaCl, 1 M Tris and mercaptoethanol)

3.3. Rearing cage and laboratory

A screen house for rearing was constructed at the glasshouse of Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. The environmental condition was ambient temperature of 29.2 °C ± 3 °C, relative humidity of 83% ± 6%. Adapting Zappalà *et al.*, (2012) protocol, improvised plastic cages were also used; dimension was 50 × 60 × 80 cm and covered with aphid-proof polyester mesh.

3.4. Quantitative survey of tomato farmers' Knowledge, Perception, and Management Preferences (KPMP) against infestation and damage of tomato leaf miners in selected states in Southwest, Nigeria

Using adapted protocol from Olaoye *et al.* (2011), a quantitative survey was carried out using a multistage method in Southwest, Nigeria (Figure 3.2). In Ogun state, there are four ADP zones (Abeokuta, Ilaro, Ijebu-ode and Ikenne), Ijebu-ode and Ilaro were purposively selected. In Ijebu-ode, two (2) block was purposively selected out of the existing six blocks. Five (5) cells were randomly selected out of the total of thirty-five cells to make a total of thirty (20) respondents out of 90 farmers.

Whereas in Ilaro, there are four (4) blocks, two (2) were purposively selected. There are thirty (30) cells, out of which four (4) randomly selected. Forty (40) farmers were randomly selected out 103 farmers in the cells. Ekiti has two (2) zones, each zone has eight (8) blocks, and each block has eight (8) cells (Omonijo *et al.*, 2014). One zone was purposively selected, three (3) blocks were selected from the eight (8) blocks, four (4) cells were selected from each of the three (3) blocks.

Then five farmers were randomly selected from each cell, a total of 60 farmers. In Oyo state, there are three zones (North, Central and South) with total of thirty (30) blocks (Daud *et al.*, 2018). Two (2) zones were selected, three blocks were purposively selected and 60 (farmers) were randomly selected from four (4) cells. Open and closed semi-structured questionnaire were administered to 180 tomato farmers (persons who plant tomato at subsistence or commercial level (Appendix) to obtain information about their Knowledge, Perception, and Management Preference (KPMP) on *Tuta absoluta*.

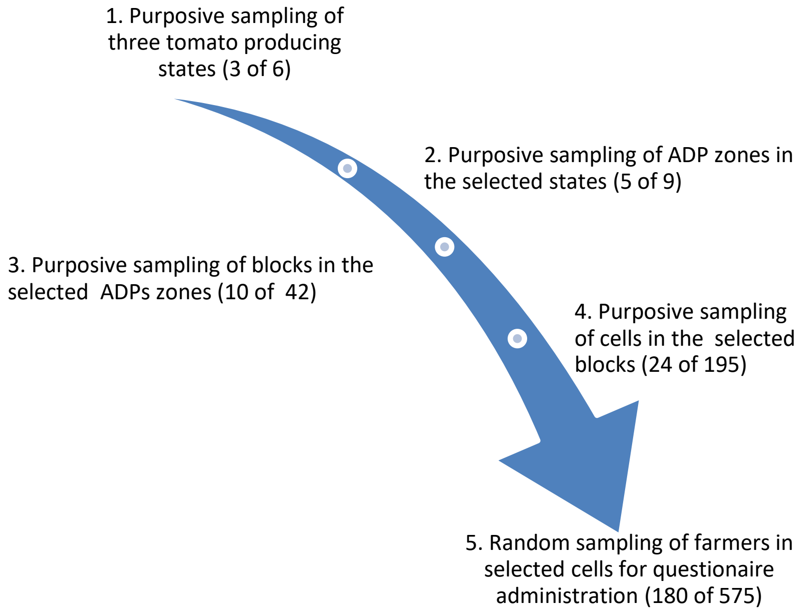

Figure 3.2: Five-stage procedure for survey of farmers' Knowledge, Perception, and Practice (KPP) against infestation and damage of *Tuta absoluta*

The instrument was tested and validated at Agricultural Extension Department of University of Ibadan, Oyo State

Data were analysed using descriptive statistics, while the means were separated by Tukey's test for pairwise comparison at $p = 0.05$.

3.5. Field evaluation of tomato accessions for occurrence and abundance of *Tuta absoluta* on twenty tomato accessions in Ibadan, Nigeria

This experiment was carried out at National Horticultural Research Institute (NIHORT)'s vegetable field, Ibadan. Twenty accessions were used for this experiment. The nursery was set up at the greenhouse in NIHORT, and the plants were transplanted to the field after three (3) weeks. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replicates. The field size was 55 m by 12 m, divided into four blocks of 5.5 m by 9.5 m. Each block was divided into 20 plots of 1 m by 1.5 m. The distance between each block was 1 m; while the edges were 0.5 m; each plot was made up of three rolls with 0.5 m by 0.5 m spacing. There were twenty plots per block, total of eighty (80) plots. Each plot had nine tomato plants making seven hundred and twenty tomato plants planted. There was no use of chemical pesticide or herbicides during the research. Agronomic activities such as weeding was manually done using hoe.

Three plants per plot were randomly selected for monitoring for infestation, sixty (60) plants per block making total of two hundred and forty (240) plants. The sampling started from the 14^{th} day-after-transplanting (DAT) till last day of harvest during the raining season. Each stand was observed from the leaf apex to the stem for the presence of *Tuta absoluta* eggs, larva, and adults. Thus, the field was assessed for the presence, abundance, and diversity of *Tuta absoluta* with *Solanum lycopersicum* during raining season in April through June 2019, and 2020.

3.5.1. Field survey of abundance and diversity of insect associated with *Solanum lycopersicum* in Ibadan, Nigeria

Identification of insects associated with *Solanum lycopersicum* in Ibadan, Southwest Nigeria was conducted on the tomato planted at the vegetable plot, NIHORT, Ibadan. In this study, three methods of trapping insects were employed namely, hand capture for wingless insects, handheld sweep net for flying insects, and light traps for nocturnal insects. The first set of field trials were conducted to assess the abundance and diversity of insects associated with *Solanum lycopersicum* during raining season in April through June 2019, followed by dry season planting in the screen house in August through November 2019.

Data obtained were pooled together for descriptive analysis.

3.5.2. Field establishment for the cultivation of tomato plants in Ibadan, Nigeria

The field experiment was established at the vegetable plot of NIHORT, Ibadan, was laid out in a Randomised Complete Block Design (RCBD) with four replications. The field size was 55 m by 12 m, divided into four blocks of 5.5 m by 9.5 m. Each block was divided into 20 plots of 1 m by 1.5 m. The distance between each block was 1 m; while the edges were 0.5 m; each plot was made up of two rolls with 0.5 m by 0.5 m spacing (Table 3.1). The schematic drawing of the layout is presented in Table 3.2.

Thorough land preparation was conducted before the trial was set up. Thereafter, the experimental plots were raised into beds to avoid flooding. Furrows were maintained to boost quick drainage of excess rainfall water and early weed control was done to improve weed control, no chemicals were used on the field, and after sowing weeding were manually done when needed. Twenty accessions of tomato sourced from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State were used for these experiments. All accessions were from *Solanum lycopersicum* and have indeterminate growth habit.

3.5.3. Sampling for the occurrence of *Tuta absoluta* in selected states in Southwest, Nigeria

The study aimed at assessing the occurrence of *Tuta absoluta* in selected states of Southwest, Nigeria to determine its pest status on tomato on the field in Southwest, Nigeria. A field survey was conducted in selected states in Southwest, Nigeria based on their production status (Table 3.3). These states (Oyo, Ogun and Ekiti) are high tomato producing states in the Southwest according to information obtained from Agricultural Development Programme, Zonal office Ibadan, Nigeria. Samplings were carried out in forty-five farms in the selected states visited for surveys. Forty tomato plants were randomly sampled in each farm visited for the occurrence of *Tuta absoluta*

Table 3.1: Layout metrics

Parameters	Measurements
Experimental area	55 m by 12 m
Experimental Block Dimension	5.5 m by 9.5 m
Experimental Plot Dimension	1 m by 1.5 m
Alley	1m
Number of replicates	4
Plots	
Number of rows	3
Row length	1.0 m
Row width	0.5 m
Inter row spacing	0.5 m
Inter plant spacing	0.5 m

Table 3.2: Experimental layout of *Solanum lycopersicum* on the field

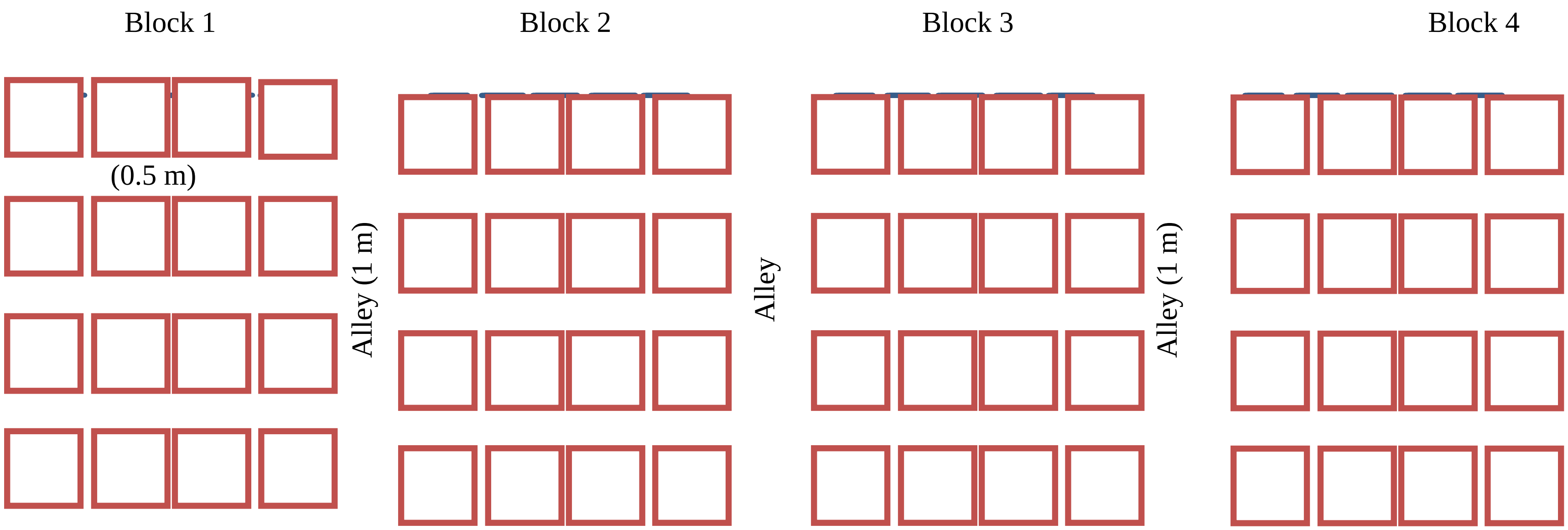

Table 3.3: Geographical locations of the surveyed areas for *Tuta absoluta* in Southwest, Nigeria

States	Local Government Areas (LGA)	GPS Coordinate	
		Latitude	Longitude
	Ogbomosho	8°08'31.79"N	4°14'42.66" E
Oyo	Oriire	8°04'23.52" N	4°14'11.69" E
	Ogo-Oluwa	7°59'24.47" N	4°12'53.39" E
Ogun	Yewa North	7°13'60.00" N	3°01'60.00" E
	Yewa South	6°53'20.44" N	3°00'50.98" E
	Ijebu-East	6°49'09.98" N	3° 55'02.32" E
Ekiti	Ikeere-Ekiti	7°29'50.93" N	5°13'49.48" E
	Ado-Ekiti	7°37'23.84" N	5°13'15.13" E
	Gbonyin	7°35'54" N	5°36'27" E

eggs, larvae, or mines. Leaflets and stems of sampled plants were inspected using a handheld lens (Magnification 50)

3.6. Molecular identification of *Tuta absoluta* in Southwest, Nigeria

Samples of *T. absoluta* (n=10) collected from a commercial screen house in Lagos State were examined at molecular level to assess the identity and variations in *T. absoluta* populations in Southwest, Nigeria at the Genetic Laboratory of NIHORT, Ibadan.

3.6.1. Total genomic DNA isolation and polymerase chain reaction (PCR) of *Tuta absoluta*

A total of ten individuals of newly emerged (24 – 30 hours) adult insect samples (male: 5 and female: 5) were collected from the culture in sterile glass vials and kept in 70% ethanol for preservation till the DNAs were isolated. The total DNA was isolated from a single insect using a method described by Dellaporta *et al.* (1983).

3.6.2. DNA extraction protocol

This extraction was carried out at the Genetic laboratory of NIHORT, Ibadan. The reagents for the DNA extraction were Proteinase K (20 mg/ml), used at 5 µl with a reaction buffer containing 200 µl of 5M extraction buffer (0.5 M EDTA, 5 M NaCl, 1 M Tris and mercaptoethanol). The Proteinase K was stored at -20 °C. Other reagents were cold isopropanol, 10 µl of sodium dodecyl sulphate (SDS), equilibrated phenol (pH 8.0), Chloroform, 70% ethanol, 1% agarose gel, ethidium bromide, and sterile distilled water. A single *Tuta absoluta* adult sample was transferred into a 1.5 ml extraction tube, then 200 µl of 5M extraction buffer (0.5 M EDTA, 5 M NaCl, 1 M Tris and mercaptoethanol) was used to grind sample thoroughly. Proteinase K (5 µl) and 10 µl of sodium dodecyl sulphate (SDS) were added thereafter. Sample was incubated in water bath at 65 °C for 30 minutes, and then 10 µl of potassium acetate was added. The sample was spun for 10 minutes at 10,000 rpm. Supernatant was decanted into new extraction tube. Cold isopropanol (300 µl) was added, and the sample was kept at 4 °C overnight. Thereafter, the sample was spun, and the supernatant decanted away without disturbing the pellets formed. The pellet was purified with 70% ethanol, before it was re-suspended with 50% sterile distilled water and the stock kept at 4 °C for 24 hours prior the amplification.

3.6.3. Quantification of the extracted DNA of *Tuta absoluta*

The quality of the extracted DNA isolated was checked with 1.2% agarose gel electrophoresis using Tris Boric acid EDTA (10 mM tris to 1 nM of EDTA) buffers at 100 V for 1-hour in 1% agarose gel pre-stained with 10 mg/ml ethidium bromide, distained in distilled water, and viewed under ultra-violet (UV)/White Light Trans-illuminator (Spectronile-Vision). Consequently, 2 µl of the extracted DNA samples of *T. absoluta* were loaded into a NanoDrop® Spectrophotometer, ND-1000 from Thermo Scientific Company, USA. The quality of the DNA was determined by quantifying the concentration in nanogram per microlitre at 260 and 280 absorbance levels. The ratio of absorbance 260:280 was calculated to determine the yield quality of the DNA from sample. The sample was stored at -20 °C for further use.

3.6.4 Dilution of DNA sample and the primers

Primers, synthesized by Inqaba Biotec, South Africa, were used for this experiment. The primers were diluted by multiplying the nano-mole value on the tube by ten and the values obtained were the volume of water added to the lyophilized content in the tube to give a total concentration of 100 µmol. From the stock tube, a working dilution was prepared using the formula below:

nmol X 10 = x of Tris-ethylenediaminetetraacetic acid (TE)

20 µLmol = 20 µL of primers: 80 µL of TE

3.6.5. Preparation of buffers for amplification and gel electrophoresis

Tris-boric-ethylenediaminetetraacetic acid (TBE) buffer was prepared by adding 10mM of Tris to 1mM of EDTA, Gel loading buffer was prepared by adding 0.25% bromophenol blue to 0.25% Xylene cyanol and 40% sucrose with water. The agarose gel used was prepared by weighing 1.2 g of agarose powder into 100 ml of TBE. The mixture was then melted in a microwave and allowed to cool off to about 40 - 45 °C and poured into a prepared gel tank tray with combs. The fragments were separated by running at 100 V for 1 hour and then viewed on an Ultraviolet light source in the dark.

3.6.6. Polymerase Chain Reaction (PCR) Amplification of the extracted DNA

The PCR was performed in a 12.5 µL reaction mixture (DNTp: 0.25 µL of 10mM; $MgCl_2$:1 µL of 25mM; 10X Buffer: 1.25 µL of 10X; Primers 0.5 µL of 20µM and Taq) using Inqaba Biotech® Taq DNA Polymaerase (recombinant). The isolated genomic DNA was used as template in PCR to amplify the COI gene using barcoding primers Lep F1:5'ATTCAACCAATCATAAAGATATTGG3' and LepR1 5' TAAACTTCTGGATGTCCAAAAATCA 3'. The PCR amplification was done under thermo-cycling condition with first denaturation at 94 ºC for 5 minutes, followed by 35 cycles consisting of 94 ºC for 30 seconds, 55 ºC for 1 minute, 72 ºC for 1.5 minutes and 72 ºC for 7 minutes was used for amplification. The PCR amplified products were subjected to electrophoresis in a 1.2% agarose gel. The agarose gel used for the electrophoresis was prepared by weighing 1.2 g of agarose powder into 100 ml of TBE, the mixture was melted in a microwave, and it was cooled to 40 - 45 ºC. The amplified product (amplicons) was purified and sequenced in a forward and reverse direction, using the PCR primers. Ten (10) µL of the amplicons was run on a 1.2% agarose gel to determine the presence or absence, and the size of the amplified DNA. The marker (100bp) used was supplied by Promega and the band size of the PCR products were between 700 bp and 750 bp. The fragments were separated by running at 100 V for 1 hour and viewed on a UV light source in the dark. The gel pictures of the PCR product were taken using Polaroid Digital Camera (6.5 megapixels).

3.6.7. Analyses of PCR of *Tuta absoluta* sample

According to Kambhampati and Smith (1995), the mitochondrial cytochrome oxidase subunit I (COI) gene has good genetic resolution that makes it suitable for PCR analysis. Sequences generated from this study was analysed using the software BIOEDIT version 7.0 program (Hall, 1999). The sequence was manually inspected for nucleotide scoring and translated into protein sequence with the aim of detecting sequencing errors. The forward sequences were then aligned to join with the reverse complements of the reverse sequences to assess fidelity of the sequences. A database search with other insect pest sequences was carried out by NCBI-BLAST program (http://blast.ncbi.nlm.nih.gov). Nucleotide (nt) sequence alignments was performed using MUSCLE program using Mega version 7 software (Tamura *et al.*, 2013). The phylogenetic tree was constructed using nucleotide sequences generated from this study and other selected *Tuta absoluta* species reported. The tree was constructed with distance/neighbour joining method with 1000 bootstrap replications.

3.7. Biology of *T. absoluta* in the laboratory

The biology of *Tuta absoluta* was studied viz-a-viz its reproductive, developmental, and behavioural biology.

3.7.1. Stock culture and mass rearing of *T. absoluta*

To raise the stock culture, a two-phase method by Omoloye (2006) was followed with modification. In the first phase larvae samples of *T. absoluta* were obtained from the field and transferred into rearing cage, a plastic cage (60 × 60 × 80) cm, at ambient conditions at 27 °C ± 3ºC temperature and 75 ± 3% relative humidity (rh) and a 16:8 h L: D regime. These were maintained on tomato plants (sweet tomato). The tomato leaves were obtained from a tomato farm at the teaching and research farm, University of Ibadan operated by an independent organic tomato farmer.

In the second phase, mated females from F1 progeny were introduced to the tomato plant under natural conditions in a screen house. The F2 emergent of the instars were carefully transferred to rearing cages and fed with fresh tomato leaves. This procedure was repeated to maintain a continuous culture for further experiments. It provided age-specific cohorts for the various experiments such as biology, resistance studies, and other bioassays.

3.8. Reproductive biology of *T. absoluta*

A batch of twenty newly emerged *T. absoluta* were placed in oviposition cages in the laboratory, the life cycle parameters, and stages of *T. absoluta* were investigated in this experiment at ambient temperature of 27 ± 3 °C and 70 ± 5% rh on the sweet tomato variety. This method was adapted from Oyedokun (2012). This experiment was to assess the oviposition, fecundity and lifespan of mated male and female adults. Pairs were observed for mating early in the morning (0500 – 0800 hours) and in the evening (1800 – 2000 hours). Pairs were separated after mating and observed for some biological parameters. Thereafter, mated males were cross mated with virgin females, and mated females. Furthermore, virgin males were cross mated with mated females. At the same, another experiment for pre-oviposition period, post-oviposition and oviposition of adult female was set up separately. Other parameters observed in the experiment were incubation period of laid eggs, percentage eclosion, as well as longevity of mated and virgin adults at different treatments (water, 5 % sugar and 10 % sugar). The protocols were adapted from Oyedokun (2012). The life table was also constructed from the data obtained using protocols described by Asamo (2016). The experiments were replicated five times in a Completely Randomized Design (CRD). Data collected the following:

Pre-oviposition period: Period between emergence and laying of first eggs

Oviposition period: The number of eggs laid per female (fecundity).

Post-oviposition period: Period after laying of eggs

Life span (longevity): virgin male x virgin female, mated male x mated female, virgin male x mated female, and mated male x virgin female

Percentage adult emergence

Life table: a life table was constructed following described protocols by Asamo (2016).

- Fecundity rate/egg to adult female birth (mx): the number of eggs laid per female was divided by 2.14 (sex ratio of 1:1.14)

- x is the age in days

- lx is the number of females surviving at the beginning of age class x, expressed as a fraction of an initial population of 1 (i.e., 100% survival).

- The net reproductive rate (R0) that is the number of times a population will multiply per generation, lxmx.

- The cohort generation time Tc is the mean age of mothers in a cohort at the birth of female offspring and is defined by

$\frac{xl_xm_x}{l_xm_x}$ or $\frac{xl_xm_x}{R_0}$.

- The innate capacity for increase (rc): the number of times a population multiplies itself per unit time and is defined as

$$\frac{\log_e R_0}{T_c}$$

- The finite rate of increase (hc): a measure of the number of times the population multiplies per day, given by erc.

- Gross reproduction rate (GRR) = a measure of the average number of females that would survive to adult, denoted by ∑mx

3.8.1. Pre-oviposition behaviour of *Tuta absoluta*

Males of the same age were introduced to females of same age in oviposition cage. Mating positions, length of copulation, and frequency of mating were observed, and documented.

3.8.2. Oviposition behaviour of *Tuta absoluta*

This is to investigate whether *Tuta absoluta* is monogamous (lays its eggs once in a lifetime after mating) or polygamous (lays egg several times after multiple mating by two or more males). The following observations were documented:

- Parthenogenesis of virgin females (n = 10)
- Fecundity of virgin males sexed with virgin females (n = 20)
- Fecundity of virgin males sexed with mated females (n = 10)
- Fecundity of mated males sexed with mated females (n = 10)
- Fecundity of mated males sexed with virgin females (n =10)
- Fecundity of mating multiple males with single female at ratio 2:1 (n = 10)
- Interaction of ovipositing females to introduced virgin males at 5th day of oviposition (n = 10)
- Fecundity of females fed on 5% sugar solution.
- Fecundity of females fed on 10% sugar solution.
- Fecundity of females fed on water only.

The eggs laid by each group on the tomato leaves were counted and recorded daily. The percentage eclosion of eggs was calculated

$$\%\, eclosion = \frac{number\ of\ emergents}{number\ of\ eggs\ laid} x\ 100$$

3.8.3. Post-oviposition behaviour

The behaviour of both sexes after mating and laying of eggs until mortality occurred was observed, described, and documented.

3.9. Developmental biology of *T absoluta* on tomato leaves

Ten pairs (male and female) of newly emerged adults of *T. absoluta* (24 hours) collected from the stock were paired for mating and oviposition for 48 hours. Subsequently, the males were removed and kept in different cages to assess their longevity. Fresh leaves of sweet tomato variety were daily replaced for oviposition; eggs laid daily were counted and recorded.

Ten freshly laid (24- 30 hours) eggs were carefully measured with an ocular micrometre screw eyepiece fitted into a microscope (Olympus Microscope) to assess the morphometrics. Ten eclosed larvae from eggs were monitored and their morphometrics was measured after being immobilised; this was replicated four times.
Growth ratio: the mean width of vertex (n=20) across the eyes of each larval instar divided by the mean width of vertex of the previous instar.

Following Samad *et al.* (2020) protocol, the progression of growth for *T. absoluta* was determined by taking the mean width of the head capsule along with the accumulated days of development of the first instars to adult moths. Emergent adults were then sexed, and pairs maintained in different cages.

The following parameters were observed, and data collected from the experiments:

i. Total number of eggs: the number of eggs laid by the adults per cage was counted daily using a hand lens.
ii. Incubation period of the eggs: the number of days from incubation of the eggs to the first larval instar emergence was recorded.
iii. Larval morphometrics (body length, abdominal width, and the head capsule width of the vertex across the eyes) were taken with the aid of micrometre eyepiece fitted into a binocular microscope.
iv. Developmental period of the instars: The developmental period was recorded as the total time taken between and within instars until adult emergence.
v. Number of instars was documented as the number of moults within the larval developmental period.
vi. Number of days to adult emergence: the mean number of days from the eggs laid to adult emergence.
vii. Total number of adult emergence from the eggs was also calculated.

3.9.1. Biology of *Tuta absoluta*

Eggs were collected from the rearing cages and placed in culture cages under ambient conditions of 27 ± 3 ºC temperature and 75 ± 3% relative humidity. Ten eggs from each cage were collected for morphometrics studies using the Olympus Microscope fitted with an ocular micrometre screw eyepiece. The experiment was carried out using Duarte *et al.* (2015) protocol.

Actual fecundity was assessed as the total number of eggs laid by a gravid female. At emergence twenty larvae were transferred into different plastic cages, these were provided with the leaves of sweet tomato variety for feeding. The leaves were changed when larvae have exited to mine into new leaves.

3.9.2. Assessment of the larval stages of *Tuta absoluta*

Thirty larvae kept in plastic dishes, fed with tomato leaves, were monitored to study their feeding habit, time interval between the larval stages (stadium), developmental process and behavioural patterns. This was replicated four times.

3.9.3. Assessment of the pupa stage of *Tuta absoluta*

Pupation was monitored for time duration and morphometrics description.

3.9.4. Assessment of the adult stage of *Tuta absoluta*

Thirty newly merged adults (male and female) were collected, sexed, and preserved in 70 % alcohol for the measurement of body length, body width, wingspan, and other morphometrics description.

3.9.5. Longevity assessment of adult *T. absoluta*

A batch of 20 newly emerged moths was monitored. The longevity of virgin males and females were then compared to those of mated males and females until death.

3.10. Evaluation of selected accessions of tomato for resistance to *T. absoluta*

The experiments were conducted at the screen house of Department of Crop Protection and Environmental Biology, University of Ibadan, and NIHORT vegetable field, Ibadan.

3.10.1. Screen house evaluation of selected accessions of tomato for resistance to *T. absoluta*

The aim of this screen house experiment was to identify potential tomato accessions resistant to *T. absoluta* that could be incorporated into breeding programme. Antixenosis, antibiosis and tolerance of tomato accessions to *T. absoluta* was investigated. The experiment was laid out in Completely Randomised Design (CRD) with three replications at the Rooftop Garden of the Department of Crop Protection and Environmental Biology. The screen house experiment was conducted using 10 kg pots filled with sterilized loamy soil, there were three replicates and infestation was through artificially conducted. Soil was obtained from the CPEB crop garden and sterilized at 200 – 300 ºC for 12 hours at CPEB, UI. No insecticide was used on any of these plots before and during the study. Data on oviposition, number of mines, and yield were taken. In addition, data on plant height, stem diameter, leaf area, number of branches, leaf stem ratio were taken too.

3.10.2. Laboratory evaluation of selected tomato accessions for antixenosis against *Tuta absoluta*

The antixenosis bioassay was studied under laboratory conditions to assess larvae feeding preference of tomato accessions (20). In a no-choice test, the adults (two days old) were placed in cages for oviposition, where they were fed on 5% sugar solution. The moths laid eggs on the leaves provided for 24 hours. Data on portions of leaves defoliated by larvae was then taken after 24, 48, and 72 hours after the 24 hours of oviposition, respectively. Damage was evaluated using mobile Easy Leaf Area application.

3.10.3. Evaluation of Tomato accessions for antibiosis to *Tuta absoluta*

The aim of this experiment was to assess the survival and development of *T. absoluta* on different tomato accessions (20) in the screen house. This was a no-choice bioassay. Two second instar larvae were collected from the culture and confined on mature green leaves within a cage covered with muslin cloth. The cages were securely tied to prevent insect escape; also, grease was applied at the sides of the cages to prevent ants from preying on the larvae. There were three replicates per accession. The morphometrics, developmental periods for each life-stages, and mortality were recorded.

3.10.4. Evaluation of selected Tomato plants accessions for tolerance to *Tuta absoluta.*

This experiment was to assess the ability of different tomato accessions (20) to withstand or recover from *T. absoluta* damage. Fifty adult pairs (25 males and 25 females) were released to oviposit on thirty potted tomato plants, there were two plants per pot, the response of the tomato plants in terms of plant regeneration, and fruiting were then observed and documented.

3.11. Identification of natural enemies

Natural enemies that preyed on the different stages of *T. absoluta* in the screenhouse were observed and documented.

3.12. Data analysis

Information from the behavioural survey was analysed using descriptive statistics.
Data for each farm was analysed for occurrence, and abundance. The total of all the various replicated sites for each state was pooled together for analysis of abundance and distribution of the insects in the states, using descriptive statistics.

Data obtained for the larvae, pupae, adult morphometrics were analysed using descriptive statistics, Analysis of Variance (ANOVA), and significant means separated by using Tukey's Studentized Range Test. These were subjected to ANOVA and the means separated by Tukey's Studentized range test for pairwise comparison at $\alpha = 0.05$.
All statistical analysis was performed using SPSS 25^{th} edition.

CHAPTER FOUR

RESULTS

4.1. Demographics of farmers surveyed

The demographics information on sex, age, marital status, educational levels, and main occupation of the respondents are reported in Table 4.1. The modal class age ranged from 40 to 49 years old (36.2 %), and 20 – 29 years had the least proportion (4.6%) of respondents. The population of male farmers was more 79.1 %, while that of female was lower (20.9%). About 88.7% of the farmers were married, 7.3% were singles, 2.6% were widowed and 1.3% divorced. The educational status showed that majority (65.3%) of the farmers had secondary school leaving certificates. The percentage of farmers with tertiary education was 11.3%, those without formal education are 5.3%, and 18.0% stopped at primary school education. Majority of the farmers, (86.0%) depend solely on their farming enterprise, while the rest have other occupations such as employment at public and private enterprise (7.0%), and few were self-employed (6.3%) (Table 4.1). A large percentage (64.5%) of the respondents practiced mixed cropping; those who are planting tomato solely were 32.2%, while just 3.3% of the farmers practiced shifting cultivation. About 25.0% of the respondents have been farming for 11 – 15 years. While less than 15% have more than 20 years' experience of farming.

4.1.1 Farmers' knowledge on *Tuta absoluta* in Southwest Nigeria

Majority of the farmers (84.2%) could identify different insect pests of tomato, and 78.8% of the respondents claimed they could detect the leaf miner on the field before causing serious damage. About 58.2 % of the respondents can identify the pest based on its damage characteristics. However, majority (89.5%) did not know the name of the pest as *Tuta absoluta*. An average of 24.3% saw it as a field pest, while 75.3% did not see it as a field pest of tomato in Southwest. However, responses from 69.5% of the respondents showed that they knew severity of damage *T. absoluta* infestation can cause (Table 4.2).

Table 4.1: Demographic data of tomato farmers surveyed

Characteristics	Frequency	Percentage	Total respondents
Sex			
Male	124	79.5	**156**
Female	32	20.5	
Age			
20 – 29	**7**	**4.6**	**152**
30 – 39	20	13.2	
40 – 49	55	36.2	
50 – 59	51	33.6	
60 >	19	12.5	
Marital Status			
Single	135	88.7	**152**
Married	11	7.3	
Widowed	4	2.6	
Divorced	2	1.3	
Educational Status			**135**
Primary	24	18.0	
Secondary	88	65.3	
Tertiary	15	11	
No Formal Education	7	5.3	
Main occupation			**139**
Farming	119	86.0	
Public employee	8	5.7	
Private employee	2	1.4	
Self-employed	9	6.3	
others	1	0.7	
Cropping system			**144**
Sole	46	31.9	
Mixed	93	64.5	
Shifting	5	3.5	
Years of farming			**155**
1 – 5	26	16.8	
6 – 10	35	22.6	
11 – 15	39	25.2	
16 – 20	32	20.7	
20 >	23	14.9	

Table 4.2: Farmers' knowledge of *T. absoluta* in some selected states of Southwest, Nigeria

Variable: Knowledge of pest	Response (%)	
	No	**Yes**
Name of the pest	89.5	10.5
Identification (pictures and damage characteristics)	21.2	78.8
Damage characteristic of *T. absoluta*	41.8	58.2
Cause of infestation	71.3	28.7
Open field pest	75.3	24.3
Severity of damage	30.5	69.5

4.1.2. Farmers' perception of *T. absoluta* damage potential in Southwest, Nigeria

Majority of the respondents, when pooled together, (93.4%) perceived that *T. absoluta* infestation is a serious threat to tomato production in Southwest, Nigeria, and a few (3.9%) were undecided, while 2.6% did not perceive it as a threat (Figure 4.1). When asked if the presence of *T. absoluta* was a serious threat, more than 50% of the respondents believed that *T. absoluta* was not a major threat to tomato plant on the field. A few (5.2%) were undecided, while 42.3 % see it as a threat to tomato in the open field (Figure 4.2). Many (75.6%) believed that the absence of resistant accessions would make *T absoluta* infestation severe. About 10.3% were undecided, while 14.1 % disagreed (Figure 4.3). About 74.5% of the respondents believed that use of synthetic pesticides was effective in controlling infestation. While 13.1% were of contrary opinion, and 12.3% were on undecided about the use of chemical pesticides (Figure 4.4).

4.1.3. Farmers' management preference of *T. absoluta* in Southwest, Nigeria

The survey showed farmers' responses on different control measures that could be taken to control *T. absoluta* in case of infestation. About 62.5% recommended the use of chemical pesticide in controlling the pest. Some others (63.3%) suggested practising crop rotation would checkmate the pest invasion. A little bit below fifty percent of the respondents (48.0%) knew about Biopesticides as a means of control, while 52.0 % are oblivious of this control measure. From the table, 69.5% believed that legumes are ineffective intercrop for controlling the pest. The use of botanicals seems more acceptable with many of the farmers (70.3), while 29.7% believed that botanicals are ineffective in controlling the pest. A portion (52.3%) of the respondents believed that soil treatment cannot be used to control infestation. Fifty-two percent (52.0%) of the respondents believed that early planting will not reduce infestation, and 69.6% agreed that uprooting and burning of infested tomato plants will control infestation. Responses on the use of biological control, biopesticides, resistant variety and use of soil treatment were less than 100 respondents (Table 4.3). On the effectiveness of control methods recommended, many of the respondents (62.5%) believed that chemical pesticides would be efficient in controlling the pest, while 52.0% are of the opinion that Biopesticides might be an efficient control measures. On use of resistance accessions, a small percentage (34.4%) believed that resistant accessions would not be efficient while 65.6% are of the contrary belief. On the efficacy of botanicals, 66.7% of the respondents are positive that they are efficient in controlling leaf miners (Table 4.3).

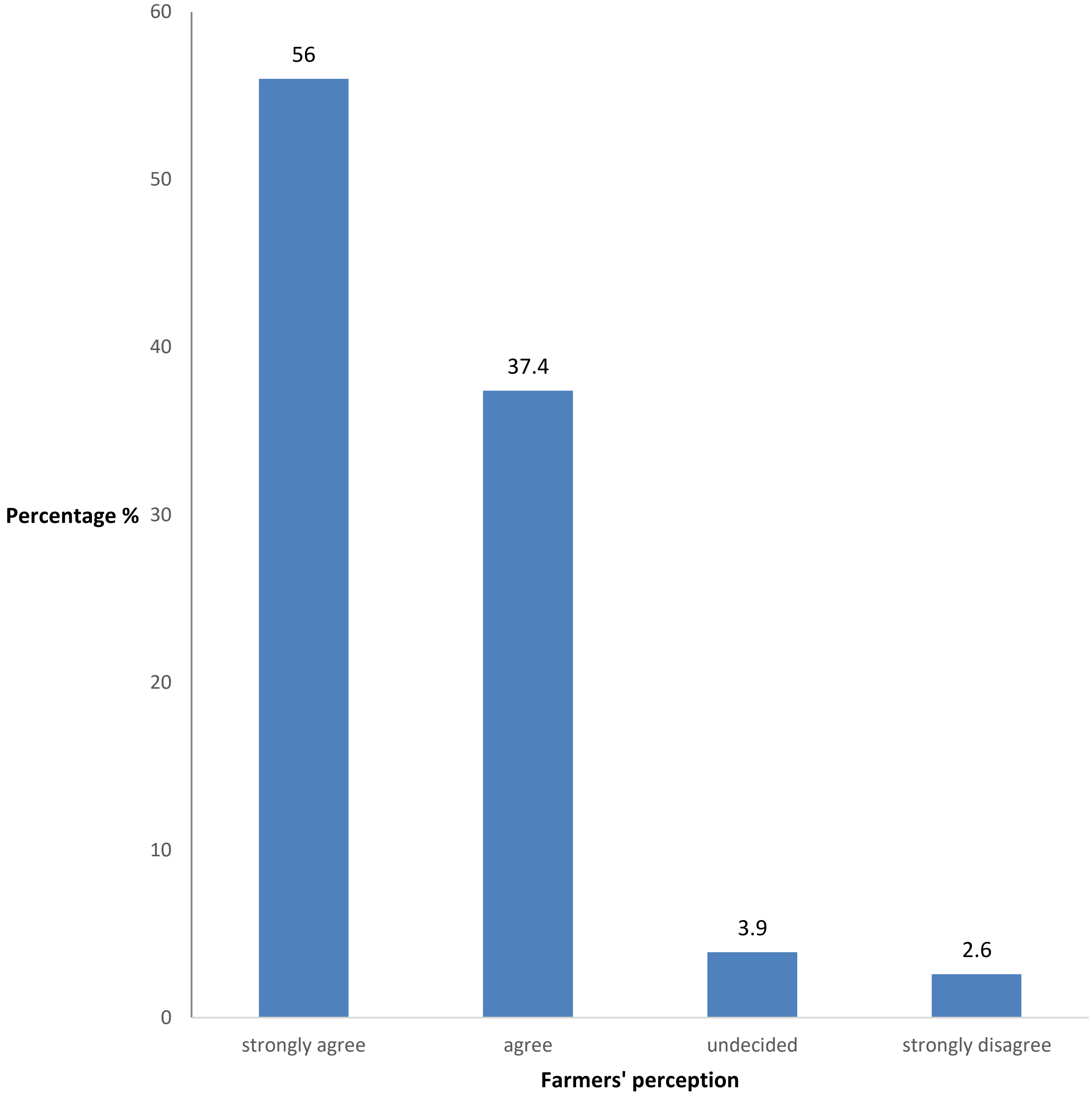

Figure 4.1: Farmers' perception of *T. absoluta* damage potential to tomato plant

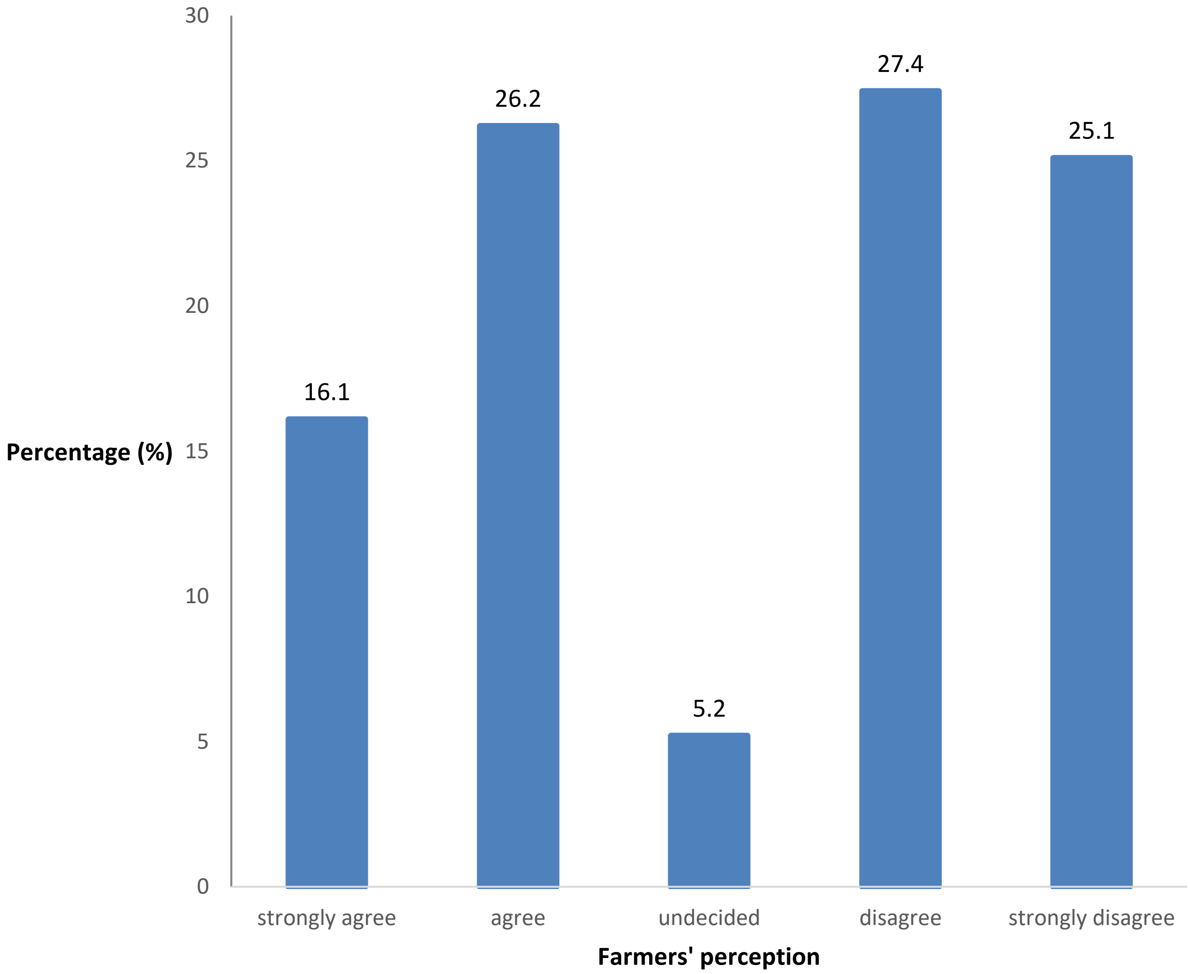

Figure 4.2: Farmers' perception of *T. absoluta* as potential serious threat to tomato plants.

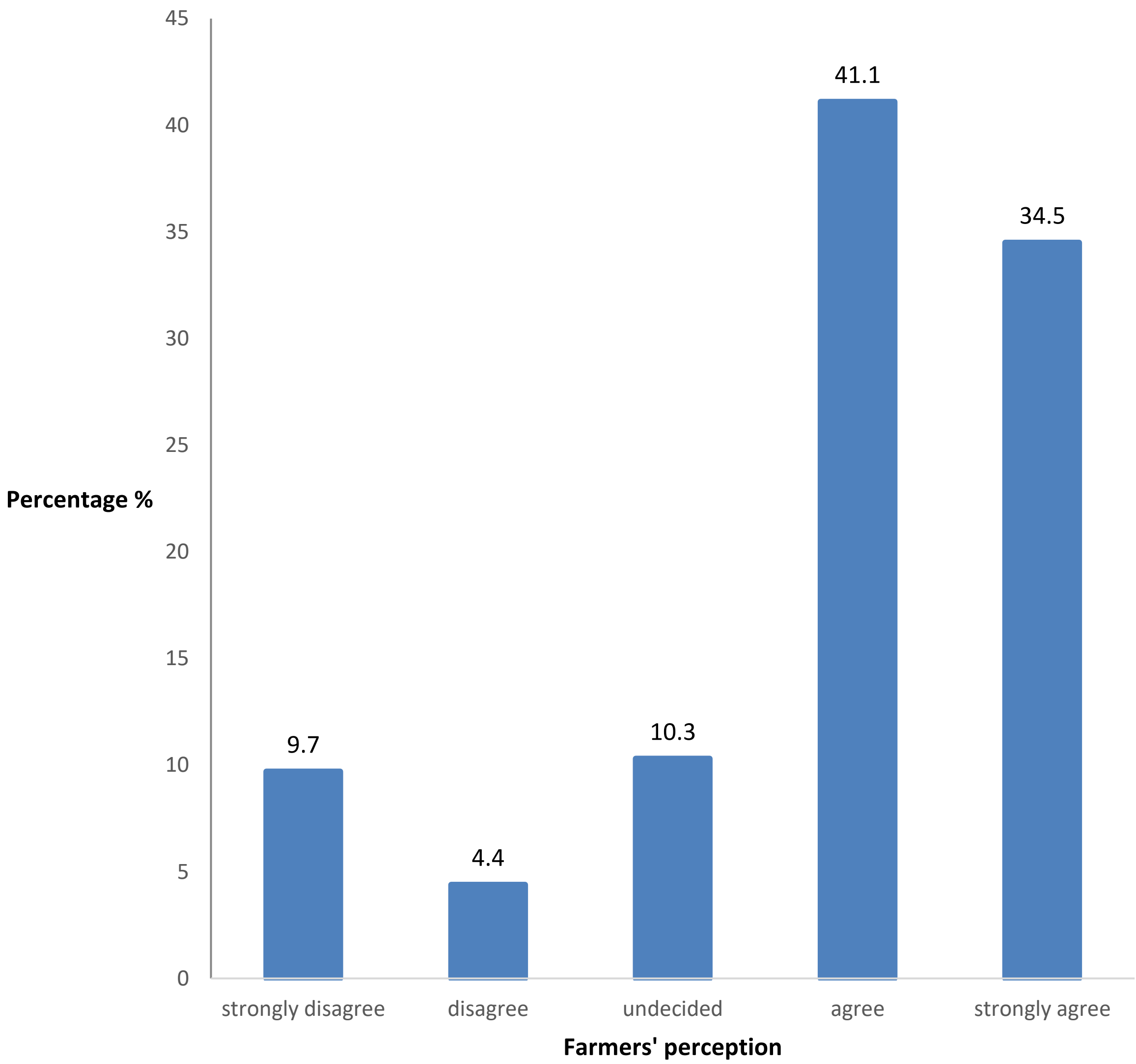

Figure 4.3: Farmers' perception of severity of damage due to lack of resistant accessions

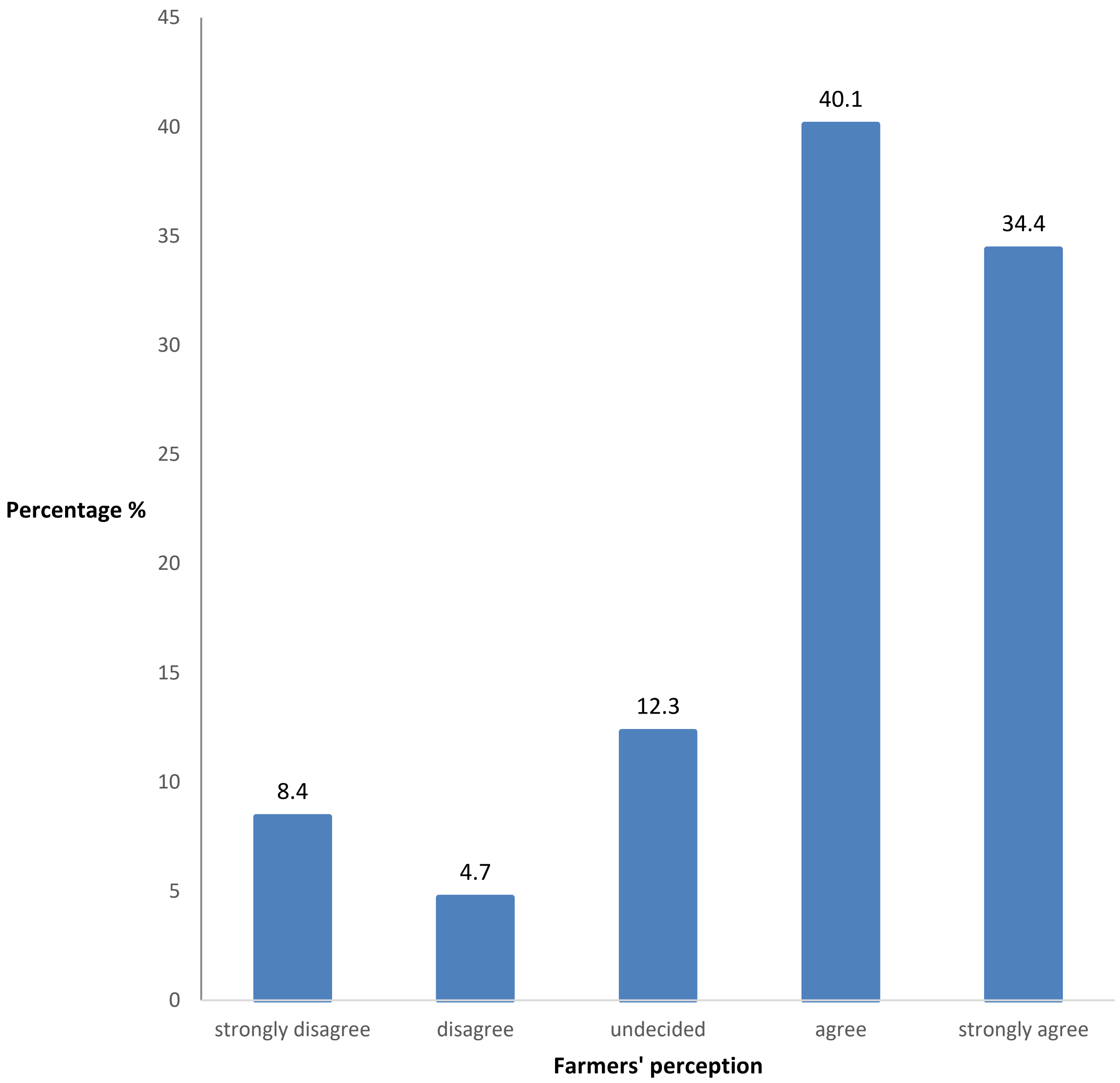

Figure 4.4: Farmers' perception on the use of synthetic pesticide to manage *T. absoluta.*

Table 4.3: Farmers' knowledge on different measures to control *Tuta absoluta*

Variable	Response (%) Yes	No	Total respondents
Management preference			
Chemical control	87.7	12.3	159
Crop rotation	63.5	36.5	148
Biological control	68.9	31.1	120
Biopesticides	48.0	52.0	94
Resistance variety	65.7	34.3	107
Physical methods	69.5	30.5	118
Botanicals	88.5	11.5	160
Soil treatment	52.3	47.7	115
Early planting	48.0	52.0	108
Intercropping with legumes	30.5	69.5	112
Effectiveness of control methods			
Chemical pesticides	62.5	37.5	130
Biopesticides	52.0	48.0	82
Resistant variety	65.6	34.4	107
Botanicals	66.7	33.3	129
Availability of control measures			
Chemical pesticides	87.9	12.1	132
Biopesticides	37.2	62.8	112
Botanicals	88.4	11.6	129
Resistant variety	28.5	71.5	100

Many of the farmers (62.8%) said they could not use Biopesticides, because they are not readily available for purchase. The respondents (71.5%) also complained that resistant accessions not readily available for cultivation. Furthermore, most of the respondents (88.4%) attested to the readily availability of botanicals for controlling insect pests. In addition, 87.7% respondents agreed that chemical pesticides are readily available for purchase (Table 4.3).

4.1.4. Relationship between level of education and farmers' responses

The level of education of the farmers was correlated with their responses on each section of the questionnaire at 95% confidence interval and means separated by Tukey HSD.

4.1.4.1 Farmers' general responses: Farmers levels of education had impact on their responses to questions asked. The correlation matrix between education level and response to different questions showed that there was a significant difference between farmers with primary and farmers without formal education. Also, there was a significant difference in responses of those with secondary education compared with those without education. There was no significant difference between farmers without formal education and those who had education up to tertiary level (Table 4.4).

4.1.4.2. Enterprise Characteristics: There was no significant difference on the level of education of farmers with their responses to questions relating to enterprise characteristics, except for those with tertiary education and primary education (Table 4.4).

4.1.4.3. Knowledge of the pest: The level of education did not reflect in the knowledge of the pest by the farmers. There was no significant difference in their knowledge of the pest based on their level of education (Table 4.4).

4.1.5. Relationship between years of farming experience and farmers' responses

A correlation between the years of farming experience was correlated with farmers' responses. The analysis showed that there was significant difference in means between years of farming experience among the farmers (Table 4.5). There was significant difference in response from those with twenty (20) years and above when compared to those with fifteen (15) years below. However, there was no significant difference between those with 20^{+} years and those with 15 to 20 years farming experience.

Table 4.4 Influence of education on farmers general responses

Variable	Highest Education	No Formal Education (NFE)	Primary	Secondary	Tertiary
Farmers general responses	NFE	--	--	--	--
	Pry Sch.	2.07407*	--	--	--
	Sec. Sch.	1.83673*	-0.23734	--	--
	Tertiary	1.70588	-0.36819	-0.13085	--
Enterprise Characteristics	NFE	--	--	--	--
	Pry Sch.	0.53704	--	--	--
	Sec. Sch.	0.48980	-0.04724	-	--
	Tertiary	0.55882	0.02179*	0.06903	--
Farmers' knowledge	NFE	--	--	--	--
	Primary	0.48712	--	--	--
	Secondary	0.67891	0.19178	--	--
	Tertiary	0.29997	-0.18716	-0.37894	--

* The mean difference is significant at 0.05 level of significance (Tukey HSD)

Table 4.5: Correlation between years of farming experience and responses of farmers

Variable	Years of farming	A	B	C	D	E
Enterprise characteristics	A	--				
	B	0.09534	--			
	C	0.01541*	-0.07993	--		
	D	-0.60126*	-0.69660*	-0.61667	--	
	E	-0.88261*	-0.97796*	-0.89802*	-0.28136	--

*The mean difference is significant at the 0.05 level (LSD)

Where A = 1-5 years, B = 6 -10 years; C = 11 -15 years; D = 16 – 20; E = more than 20 years

4.2. Occurrence and abundance of *Tuta absoluta* on tomato plant in Ibadan, Nigeria

There was no *T. absoluta* infestation observed on tomato planted in the open field in Ibadan throughout the period of the experiment. Weekly examination for *Tuta absoluta* infestation yielded no positive results. With the absence of infestation, it was difficult to evaluate resistance to infestation on the open field.

4.3. Yield of tomato accessions from the field trial in Ibadan, Nigeria

Table 4.6 shows the data on yield of tomato planted in the open field. NGB00718 had the highest weight/fruit at 23.7 g/fruit and 26.1 tonnes/ha, followed by NGB00716 at 20.5 g/fruit but total harvest per was 4.9 tonnes/ha. The weight of fruit of other accessions in the range of 15 g - 20 g/fruit are NGB00711 (18 g/fruit), NGB00717 (16.8 g/fruit), NGB00724 (16.4 g/fruit), NGB00715 (15.4 g/fruit), while NGB00737 and NGB00749 had (15 g/fruit) each. Accessions with fruit weight below 15 g/fruit but above 10 g/fruit includes NGB00696 (12.6 g/fruit), NGB00714 (10 g/fruit), and NGB00720 (11 g/fruit). The smallest weight was on accessions NGB00725 and NGB00741 at 2 g/fruit. The number of fruits per truss varies across the accessions, accession NGB00726 had the highest fruits per truss at 16 fruits per truss, and the next was NGB00725 at 15 fruits per truss. Accessions NGB00711 and NGB00741 had 11 fruits per truss, while accessions NGB00714 and NGB00718, and NGB00734 had 10 fruits per truss. The least fruit per truss was on NGB00719 at 6 fruits per truss. The harvesting started from 5 weeks after transplanting and lasted for seven weeks; harvesting was done weekly. The total harvests from the accessions were pooled together for analyses. Accessions NGB00718 and NGB00711, had yields of 26.1 tonnes/hectare and 20.4 tonnes/hectare respectively. These were the only two accessions that had yields exceeding 10 tonnes/ha, other accessions were below 10 tonnes/ha.

Twelve accessions were in the range of 5 tonnes/hectare to 10 tonnes/hectare, NGB00719 (9.8 tonnes/hectare), NGB00737 (8.3 tonnes/ha), NGB00741 (7.9 tonnes/ha), NGB00720 (7.9 tonnes/ha), NGB00721 (7.2 tonnes/ha), NGB00749 (7.1 tonnes/ha), and NGB00717 (7.0 tonnes/hectare), In addition, were accessions NGB00724 (6.8 tonnes/ha), NGB00715 (6.4 tonnes/hectare), NGB00735 (6.1 tonnes/ha), NGB00734 (5.7 tonnes/ha), and NGB00729 (5.6 tonnes/ha). The remaining six accessions: NGB00696, NGB00714, NGB00716, NGB00725, NGB00726 and NGB00746 yielded below five tonnes/hectare.

Table 4.6: Yield of twenty tomato accessions planted in the open field in Ibadan

Accessions	Weight/fruit (grams)	Fruit/Truss	Harvest (kg/plot)	Variance	Harvest (tonnes/hectare)
NGB00696	12.6	9	29.1	30.8	4.3
NGB00711	18.0	11	136.8	373.25	20.4
NGB00714	10.0	10	18.1	49.58	2.7
NGB00715	15.4	9	43.4	158.32	6.4
NGB00716	20.5	8	32.4	17.71	4.9
NGB00717	16.8	9	47.1	307.56	7.0
NGB00718	23.7	10	174.6	501.13	26.1
NGB00719	5.4	6	65.3	80.51	9.8
NGB00720	11.0	7	53.2	282.84	7.9
NGB00721	13.5	12	48.5	152.26	7.2
NGB00724	16.4	14	45.8	158.72	6.8
NGB00725	2.0	25	21.8	9.93	3.2
NGB00726	4.0	16	20.6	10.48	3.1
NGB00729	5.0	14	37.8	145.71	5.6
NGB00734	5.4	11	38.2	15.76	5.7
NGB00735	9.0	11	41.1	95.34	6.1
NGB00737	15.0	11	55.5	61.11	8.3
NGB00741	2.0	23	52.6	178.21	7.9
NGB00746	8.0	17	29.9	21.68	4.5
NGB00749	15.0	14	47.6	64.40	7.1

4.4. Occurrence, abundance, and distribution of *Tuta absoluta* and other insect pests of tomato in Southwest, Nigeria

There was no incidence of *T. absoluta* infestations on the open fields surveyed during the cause of this research. Screen houses that experienced the infestation in 2016, have stopped tomato production too. However, another major pest of importance reported were *Zonocerus variegates* (22.1%), white fly *Bemisia tabaci* (1.5%), and mole crickets *Gryllotalpa spp* (15.3%). Furthermore, serpentine miners (1.5%) and aphids (17.6%) were also recorded as pests of importance. The main pest reported was *Helicoverpa armigera* making up 38.5% of all reported insect pests (Figure 4.5).

Figure 4.6 shows the presence and frequency of the insect pest sampled per local government area. Ogbomosho LGA had the highest incidence of *Helicoverpa armigera,* followed by Ogo-oluwa and Ekiti-ado LGAs sharing second position closely, while Orire LGA was the third. Meanwhile no incidence of *Helicoverpa armigera* was recorded in Yewa and Ijebu East LGAs. The three LGAs sampled in Ogun states (Yewa north, Ijebu-East and Yewa south) had the highest incidence of locust infestation, while Ekiti-Ado and Ekiti-Gbonyin had the least infestation. Grasshopper population was highest in Ekiti state, and none was recorded in Ogun State and Oyo State.

Table 4.7 shows the occurrence of insect pests and their importance to the farmers in each of the different LGAs. To all the farmers surveyed cotton bollworm is an important insect pest of tomato. In Ogun State mite, cricket, tomato leaf miner, and termites were reported as important but do not pose serious threat to production. Aphids and whiteflies are important to production. In Oyo State whitefly was not an important insect pest. Aphid is very important to production in Ogo-Oluwa and Ogbomosho south LGAs. Grasshopper was reported as an important insect pest to farmers in Ogo-Oluwa LGA farmers. In Ekiti State, apart from cotton bollworm, grasshoppers were reported to be of important to production in Ado Ekiti and Ikere Ekiti LGAs. Tomato leaf miner is regarded as important but not a threat in all the LGAs. Aphids and whiteflies were also reported as important but not a threat in Ogun State. However, aphids are reported to be important to farmers in Ogo-Oluwa and Ogbomosho south LGAs, Oyo state. Farmers surveyed in Ogun State reported that locust is an important insect pest to tomato production.

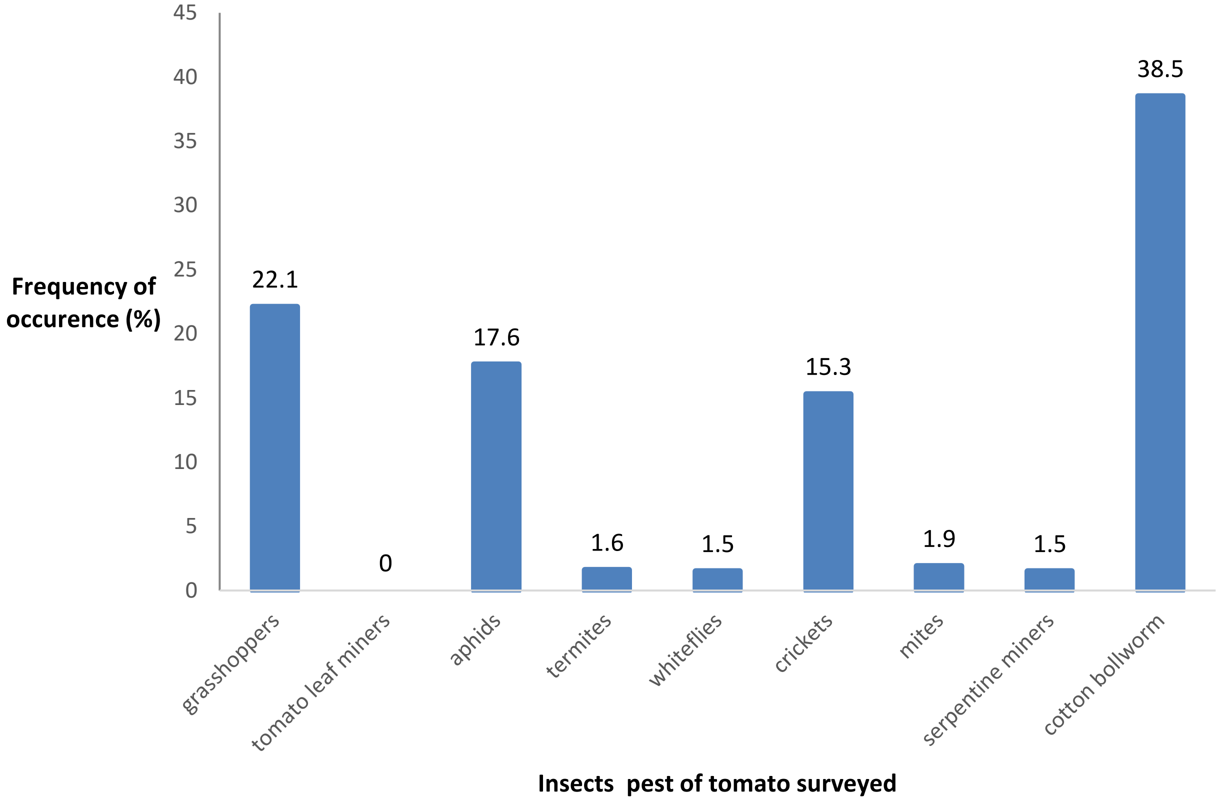

Figure 4.5: Insect pests recorded from field observations in the three major tomato-producing states in Southwest, Nigeria.

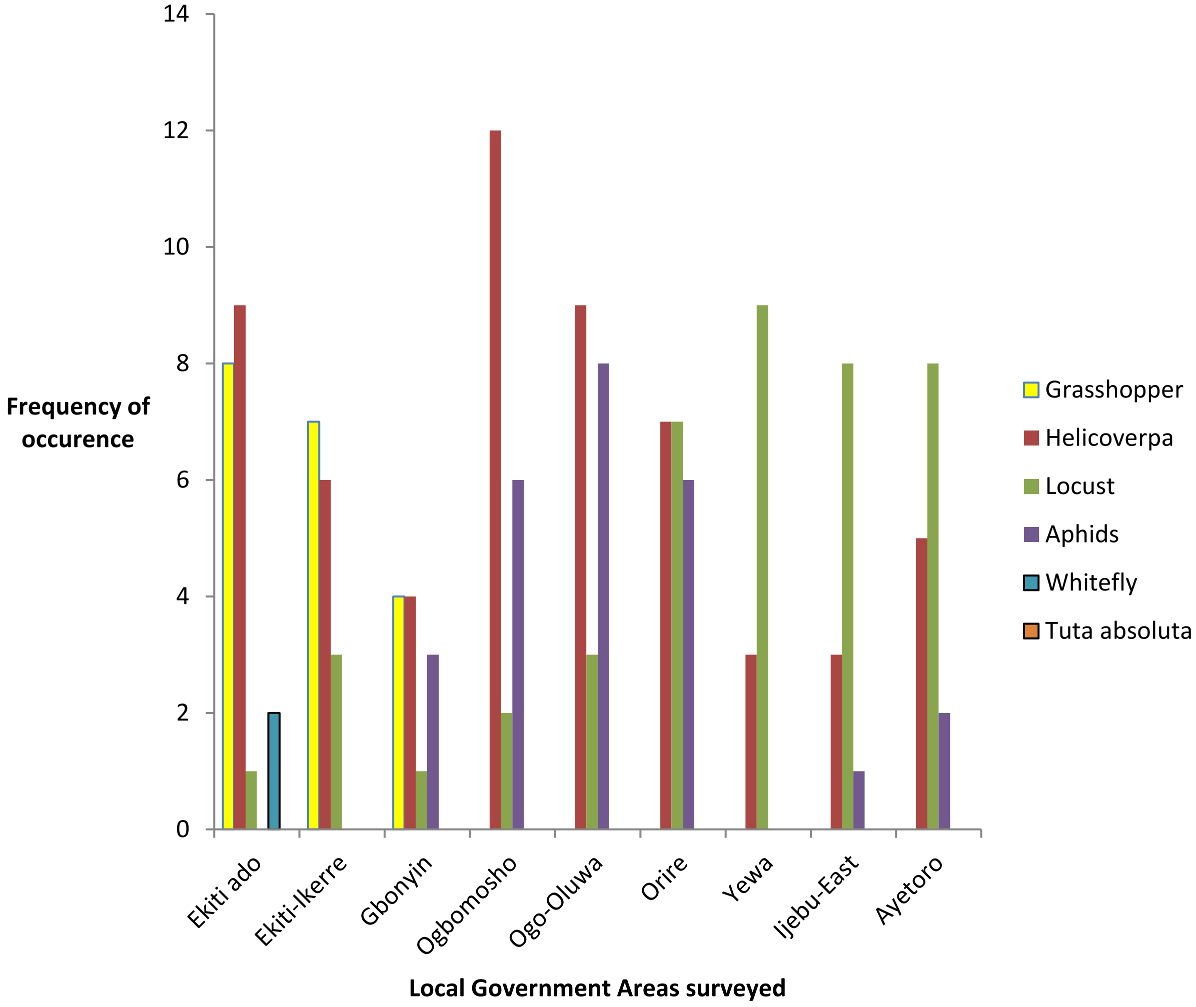

Figure 4.6: Occurrence of different insect pests surveyed in different LGAs

Table 4.7: Importance of insect pest to farmers in the different local government areas surveyed

Insect Pest	Ogun			Oyo			Ekiti		
	YN	YS	IE	OO	OS	Or	EA	EG	EI
Cricket	+	+	+	+	+	+	+	+	+
Whitefly	++	++	++	-	-	-	-	-	-
Aphid	++	++	++	+++	+++	++	-	+	-
Tomato leaf miner	+	+	+	+	+	+	+	+	+
Cotton bollworm	++	++	+++	+++	+++	+++	+++	+++	+++
Mite	+	+	-	-	+	+	-	+	-
Locust	+++	+++	++	-	+	-	-	+	-
Grasshopper	++	+	+	++	+	+	++	+	++
Termite	+	+	+	-	+	-	-	-	-

Note: Yewa North (YN); Yewa South (YS); Ijebu-East (IE); Ogo-Oluwa (OO); Ogbomosho South (OS); Orire (Or), Ekiti-Ado (EA), Ekiti-gbonyin (EG); and Ekiti-Ikere (EI)

Legend:

- \- Not important
- \+ Important but not a threat
- ++ Important
- +++ Very important

4.5. Molecular identification of *T. absoluta* in Southwest, Nigeria

A sample of *T. absoluta* populations was characterised using the whole genome sequence of the insect sample via the following procedures: A cytochrome oxidase subunit I (COI) gene DNA fragment of ~700 bp was amplified by PCR using primers LepF1/LepF2 (Figure 4.7). The partial nucleotide sequence generated from this study was 684 bp. The database searches with other Insect pest sequences carried out by NCBI-BLAST program (http://blast.ncbi.nlm.nih.gov) revealed that the sequence generated from this study has 99% identity with *Tuta absoluta* cytochrome oxidase subunit 1 (COI) gene from the republic of Congo and Nigeria with NCBI accession number MG693217 and MK189162, respectively. Two phylogenetic trees were constructed; the first was to see the relationship of our sequence with some other species in the Gelechidae family.

The other tree was constructed to compare our sequence to eight public sequences, accession numbers KY212126, MT021750, KU565497, KY129657, MN759250, MK189159, GU353337 and MG596098 (http://www.ncbi.nlm.nih.gov/). The sequences were used for comparison, and they correspond to partial sequences of cytochrome oxidase subunit I (COI) gene from different South Africa, USA, Kenya, Egypt, Togo, Nigeria, Spain, and Brazil, respectively. The phylogenetic tree constructed to compare the sequence from this study and other sequences in the GenBank showed that the tree separates into two distinct clades. The maximum-likelihood genealogies of nucleic acid sequence demonstrated that the isolate from this study clustered within a biphyletic clade that comprises sequences of *Tuta absoluta* from South Africa (KY212126), USA (MT021750), Kenya (KU565497), Egypt (KY129657), Togo (MN189159) and Nigeria (MK189159) in a clade. However, *Tuta absoluta* from Spain (GU35337) and Brazil (MG59098) were found branching off from the same root on the other clade (Figure. 4.8).

Other species in the Gelechidae family found on that clade included *Sitotroga cerealella, Tecia solanivora, Phthorimaea operculella, Chionodes fructuarius, Chrysoesthia sexguttella and Gelechia senticetella* among others. Figure 4.9 shows that the Brazilian variant of *Tuta absoluta* stands alone, while that from Spain shares same clade with other specimens reported from USA, South Africa, Kenya, Egypt, Togo, and Nigeria. Sequences submitted from Nigeria were from Kadawa, Kano State, the sequence from the research is the first sequence submitted from Southwest Nigeria.

Lep F1:	**5’ATTCAACCAATCATAAAGATATTGG3’**
LepR1	**5’TAAACTTCTGGATGTCCAAAAATCA3’**

Figure 4.7: Primers used for the molecular characterization of *Tuta absoluta*

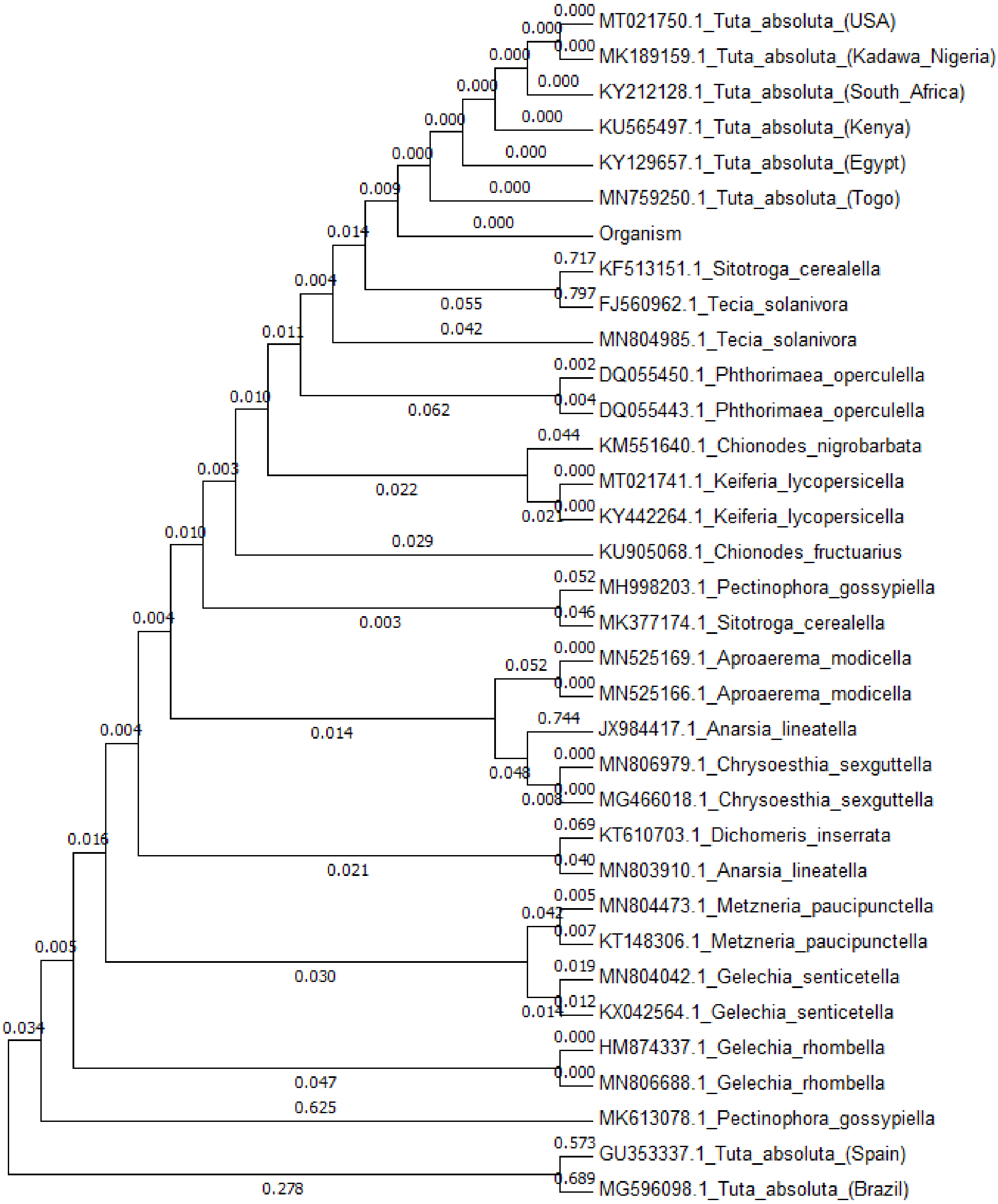

Figure 4.8: **Phylogenetic tree depicting evolutionary relationship among the sequence derived in the study and other species in the Gelechiidae family**

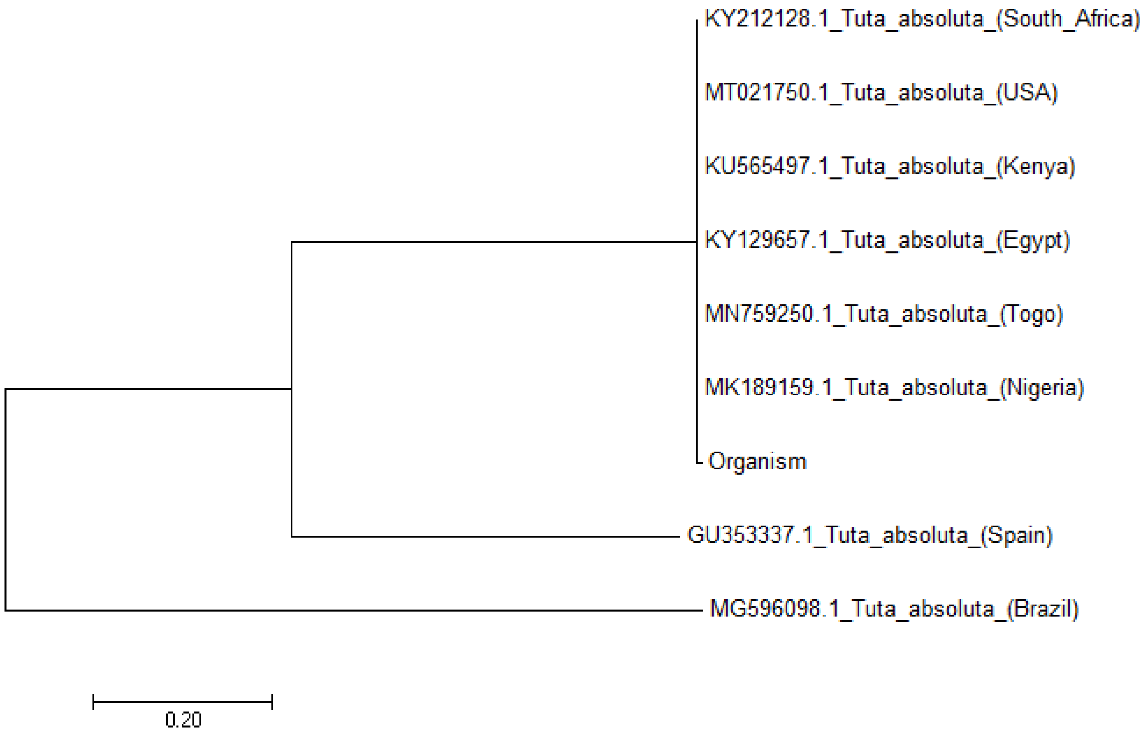

Figure 4.9: Unrooted maximum likelihood phylogenetic tree depicting evolutionary relationships among sequence derived in this study and 8 others from the GenBank.

4.6. Biology of *Tuta absoluta*

The mean life cycle (egg to adult) of *T. absoluta* was 20.9 days as presented in the Table 4.8. *T absoluta* passed through complete metamorphosis of four larval instars, pupa stage before adult emergence. The description of the life stages of *T. absoluta* is presented below.

4.6.1. Description of eggs of *T. absoluta*

Freshly deposited eggs are oval, creamy white in colour with translucent, smooth, and shinning feel when viewed under a microscope. The eggs were laid singly on the adaxial side of the leaves, at such an angle that allows for the exposure of the respiratory horns. The size of the egg was 0.44 ± 0.001 mm in length and the breadth was 0.22 ± 0.002 mm at the widest point of measurement (Table 4.8). The mean development period was 4.12 ± 0.016 days at temperature of 27.3 ±3 ºC, and 75.3 ± 1.6% relative humidity. They changed to black just before hatching after 3 - 5 days of incubation at 27 – 29 ºC. The eggs were laid daily for more than 7 days, 24 hours after adult emergence, in mated and virgin females.

4.6.2. Description of first instar larva

Newly hatched larvae, eclosed from eggs after four (4) days, have soft creamy coloured body with a light brown head capsule. The first larval instar stage lasted an average of 2.9 ± 0.08 days, with a mean head capsule size of 0.15 ± 0.001 mm, and average body length and body width of 1.49 ± 0.015 mm and 0.28 ± 0.001, respectively (Table 4.9).

The newly hatched larva spends few minutes (15 ± 4.5 min) circling on the leaf surface before finally eating in on a spot to penetrate the leaf, which took 25 ± 7.5 min, total 40 minutes to tunnel fully into the leaf.

4.6.3. Description of second instar larva

The second instar stage lasted 2.5 ± 0.00 days; the head capsule was 0.23 ± 0.002 mm, and the length and width of the body was 2.82 ± 0.022 mm and 0.36 ± 0.11 mm, respectively (Table 4.9).
The body was creamy white; the head had a darker brown colouring than the first instar stage. Larvae remain in the mines feeding on the mesophyll, and less exposed to predation. The body segmentation and tubercles were becoming visible at the stage.

Table 4.8: Developmental biology of *Tuta absoluta* eggs in Ibadan, Nigeria (N=20)

Parameters	Mean ±SE	Range
Incubation period	4.12 ± 0.016	3.9 – 4.3
Egg diameter (mm)	0.22 ± 0.01	0.1 – 0.26
Eggs laid	125.5 ± 3.58	115 – 132
Eclosion (%)	74.80 ± 2.30	63 – 105

Incubation and egg eclosion were at 27.3 ±3 ºC, 75.3 ± 1.6% relative humidity, and 12:12 hours light and dark photoperiods in the laboratory.

SE= Standard error of the mean.

Table 4.9 Daily Morphometrics of *Tuta absoluta* larvae reared on sweet-tomato leaves in Ibadan, Nigeria (n=10)

Days after larval eclosion	Larval stage	Mean (mm) + SE (range)		
		Body length	Body width	Head capsule
0	1st	0.41 ± 0.03 (0.2 -0.6)	0.10 ± 0.01 (0.07-0.13)	0.05 ±0.02
1		0.90 ± 0.01 (0.6–1.3)	0.15 ± 0.03 (0.10-0.18)	0.09 ±0.02
2		1.21 ± 0.05(1.0– 1.27)	0.22 ± 0.05 (0.18-0.27)	0.12 ±0.01
3		1.49 ± 0.05(1.32-1.54)	0.28 ± 0.04 (0.25-0.33)	0.15 ±0.01
4	2nd	1.78 ± 0.09(1.66-2.20)	0.31 ± 0.05(0.28-0.33)	0.17 ±0.05
5		2.34 ± 0.15(2.27-2.59)	0.33 ± 0.02 (0.31-0.40)	0.19 ±0.02
6		2.82 ± 0.22(2.56-2.90)	0.36 ± 0.01 (0.31-0.47)	0.23 ±0.03
7	3rd	3.43 ± 0.19(3.16-3.76)	0.40 ± 0.12 (0.35-0.43)	0.28 ±0.05
8		4.18 ± 0.15(3.95-4.21)	0.43 ± 0.16 (0.38-0.49)	0.34 ±0.06
9		5.69 ± 0.45(5.28-6.55)	0.48 ± 0.14(0.46-0.52)	0.39 ±0.09
10	4th	6.80 ± 0.22(6.62-7.36)	0.53 ± 0.22(0.49-0.57)	0.45 ±0.14
11		7.44 ± 0.36(7.22-7.70)	0.60 ± 0.38(0.55-0.65)	0.50 ±0.20
12		8.00 ± 0.50(7.90-8.24)	0.67 ±0.014(0.63-0.76)	0.56 ±0.12

4.6.4. Description of third instar larva

The third instar had a greenish colouration of the body, while the sides were pinkish in colouration. The head was darker with capsule measuring 0.34 ± 0.006 mm, the body length was 5.69 ± 0.047 mm, and body width was 0.48 ± 0.006 mm. The third instar stage lasted 2.53 ± 0.00 days (Table 4.9).

4.6.5. Description of fourth instar larva

The larva at this stage has a greenish and light pink colouration. The body length of the fully-grown larvae was 8.0 ± 0.004 mm, while the abdominal width was 0.67± 0.014 mm. The head capsule was 0.56 ± 0.012 mm and the growth period lasted 3.1 ± 0.01 days (Table 4.9). The body colour changes with maturity to greenish, as it nears pupation it becomes pinkish in colour. To pupate, most of the larvae drop by silk threads to the ground, others remained on the leaves, while a few others remained in the mines. The fully grown larva weaves a cocoon around itself, entering a non-feeding stage, as it prepares itself for pupation. The total larval developmental stages took 12.2 ± 0.1 days at 29.3 ± 2.17 (RH 75.3 ± 1.6%). They went through four instar stages: When shedding its skin to change from one instar stage to another, the colour of the larvae will change to whitish/creamy colour. The body will be compressed and attached at the rear to the surface of the leaves or any other support, while forcing itself out of the old skin.

4.6.6. Description of pupa

The larvae pupate after the fourth instar; the pre-pupa stage is the stage when the larva weaves a cocoon around itself on the leaflet, inside the mine or inside soil, and under bucket rims in the screen house when it is not in a mine. The pre-pupa stage is a non-feeding stage, just like the pupal stage. At the pupal stage, the initial colour was green, and it turns to brown with maturity. The shape is cylindrical; the outline of the shape of the head and wings are visible. The time of development was 7.4 ± 0.03 days for males, while the females had a shorter developmental period at 6.5 ± 0.04 days, hence, the emergence of females before the males. The body morphometrics differ with sex; the male had a smaller body weight at 3.18 ± 0.02 mg, a shorter body length at 3.57 ± 0.03 mm and body width of 0.51 ± 0.04, whilst the female weighed 4.20 mg, at a body length of 4.00 ± 0.08 mm (Table 4.10). Sex determination was achieved by viewing the ventral surfaces of the 8th, 9th, and 10th abdominal segments. There were ten (10) visible abdominal segments on the pupa,

Table 4.10: Developmental biology of *Tuta absoluta* pupae (male and female): Morphometrics and duration of stage in days

	Mean ± SE (Range)			
Sex	**Body length (mm)**	**Body width (mm)**	**Body weight (mg)**	**Duration (days)**
Male	3.37 ± 0.03a (2.43 – 4.54)	0.51 ± 0.04a (0.38 – 0.76)	3.18 ± 0.02b (2.88 – 4.22)	7.4 ± 0.03a (5.65 – 8.00)
Female	4.00 ± 0.08a (3.00 – 4.85)	0.64 ± 0.06a (0.49 – 0.70)	4.20 ± 0.06a (3.32 – 5.00)	6.5 ± 0.04b (5.80 – 7.50)

Means ± SE values in same column followed with same letter are not significantly different at 5% level of significance according to Turkey's Honestly Significant Difference (n =20) the 10th segment included the cremaster and had an anal opening. Female pupae had a longitudinal suture or slit in the middle of the 8^{th} abdominal segment in between the two small tubercles, while the male pupae had a suture in the middle of the 9th abdominal segment. The wings and legs casings end at the 5th abdominal segment in the females but extend to the 6^{th} abdominal segment in the male pupae (Plate 4.1).

4.6.7. Description of adult *Tuta absoluta*

The emerged adults were silvery brown in colour, with black spots on the forewings. The female had a bigger and wider abdomen in comparison to the slender male's abdomen. The antennae are filiform, long, and banded in black and brown colouration which lie backwards when at rest. The males had wings span of 9.05 ± 0.45 mm, while the females had an average of 9.55 ± 0.06 mm. The body length was 5.57 ± 0.04 mm for the female, while it was 5.30 ± 0.040 mm for the males. The wing apex is fringed, brownish in colour with silver scales having black spots on the forewings. The wings' length was 9.05 ± 0.45 mm in the males, and 9.55 ± 0.06 mm in the females, and sex ratio was male to female (1:1.5) (Table 4.11). There was no significant difference in the values obtained for both sexes. There was significant difference in the life span of the moths at $p > 0.05$. Virgin males and females, lived up to 7.3 ± 0.20 days and 12.9 ± 0.55 days, respectively. They have suctorial mouthparts modified for sucking nectar and sap from flowers. The labial palp is prominent, curved, with the apical segment long and acute. The complete life cycle from eggs too adult is presented in Plate 4.2.

4.6.8. Fecundity and eclosion of adult *Tuta absoluta*

Mean fecundity and eclosion of *T. absoluta* is shown on Table 4.12. The results showed that the fecundity of the mated virgin pairs was 125.5 ± 3.58, with eclosion of 94.6 ± 3.53 and the percentage eclosion was 74.80%. The fecundity of mated females paired with virgin males had 50.1 ± 6.8 fecundity, with 33.2 ± 1.49 hatched eggs at 66.82 % eclosion. The fecundity of mated males and virgin females was 101.7 ± 2.49, hatchability was 77.1 ± 3.07 and percentage eclosion was 75.77%. Mated males paired with mated females had a fecundity value of 49.4 ± 9.95; eclosion was 29 ± 7.33 at 59.53 % percentage eclosion. When single virgin females were paired with two virgin males the mean fecundity was 127.9 ± 15.29, with mean eclosion of 92.4 ± 3.01, and percentage eclosion of 73.36%. Also, when virgin females were mated again at 5 days after ovipositing, the mean fecundity was 174.5 ± 12.79, with mean fecundity of 134.7 ± 2.63, and percentage eclosion

Plate 4.1: Pupae of *Tuta absoluta* (A) female (B) male

Table 4.11: Morphometrics, lifespan and sex ratio of *Tuta absolute* moths (male and female)

Sex	Mean ± SE (Range) Body length (mm)	Body width (mm)	Wingspan (mg)	Lifespan (days)	Sex ratio
Male	5.30 ± 0.04a (4.58 – 5.55)	0.49 ± 0.05a (0.40 – 0.56)	9.05 ± 0.45a (8.80 – 9.10)	7.3 ± 0.20b (6.50 – 7.85)	1
Female	5.57 ± 0.04a (5.00 – 6.40)	0.55 ± 0.07a (0.48 – 0.65)	9.55 ± 0.06a (9.00 – 10.50)	12.9 ± 0.55a (10.30- 13.50)	1.5

Means ± SE values in same column followed with same letter are not significantly different at 5% level of significance according to Turkey's Honestly Significant Difference (n = 20)

Egg singly laid

Larval (four stages involved)

Pupa

Adult moths

Plate 4.2: The developmental stages of *Tuta absoluta*

Table 4.12: Fecundity and eclosion (%) of *Tuta absoluta* at different mating and treatment regimes

Treatment	Fecundity Mean ± SE	Eclosion Mean ± SE	Percentage (%)	
			Eclosion	Mortality
Virgin female	94.4 ± 1.21c	31.3 ± 2.60	33.16	66.84
Virgin male X virgin female	125.5 ± 3.58b	94.7 ± 3.53	74.80	25.20
Virgin male X mated female	50.1 ± 6.84d	33.2 ± 1.49	66.82	33.18
Mated male X virgin female	101.7 ± 2.49c	77.1 ± 3.07	75.77	24.23
Mated male X mated female	49.4 ± 9.95d	29.4 ± 7.33	59.53	40.47
Virgin males X virgin female (2:1)	127.9 ±15.29b	92.4 ± 3.01	73.36	26.64
Multiple mating X virgin female (2nd mating 5th day of oviposition)	174.5 ±12.8a	134.7 ± 2.63	76.13	23.87
Female fed on 5% sugar solution	115.4 ±1.58ac	97.0 ± 8.77a	83.94	16.06
Female fed on 10% sugar solution	123.4 ± 4.22b	96.7 ± 1.86	78.34	21.66
Females fed on water alone	117.6 ± 7.41c	84.2 ± 2.78	71.74	28.26

n= 10 treatment. Mean ± SE followed by the same letter in the same column are not significantly different ($p > 0.05$) Tukey's Honestly Studentized Range (HSD) test.

was 76.13 %. With change in diet at 10% sugar solution, the mean fecundity of virgin males paired with virgin females were 123.0 ± 4.22%, the mean hatchability was 96.7 ± 1.86%, while the percentage hatchability was 78.34%. At 5% sugar solution, virgins had a mean fecundity of 115.4 ± 1.58, with mean eclosion of 97.0 ± 2.77%; the percentage that eclosed was 83.94%. When fed on water the females laid an average of 117.6 ±7.41, with 84.2 ± 2.78 successfully hatched, at a percentage of 71.74%. There was significant difference ($p>0.05$) with mean fecundity, mean eclosion, and percentage eclosion of virgin females paired with multiple males compared to one female paired with one virgin male.

4.6.9. Parthenogenicity in *Tuta absoluta*

Unmated females laid 94.4 ± 7.21 eggs and 31.3 ± 2.60 successfully hatched, thus making an eclosion percentage of 33.16%. Both male and female offspring were produced at a sex ratio of 1:1.8.

4.6.10. Damage by larvae of *Tuta absoluta*

The larval stage is the damaging stage of the *T. absoluta* on tomato plants. The larvae fed on the chloroplast of the leaves having borne a hole through the mesophyll. This feeding created conspicuous mines and galleries on the leaves, fruits, and stem (Plate. 4.3). The resulting mines and galleries on the leaves caused reduction of the photosynthetic ability of the leaves, thus the leaves dried out early (Plate 4.4). This affected other aspects of tomato growth like flowering, and fruiting, when the attack occurred at the early stage of tomato growth. However, some accessions tolerated the infestation and produced fruits. The fruits produced were attacked. The larvae began mining into the fruits from the crown and fed on the flesh of the fruit from inside. This led to secondary infestation by disease causing organisms, leading to fruits rot.

4.6.11. Longevity of adult *Tuta absoluta*

Longevity of *T. absoluta* moths was affected by sex, feeding and mating. Virgin males and females lived up to 7.3 ± 2.13 and 12.9 ± 3.55 days, respectively, the values were significantly different ($p>0.05$) for males and females (Table 4.13a). The effect of feeding observed showed there was increase in the longevity of the female moths that were fed on 5% and 10% sugar solution. Whilst the males lived up to 7.5 ± 0.17 days at 5% sugar solution and 7.8 ± 0.34 at 10% sugar solution (Table 4.13b). Mated males and females had an average

Plate 4.3: Tomato leaf damaged by larvae of *Tuta absoluta*

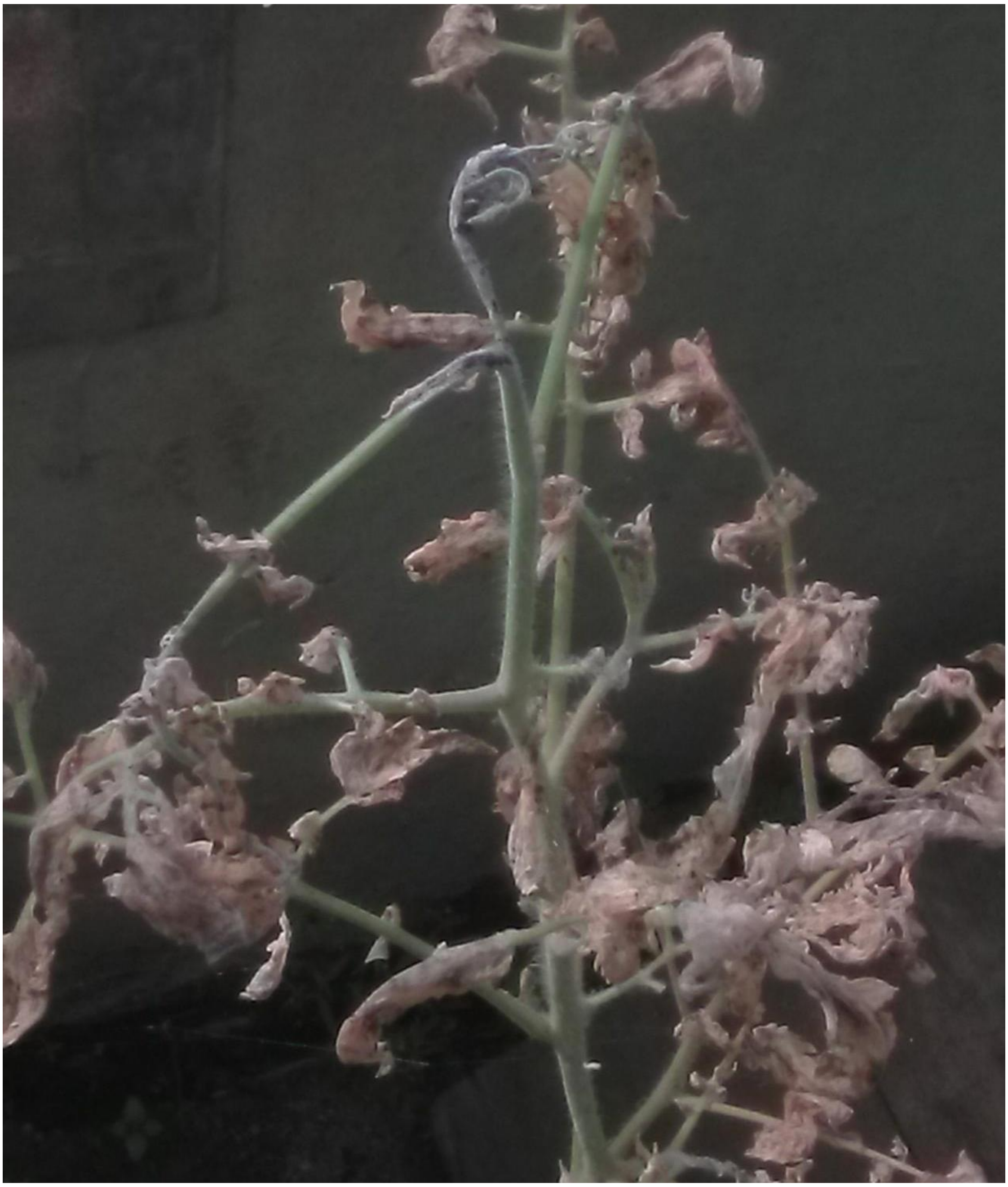

Plate 4.4: The total defoliation of a tomato plant by larvae of *Tuta absoluta* in the screen house

Table 4.13a: Effect of sex on the longevity of *Tuta absoluta* moths

Sex of moth	Mean ± SE (days)	Range
Male	7.3 ± 0.13b	6-8
Female	12.9 ± 0.55a	11- 13

N = 10. Mean ± SE followed by the same letter in the same column is not significantly different ($p > 0.05$) Tukey's Honestly Studentized Range (HSD) test.

Table 4.13b: Effect on feeding on the longevity of *Tuta absoluta* moths (n =20)

Sex of moth	Feeding status	Mean ± SE	Range
Male	Water	7.3 ± 0.13b	6 – 8
	5 % sugar solution	7.5 ± 0.17b	6 – 9
	10 % sugar solution	7.8 ± 0.34b	6 - 8
Female	Water	13.0 ± 0.51a	11 – 16
	5 % sugar solution	13.9 ± 0.37a	12 – 17
	10 % sugar solution	14.1 ± 0.30a	12 - 17

Mean ± SE followed by the same letter in the same column is not significantly different ($p > 0.05$) Tukey's Honestly Studentized Range (HSD) test longevity of 7.0 ±0.15 and 20.5 ± 3.44 days, respectively. The adult males and females fed on water solution lived up to 7.3 ± 2.13 and 13.0 ± 2.51days, respectively. Those fed on 10 % sugar solution lived up to 7.3 ± 0.09 days and 14.1 ± 0.30 days for males and females, respectively. There was significant difference in the values obtained for males and females at $p>0.05$. Mating affected longevity of *T. absoluta* moths. Males lived up to 7.0 +0.15 days while the females lived up to 20.5 + 0.44 days. These values significantly different at $p>0.05$ (Table 3.14c).

The interaction of sex, feeding and mating on longevity showed that mated females lived up to 20.5 ± 3.44 days, when fed with water alone (Table 4.13d). However, mated females lived up to 21.3 ± 4.28 days on 5% sugar solution diet. At 10% sugar solution diet mated females had longevity value of 20.5 ± 0.41 days. The mated males fed on water lived up to 6.9 ± 0.16 days, while the females lived up to 20.5± 0.44. Mated males fed on 5% sugar diet they lived up to 7.2 ± 0.08 days, while the females lived up to 21.3± 0.28. Those fed on 10% sugar solution; longevity was 6.9 ± 0.11 and 20.5 ± 0.41 days for mated males and females, respectively.

There was no significant difference between virgin males and females fed on water alone and those fed on 5 % sugar solution. There was however a significant difference in the longevity of mated females compared to virgin females fed on water and 5% sugar solution. There was no significant difference between mated females fed of 5% and 10% sugar solution. There was significant difference in the longevity of males and females, irrespective of the treatment given them. In addition, there was significant difference in those fed with sugar diet and those not reared on sugar diet.

4.7. Survival and mortality table, and life table for *Tuta absoluta* fed on tomato leaves

Table 4.14 shows the survival and mortality table for *Tuta absoluta* (80 eggs) reared on tomato leaves (Sweet tomato c.v.). The overall mortality was 32.50% with 67.5% survival. The breakdown of the result is as follows. The eggs that hatched into 1st instar larvae were 54 in number, while 26 eggs did not hatch. For first instar larvae, 47 (87.04%) survived to the second larval instar stage with12.96% mortality recorded. At 2nd instar stage 10.64 % mortality was recorded, while 89.34% survived to the third larval instar stage level. Third instar stage began with 42 (89.36%) larvae from the 2nd instar larvae stage.

Table 4.13c: Effect of mating on longevity of *Tuta absoluta* moth (n =20)

Sex of moth	Mean ± SE	Range
Male	7.0 ± 0.15b	6 – 8
Female	20.5 ± 0.44a	18 – 22

Mean ± SE followed by the same letter in the same column is not significantly different ($p > 0.05$) Tukey's Honestly Studentized Range (HSD)

Table 4.13d. Effect of sex, feeding, and mating on longevity of *Tuta absoluta* moths (n =20)

Sex of moth	Feeding status	Mean ± SE	Range
Male	Water	6.9 ± 0.16b	6 – 8
	5 % sugar solution	7.2 ± 0.08b	6 – 9
	10 % sugar solution	6.9 ± 0.11b	6 – 8
Female	Water	20.5 ± 0.44a	17 – 22
	5 % sugar solution	21.3 ± 0.28a	18 – 23
	10 % sugar solution	20.5 ± 0.41a	18 – 23

Mean ± SE followed by the same letter in the same column is not significantly different ($p > 0.05$) Tukey's Honestly Studentized Range (HSD) test

Table 4.14: Mortality and survival table *Tuta absoluta*

Parameter		Surviving at start (lx)	Number dying (dx)	Mortality (%)	Survival (%)
Eggs		80	26	32.50	67.5
	1st	54	07	12.96	87.04
Larvae	2nd	47	05	10.64	89.36
	3rd	42	02	4.76	95.24
	4th	40	04	10.00	90.00
Pupae		36	04	11.11	88.89
Adult		32			
Adult emergence					
		Male		Female	
		13		19	

Mortality recorded at this stage was 4.76 % (2). Thus, 40 (95.24%) larvae survived till 4^{th} instar larval stage. The highest survival rate was recorded at third instar stage (Table 4.14). The 4th instar larval stage recorded 10% (4) mortality hence only 36 (90%) of the larvae survived to pupal stage. There was 11.11% (4) mortality in the pupal stage, 36 (88.89%) pupae survived to adult emergence. From the 36 pupae that survived to adult emergence seventeen (17) were males emerged and twenty were females (21). The first instar stage had the highest mortality rate at 12.96%, while survival rate was 87.04% (Table 4.14).

Table 4:15 shows the age-specific life table of *Tuta absoluta* (n=20), from which the following were calculated: Net reproductive rate (NRR) R_0 was 46.01, Cohort generation time (T_c) was 23.68 days, Innate capacity for/Intrinsic rate of increase (r_c) was 0.16, and the Doubling Time (DT) was 2.53 days. Furthermore, the following were also calculated; Corrected generation time (T) was 23.93 days. While Gross Reproduction Ratio (GRR) was 46.68, Finite capacity for increase (h_c) was 1.17 and the weekly multiplication of population was 3.00.

4.8. Behavioural biology of *Tuta absoluta*

Adults *T. absoluta* were not active during the day, hiding under leaf canopy, resting on the cage and the mesh covering. They become active from sunset (1600hours) to early morning around 0800 hours. However, they tend to stop activities at night in the presence of light. Table 4.16 shows information on pre-oviposition, oviposition, and post-oviposition of *T. absoluta* the following observation were made:

4.8.1. Pre-oviposition behavioural biology of *Tuta absoluta*

The adults rested on the wall of the cage, net and many hid under the tomato canopy during the day, they did not move unless when disturbed. No mating occurred for more than 24 hours (1.9 ± 0.01 days). The males become active in the early hours around 0600 – 0900 hours and late in the evening 1800 – 2000 hours, flying frantically about the females until they succeed in mating with the females. The mating pair faces opposite direction while mating. The pair then lie still mating for 90 ± 8.9 minutes, mating occurs mainly in the earlier morning, and randomly in the evening too.

4.8.2. Oviposition behavioural biology of *Tuta absoluta* in the laboratory

Mating pair faced opposite direction while mating, they will not separate even when disturbed

Table 4:15. Age specific life table for *Tuta absoluta*

Age in days (x)	**Proportion surviving (l_x)**	**No. of eggs laid (Mean ± S.E.)**	**Fertility/fecundity (m_x)**	**Product ($l_x m_x$)**	**$xl_x m_x$**	**Death rate**
0 -20	Immature stages	0	0	0	0	
21	1	11.6 ± 0.37	4.64	4.6	97.4	0
22	1	29.0 ± 0.56	11.6	11.6	255.2	0
23	1	23.9 ± 0.32	9.56	9.6	219.9	0
24	1	17.7 ± 0.46	7.08	7.1	169.9	0
25	1	12.0 ± 0.46	4.8	4.8	120.0	0
26	1	7.2 ± 0.38	2.88	2.9	75.4	0
27	0.9	6.1 ± 0.31	2.44	2.2	59.4	0.1
28	0.9	4.2 ± 0.16	1.68	1.5	42.0	0.1
29	0.9	3.6 ± 0.23	1.44	1.3	37.7	0.1
30	0.8	1.0 ± 0.20	0.40	0.3	9.0	0.2
31	0.8	0.3 ± 0.13	0.12	0.1	3.1	0.2
32	0.6	0.1 ± 0.05	0.04	0.01	0.64	0.4
33	0.6	0.0 ± 0.00	0	0	0	0

Net reproductive rate (NRR) R_0 = **46.01**

Cohort generation time (T_c) = **23.68**

Innate capacity for/Intrinsic rate of increase (r_c) = **0.16**

Doubling Time (DT)= **2.53**

Corrected generation time (T) days= **23.93**

Gross Reproduction Ratio (GRR) = **46.68**

Finite capacity for increase h_c = **1.17**

Weekly multiplication of population = **3.00**

Table 4.16: Data on pre-oviposition, oviposition, and post-oviposition of *Tuta absoluta*

Parameter	Pre-oviposition (days)	Oviposition (days)	Post-oviposition (days) Male	Post-oviposition (days) Female	Fecundity	Eggs (hatched)	Adult longevity (days) Male	Adult longevity (days) Female
Average	1.9	10.0	1.5	3.7	106.3	90.8	7.7	15.54
Standard error	0.01	0.16	0.10	0.16	1.56	1.40	0.39	0.202
SD	0.038	0.694	0.532	0.731	6.959	6.28	1.74	0.905
SV	0.001	0.482	0.093	0.342	48.43	39.46	3.01	0.819
Range	1.77 – 1.98	9.21 - 11.21	1.22 -2.32	2.3 – 4.11	92 - 120	78 – 100	5.56 - 12.02	14.21 – 17.02
Confidence Interval (95%)	0.018	0.325	0.422	0.321	2.213	2.942	0.81	0.434
Count	20	20	20	20	20	20	20	20

but fly away in same direction while still locked to each other. Eggs are laid singly on leaves and net; they are creamy in colour and hatch in about four days (4.2 ± 0.1 days). The eggs were preferably laid on the underside of the leaflets. More than 70% of the eggs laid were in the first 7 days, the second day has the highest eggs laid, which then declines with the days (Figure 4:10). Female *T. absoluta* are polygamous in nature, as they are observed mating several times with virgin and mated males.

The virgin female mated with virgin male went through the oviposition period for an average of 10.0 ± 0.16 days (Table 4.16). Mated females laid eggs again after mating with introduced mated males with few eggs laid. Unmated males paired with oviposited females after the fifth day of oviposition resulted in mating and increased oviposition. When mated males were introduced to virgin females, they mated, and eggs were laid. In all the experiments with virgin male and female, oviposition was low on the first day, an average of 9.8 eggs were laid, number of eggs peaked on the second day at an average of 26.2 eggs laid and gradually dropped with increase in days until the 12th day when an average of 0.3 eggs were laid (Figure 4.10). Eggs hatchability was 90.8% (Table 4.16).

4.8.3. Post-oviposition behavioural biology of *Tuta absoluta* in the laboratory

After mating, the pairs separate; fly away from the site of mating. On arrival at the other spot, they lay still again till evening when they become active. The males die within 7.7 ± 0.39 days while the females lived up to 2.5 ± 0.20 after oviposition (Table 4.17).

4.9. Natural enemies of *Tuta absoluta* in the screen house and laboratory

During this study, the life stages of *Tuta absoluta* were parasitized and predated on in the screen house. Table 4.17 shows the natural enemies observed and identified in the screen house during this study. The egg and instar stages were affected by ants, such as the Ghost ant (*Tapinoma melanocephano*) (Fabricius), black ants *Tapinoma melanocephalum*, and trap-jaw ants *Odontomachus clarus*. Predators such as skinks, tropical house gecko and lizards were observed preying on the 4th instar larval stage, pupae, and the adult moths in the screen house. Other natural enemies observed in the screen house was different praying mantis and spider species which preyed on the adult moths (Plate 4:5). The major natural enemies observed in the laboratory was the tiny yellow ants; they fed on the eggs, and pupal stage.

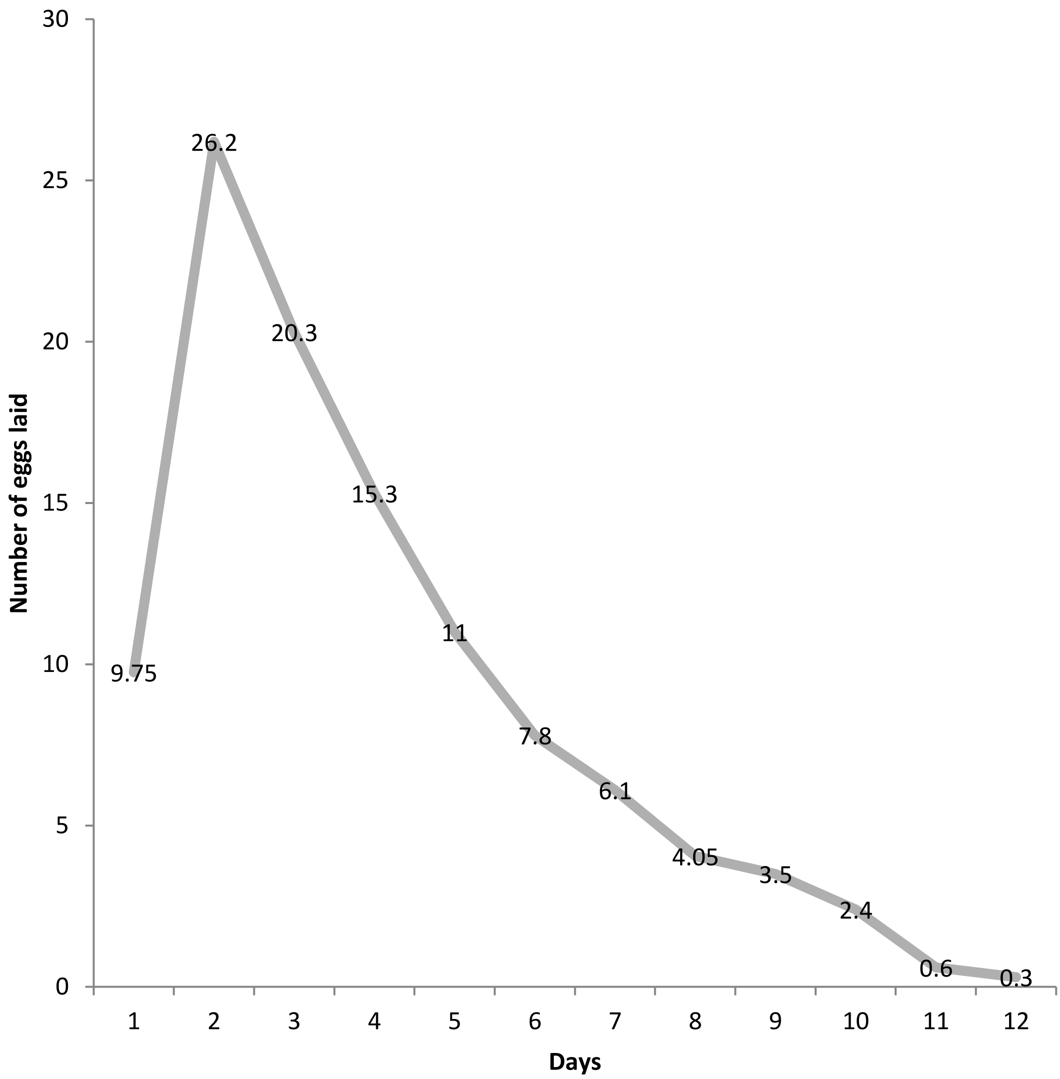

Figure 4.10: Actual daily fecundity of *Tuta absoluta* at 27.3 ± 2.17 ºC

Table 4.17: Natural enemies of *Tuta absoluta* observed in the screen house

Life stage	Organism	Order/Family	Status
Eggs	*Tapinoma melanocephalum* (Black ant)	Hymenoptera: Formicidae	Predator
1st instar	*Tapinoma melanocephalum* (Black ant)	Hymenoptera: Formicidae	Predator
2nd instar	None observed	-	-
3rd instar	*Tapinoma melanocephalum* (Black ant)	Hymenoptera: Formicidae	Predator
4th instar	*Tapinoma melanocephalum* (Black ant)	Hymenoptera: Formicidae	Predator
	Oecophylla longinoda (Weaver ant)	Hymenoptera: Formicidae	Predator
	Dinoponera australis (Giant tropical ant)	Hymenoptera: Formicidae	Predator
	Odontomachus clarus(trap-jaw ants)	Hymenoptera: Formicidae	Predator
Pupa	*Tapinoma melanocephalum* (Black ant)	Hymenoptera: Formicidae	Predator
	Oecophylla smaragdina (Weaver ant)	Hymenoptera: Formicidae	Predator
	Dinoponera australis (Giant tropical ant)	Hymenoptera: Formicidae	Predator
	Odontomachus clarus(trap-jaw ants)	Hymenoptera: Formicidae	Predator
	Coleomegilla maculata (lady beetle)	Coleoptera: Coccinellidae	Predator
	Hemidactylus lurcicus (house gecko)	Squamata: Gekkonidae	Predator
Adult	*Icius insolidus* (jumping spider)	Araneae/Salticidae	Predator
	Heliophanus sp. (jumping spider)	Araneae/Salticidae	Predator
	Hemidactylus lurcicus (house gecko)	Squamata: Gekkonidae	Predator
	Agama agama (Lizard)	Squamata: Agamidae	Predator
	Trachyleplis striata (Skinks)	Squamata: Scincidae	Predator
	Sphodromantis gastrica (Praying mantis)	Mantodea: Mantidae	Predator

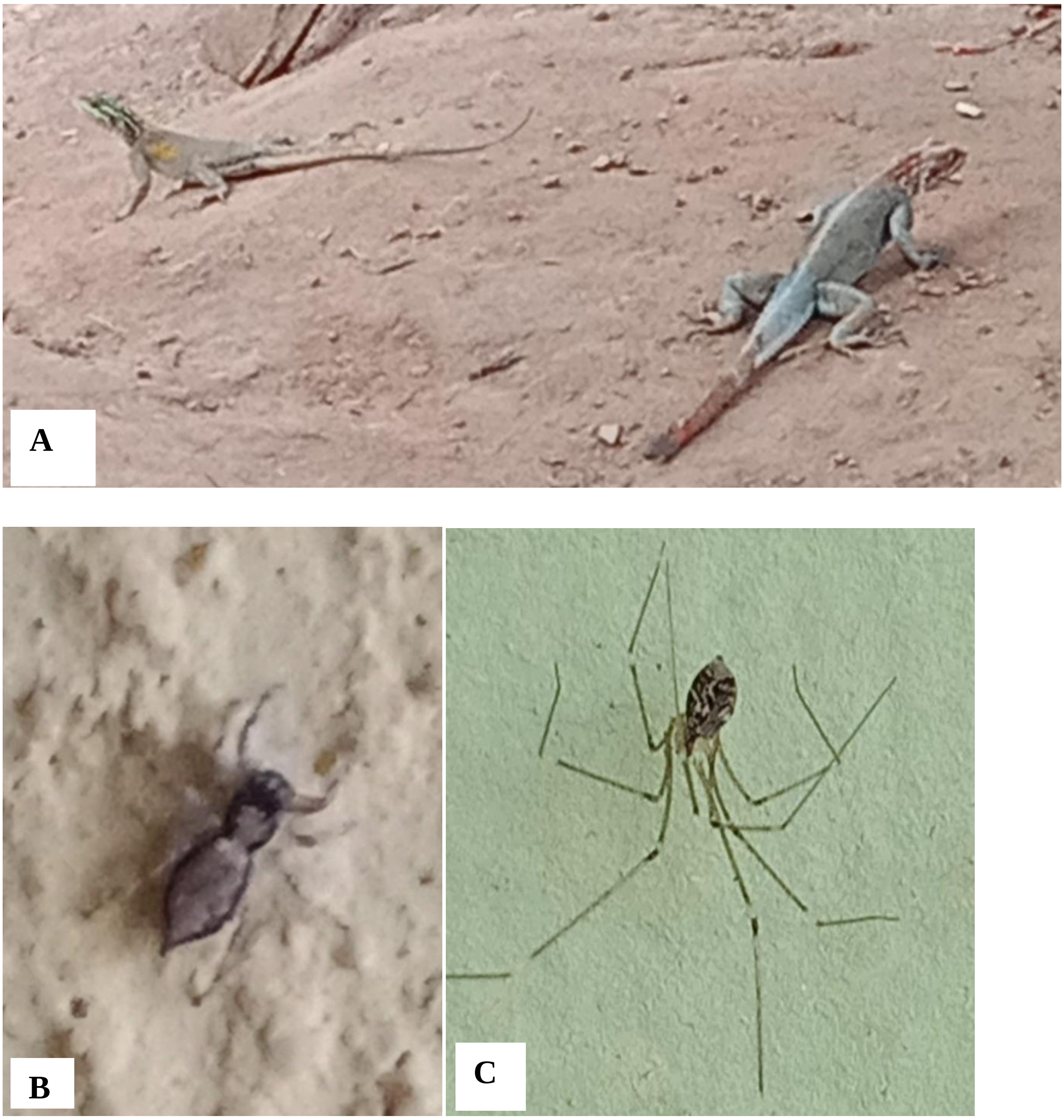

Plate 4:5: Observed natural enemies: A: ***Agama agama B: Heliophanus*** **sp. C:** ***Icius insolidus***

4.10. Growth characteristics of tomato planted in the screen house

Tables 4.18 to 4.20 showed the growth characteristics of the twenty accessions planted in the screen house. The growth was progressive across all the accessions. Only seven accessions had branched out by the 14th day after transplant, NGB00715, NGB00717, NGB00719, NGB00721, NGB00726, NGB00729, and NGB00735. By the 4th week after transplanting, as shown in Table 4.19, number of leaves had increased with increase in the number of branches and leaf area. The accession with the highest height was NGB00716 (74.23 ± 3.22cm), the shortest accession was NGB00714 at 35 ± 0.83 cm. Highest number of leaves were found on NGB00746 (42.00 ± 0.5 leaf/plant), while the lowest was on NGB00696 (14.33 ± 0.67 leaf/plant). The leaf area was widest in accession NGB00729 (96.81 ± 8.59 cm^2), while accession NGB00711 and NGB00746 both had leaf area 42.01 ±3.94 cm^2 each. Table 4.20 shows growth performance of the accessions by 6th week after transplant. Plant height was highest in accession NGB00735 (98.2 ± 0.93 cm), accession NGB00714 had the shortest height (54.67 ± 0.53 cm). Number of leaflets per leaf was highest in accession NGB00746 (24.5 ± 1.45 leaflets/leaf). Accession NGB00726 had 22.0 ± 1.50 leaflet/leaf, NGB00715 had 19.5 ± 0.29 leaflet/leaf, and the lowest leaflets/leaf was on NGB00714 (10.0 ± 0.23 leaflets/leaf). At 14 days after transplanting (DAT), the stem girth of accession NGB00737 was the thickest (0.42 ± 0.00), followed by accessions NGB00746 (0.41 ± 0.01), and NGB00714 (0.39 ± 0.27). The smallest stem girth was (0.31 ± 0.01) By 28 DAT, accession NGB00721 (0.81 ± 0.03), while the smallest was accession NGB00714 (0.61 ± 0.00). The branches were documented by the 28 DAT, accession NGB00746 (7.00 ± 0.58) recorded the highest number of branches, while accession NGB00714 (1.33 ± 0.88). By 42 DAT accessions NGB00746 and NGB00749 had the highest number of branches 8.00 ± 0.58 each.

4.11. Resistance studies: Choice and no-choice tests

Resistance studies conducted included the effect of different accessions on the development of *T. absoluta* in a choice and no-choice tests. Data on antibiosis, antixenosis, and tolerance through yield, and the results are presented

4.11.1. Choice test: Oviposition of *Tuta absoluta* on different accessions

Table 4.21 shows the average number of eggs laid per leaflet on different accessions in a choice test experiment within 24 hours. The highest number of eggs laid per leaflet was on NGB00746

Table 4.18: Growth parameters of tomato accessions at 14 days after transplanting (DAT)

Accessions	Plant height	No of leaves	Stem girth (cm)	Leaf area (cm^2)
NGB00696	25.9 ± 0.40	8.3 ± 0.33	0.3 ± 0.01	19.5 ± 1.93
NGB00698	17.9 ± 0.53	7.7 ± 0.67	0.3 ± 0.02	36.6 ± 4.71
NGB00711	25.5± 1.21	9.3 ± 0.88	0.4 ± 0.35	13.3 ± 2.19
NGB00714	17.7 ± 0.38	8.3 ± 0.67	0.4 ± 0.27	25.9 ± 2.90
NGB00715	35.7 ± 2.34	9.0 ± 0.58	0.3 ± 0.02	34.4 ± 3.58
NGB00716	35.7 ± 0.75	9.0 ± 0.58	0.3 ± 0.01	20.7 ± 0.61
NGB00717	29.4 ± 2.61	10.0 ± 0.58	0.3 ± 0.03	33.7 ± 2.57
NGB00719	27.4 ± 2.04	10.7 ± 0.88	0.4 ± 0.02	36.1 ± 2.59
NGB00720	23.7 ± 2.04	9.7 ± 0.67	0.4 ± 0.04	20.9 ± 5.74
NGB00721	24.9 ± 2.43	11.0 ± 1.00	0.3 ± 0.02	10.8 ± 2.02
NGB00724	23.1 ± 0.75	8.3 ± 0.33	0.4 ± 0.02	21.5 ± 0.50
NGB00725	22.7 ± 0.58	10.7 ± 0.33	0.4 ± 0.02	37.9 ± 1.33
NGB00726	26.9 ± 1.05	10.0 ± 0.58	0.3 ± 0.06	34.3 ± 0.89
NGB00729	36.4 ± 1.44	13.0 ± 0.58	0.3 ± 0.04	32.7 ± 1.28
NGB00734	28.9 ± 1.96	11.3 ± 0.33	0.4 ± 0.02	35.7 ± 2.85
NGB00735	33.3 ± 1.35	11.7 ± 0.33	0.4 ± 0.03	15.4 ± 0.94
NGB00737	31.8 ± 0.44	8.7 ± 0.33	0.4 ± 0.00	34.8 ± 0.34
NGB00741	25.0 ± 0.32	8.3 ± 0.33	0.4 ± 0.02	40.9 ± 2.34
NGB00746	33.8 ± 0.56	8.7 ± 0.33	0.4 ± 0.01	27.4 ± 1.15
NGB00749	28.9 ± 0.71	10.0 ± 0.58	0.4 ± 0.01	38.7 ± 1.02

n=5, Mean ± SE

Table 4.19: Growth parameters of different accessions of tomato at 28 days after transplanting (DAT)

Accessions	Plant height (cm)	Leaf/plant	Branches	Stem girth (cm)	Leaf area(cm^2)
NGB00696	56.9 ± 0.27	14.3 ± 0.67	1.7 ± 0.33	0.7 ± 0.01	47.1 ± 0.31
NGB00698	44.0 ± 0.49	18.7 ± 0.33	1.7 ± 0.33	0.6 ± 0.00	69.1 ± 3.68
NGB00711	49.0 ± 3.60	24.0 ± 2.52	1.7 ± 0.33	0.7 ± 0.04	42.0 ± 3.94
NGB00714	35.7 ± 0.83	30.0 ± 2.08	1.3 ± 0.88	0.6 ± 0.00	64.2 ± 1.72
NGB00715	67.2 ± 1.03	19.0 ± 0.58	2.3 ± 0.33	0.6 ± 0.02	82.7 ± 0.83
NGB00716	74.2 ± 3.22	19.3 ± 0.33	2.7 ± 0.33	0.6 ± 0.01	56.1 ± 1.57
NGB00717	61.3 ± 2.51	21.0 ± 0.58	4.7 ± 0.33	0.7 ± 0.03	64.5 ± 3.98
NGB00719	60.9 ± 1.87	22.0 ± 0.58	3.0 ± 0.01	0.6 ± 0.05	87.0 ± 5.98
NGB00720	47.6 ± 0.12	17.7 ± 0.67	3.3 ± 0.67	0.7 ± 0.04	49.1 ± 9.46
NGB00721	46.6 ± 1.73	30.7 ± 2.73	1.7 ± 0.33	0.8 ± 0.03	44.3 ± 0.01
NGB00724	45.7 ± 0.75	12.3 ± 0.88	3.0 ± 0.58	0.7 ± 0.03	47.2 ± 1.77
NGB00725	44.4 ± 1.90	15.7 ± 0.88	3.3 ± 0.33	0.6 ± 0.03	59.1 ± 3.90
NGB00726	56.1 ± 1.94	18.7 ± 1.20	3.3 ± 0.33	0.6 ± 0.01	77.7 ± 2.45
NGB00729	65.5 ± 3.10	27.7 ± 2.73	4.0 ± 0.58	0.7 ± 0.02	96.8 ± 8.59
NGB00734	49.0 ± 1.82	20.7 ± 0.88	2.7 ± 0.33	0.7 ± 0.02	80.2 ± 2.36
NGB00735	65.4 ± 0.50	20.0 ± 0.58	2.7 ± 0.67	0.6 ± 0.01	48.2 ± 1.26
NGB00737	65.6 ± 2.07	16.0 ± 1.15	4.7 ± 0.33	0.7 ± 0.00	47.1 ± 0.31
NGB00741	52.6 ± 0.55	15.3 ± 0.88	5.0 ±0.58	0.7 ± 0.02	69.1 ±3.68
NGB00746	43.7 ± 0.81	42.0 ± 2.31	7.0 ±0.58	0.7 ± 0.01	42.0 ±3.94
NGB00749	57.9 ± 1.22	17.0 ± 0.58	6.0 ±1.53	0.7 ± 0.01	64.2 ±1.72

n =5, Mean ± SE

Table 4.20: Growth parameters of different accessions of tomato at 42 days after transplanting

Accessions	Plant height (cm)	No of leaves	Leaflets/leaf	Branches	Stem girth (cm)	Leaf area (cm^2)
NGB00696	83.9 ± 2.74	22.7 ± 1.45	10.5 ± 0.31	4.0 ± 0.58	1.0 ± 0.01	144.2 ± 6.13
NGB00698	74.1 ± 0.47	25.3 ± 0.33	12.7 ± 0.12	3.3 ± 0.33	1.0 ± 0.01	151.4 ± 7.20
NGB00711	72.8 ± 6.13	31.0 ± 5.51	10.4 ± 0.43	3.3 ± 0.67	1.0 ± 0.02	107.1 ± 3.58
NGB00714	54.7 ± 0.53	26.0 ± 2.52	10.0 ± 0.23	4.0 ± 0.58	1.0 ± 0.01	102.9 ± 1.84
NGB00715	85.5 ± 2.80	24.3 ± 1.76	19.5 ± 0.29	5.0 ± 0.58	1.0 ± 0.02	163.2 ± 0.65
NGB00716	90.8 ± 6.01	16.7 ± 1.20	14.5 ± 0.11	5.0 ± 0.58	1.0 ± 0.00	107.7 ± 0.99
NGB00717	78.3 ± 4.02	19.3 ± 1.45	15.0 ± 0.56	5.0 ± 0.58	1.0 ± 0.01	143.7 ± 3.98
NGB00719	77.6 ± 11.00	19.7 ± 0.33	14.5 ± 0.31	4.0 ± 0.58	1.0 ± 0.03	129.2 ± 2.98
NGB00720	80.0 ± 3.45	15.0 ± 0.58	15.0 ± 0.12	3.7 ± 0.33	1.0 ± 0.02	158.3 ±15.07
NGB00721	67.4 ± 1.82	25.7 ± 2.19	12.7 ± 1.00	3.3 ± 0.33	1.1 ± 0.01	121.3 ± 3.54
NGB00724	76.0 ± 0.80	12.3 ± 0.88	11.0 ± 0.21	3.0 ± 0.58	1.0 ± 0.02	161.9 ± 0.12
NGB00725	65.1 ± 0.27	15.7 ± 0.88	18.0 ± 0.91	3.3 ± 0.33	1.0 ± 0.01	170.1 ± 5.96
NGB00726	93.7 ± 2.60	23.7 ± 1.76	22.0 ± 1.50	3.7 ± 0.67	1.1 ± 0.02	173.7 ± 2.86
NGB00729	98.5 ± 0.53	23.7 ± 1.76	12.3 ± 0.32	4.0 ± 0.58	1.0 ± 0.02	157.6 ± 3.08
NGB00734	75.9 ± 0.20	17.3 ± 1.20	15.0 ± 0.12	5.0 ± 0.58	1.3 ± 0.02	158.5 ± 4.67
NGB00735	98.2 ± 0.93	19.4 ± 0.34	16.0 ± 0.13	5.3 ± 0.33	1.1 ± 0.01	117.8 ± 7.20
NGB00737	88.6 ± 0.58	16.0 ± 1.15	18.0 ± 0.78	4.7 ± 0.33	1.0 ± 0.03	144.2 ± 6.13
NGB00741	77.6 ± 0.61	13.4 ± 1.67	15.2 ± 0.15	5.0 ± 0.58	1.1± 0.02	151.4 ± 7.20
NGB00746	65.0 ± 0.55	42.0 ± 2.31	24.5 ± 1.45	8.0 ± 0.58	1.0 ± 0.01	93.8 ± 15.13
NGB00749	87.8 ± 0.96	17.0 ± 0.58	13.7 ± 0.42	8.0 ± 0.58	1.2 ± 0.04	102.9 ± 1.84

n =5, Mean ± SE

Table 4.21: Oviposition, hatchability, and adult emergence of *Tuta absoluta* on tomato accessions in a choice test

Accession	Oviposition	Larvae emergence	Larval survival (%)	Adult emergence	Adult emergence (%)
NGB00696	5.8 ± 0.58b	4.2 ± 0.45	72.41	1.4 ± 0.51	33.33
NGB00698	4.2 ± 0.37c	2.2 ± 0.58	52.38	0.6 ± 0.25	27.27
NGB00711	5.2 ± 0.73b	2.6 ± 0.51	50.00	0.8 ± 0.37	30.77
NGB00714	4.6 ± 0.51c	3.2 ± 0.37	69.57	0.6 ± 0.25	18.75
NGB00715	3.8 ±0.37cd	2.6 ± 0.51	68.42	0.6 ± 0.25	23.08
NGB00716	3.8 ±0.37cd	1.8 ± 0.37	47.37	0.8 ± 0.37	44.44
NGB00717	3.6±0.25cd	2.2 ± 0.37	61.11	0.8 ± 0.49	36.36
NGB00719	4.0 ± 0.71c	2.2 ± 0.37	55.00	0.8 ± 0.37	36.36
NGB00720	4.0 ± 0.32c	2.2 ± 0.20	55.00	0.8 ± 0.49	36.36
NGB00721	4.2 ± 0.37c	1.8 ± 0.20	42.86	0.8 ± 0.49	44.44
NGB00724	3.4±0.51cd	1.4 ± 0.40	41.18	0.8 ± 0.71	57.14
NGB00725	3.0 ± 0.32d	1.8 ± 0.37	60.00	0.8 ± 0.37	44.44
NGB00726	3.8±0.37cd	1.6 ± 0.25	42.11	1.0 ± 0.45	62.50
NGB00729	2.8 ± 0.58d	1.2 ± 0.49	42.86	0.6 ± 0.25	50.00
NGB00734	5.6 ± 0.51b	2.8 ± 0.74	50.00	0.8 ± 0.37	28.57
NGB00735	3.2 ± 0.37d	1.4 ± 0.4	43.75	0.4 ± 0.25	28.57
NGB00737	3.8±0.37cd	1.8 ± 0.58	47.37	0.6 ± 0.25	33.33
NGB00741	3.4 ± 0.52d	2.0 ± 0.45	58.82	0.8 ± 0.37	40.00
NGB00746	6.2 ± 0.66a	2.8 ± 0.66	45.16	1.0 ± 0.45	35.71
NGB00749	4.6 ± 0.54c	2.6 ± 0.60	56.52	0.8 ± 0.37	30.77

Means followed by the same letter (s) in the same column is not significantly different ($p>0.05$) following Tukey's Studentized Range (HSD) Test

(6.2 ± 0.66), followed closely by NGB00696 (5.8 ± 0.58), NGB00734 (5.6 ± 0.51) and NGB00711 (5.20 ± 0.73eggs/leaflet). The lowest number of eggs was laid on accession NGB00729 (2.8 ± 0.58). Percentage survival for larvae was 72.41% on NGB00696, 69.57% on NGB00714 and 68.42% for NGB00715. Survival rate on eight of the accessions, NGB00716, NGB00721, NGB00724, NGB00726, NGB00729, NGB00735, NGB00737, and NGB00746, was less than 50%. Adult emergence was low (< 50%) in most of the accessions and the lowest was on accession NGB00714 (18.75%). Adult emergence was more on accessions (NGB00726 and NGB00724) high at 62.50% and 57.14 % respectively.

4.11.2. No Choice test: Oviposition of *Tuta absoluta* on different accessions

In a no-choice test, the eggs laid per leaflet on each accession was highest on NGB000746 with 11.4 ± 1.03 eggs per leaflet; followed by NGB00737 (10.8 ± 1.39), NGB000696 (10.6 ± 0.68), NGB000741 (9.8 ± 0.66), and NGB000736 (9.6 ± 0.51). The accession with the lowest number of eggs was NGB000724 with 6.2 ± 3.43 eggs per leaflet (Table 4.22).

Eclosion (%) was highest in accession NGB00716 (80.00%), followed by NGB00741 (79.59%), NGB00737 (77.78%), NGB00734 (72.91%) and NGB00714 (71.96%). Seven accessions had less than 50% eclosion (%) which include accessions NGB00717 (47.06%), NGB00719 (44.44%), NGB00720 (47.50%), NGB00721 (43.18%), NGB00725 (48.39%), NGB00725 (41.05%) and NGB00749 (45.00%). The remaining eight accessions (NGB00696, NGB00698, NGB00711, NGB00715, NGB00726, NGB00729, NGB00735 and NGB00746) ranged between 50% to 69%.

Adult emergence was highest in accessions NGB00696 (56.76%) and NGB00725 (50.00%), other accessions recorded less than 50% adult emergence (%) per leaflet. Total days to adult emergence in days varied with accessions. The longest day was on accessions NGB00724 (25.31), NGB00749 (25.29) and NGB00725 (25.11).

Sex ratio was affected by the different accessions. Eighteen of the accessions had more females than males, while accessions NGB00749 had a sex ratio 1:1 (male:female), and accession NGB00729 had fewer female adult released (1:0.7). The accessions with the highest female sex ratio was NGB00721 with 1:2.5 sex ratio for males and females, while accessions NGB00698 and NGB000719 had sex ratios 1:2 for male and females. The remaining accessions ranged between 1:1.1 to 1: 1.8 (male:female) sex ratios.

Table 4.23 shows the leaf damage by second instar larval across the accessions at 8 hours, 16

Table 4.22: Oviposition, hatchability, and adult emergence of *Tuta absoluta* on tomato accessions in a no-choice test

Accession	Oviposition	Larvae emergence	Eclosion %	Adult emergence	Adult emergence %	Total Days to Emergence (days)	Sex ratio	
							Male	Female
NGB00696	10.6 ±1.52a	7.4 ±2.51	69.81	4.2±0.37	56.76	21.21	1	1.3
NGB00698	8.6 ±1.82a	4.6 ±0.68	53.49	2.2±0.58	47.83	22.93	1	2
NGB00711	9.2 ±3.27a	5.4 ± 0.81	58.70	2.0±0.55	37.04	23.87	1	1.3
NGB00714	8.6 ±1.52a	5.8 ±0.97	71.96	2.2±0.74	37.93	24.67	1	1.4
NGB00715	9.2 ±1.92a	5.8 ±0.97	63.04	2.2±0.66	37.93	23.54	1	1.8
NGB00716	9.0 ±1.87a	7.2 ± 0.66	80.00	2.8±0.37	38.89	24.25	1	1.3
NGB00717	6.8 ±1.3ab	3.2 ± 0.37	47.06	1.4±0.40	43.75	24.05	1	2.5
NGB00719	9.0 ±1.58ab	4.0 ±0.84	44.44	1.6±0.25	40.00	24.09	1	2
NGB00720	8.0 ±1ab	3.8 ±0.37	47.50	1.4±0.40	36.84	24.38	1	1.3
NGB00721	8.8 ±0.84ab	3.8 ±0.49	43.18	1.4±0.40	36.84	25.17	1	2.5
NGB00724	6.2 ±0.84bc	3.0 ±0.32	48.39	1.0±0.32	33.33	25.31	1	1.4
NGB00725	7.8 ±0.84abc	3.2 ±0.37	41.03	1.6±0.40	50.00	25.11	1	1.4
NGB00726	7.8 ±2.39abc	4.2 ±0.58	53.85	1.4±0.40	28.57	22.99	1	1.3
NGB00729	7.2 ±1.1abcd	3.6 ±0.68	50.00	1.0±0.45	27.78	22.93	1	0.7
NGB00734	9.6±1.14abcd	7.0 ±1.38	72.91	2.4±0.68	34.29	24.71	1	1.4
NGB00735	7.8 ±1.3abcd	4.2 ±0.66	53.84	1.4±0.60	33.33	23.64	1	1.4
NGB00737	10.8±3.11abd	8.4 ±0.98	77.78	3.2±0.86	38.10	23.95	1	1.7
NGB00741	9.8±1.48abcd	7.8 ±0.66	79.59	3.6±0.51	46.15	23.91	1	1.1
NGB00746	11.4 ±2.3a	7.2 ±0.66	63.16	2.8±0.37	38.89	24.38	1	1.3
NGB00749	9.2±1.92abcd	4.2 ±0.37	45.65	1.2±0.49	28.57	25.29	1	1

n=5, Means followed by the same letter (s) in the same column are not significantly different ($p > 0.05$) following Tukey's Studentized Range (HSD) Test

Table 4.23: Leaf damage assessment of second larval stage on different accessions

Accession	8hrs (Mean % ± SE)	16hrs (Mean %± SE)	24hrs (Mean % ± SE)
NGB00696	2.2 ± 0.28a	4.3 ± 0.23a	6.5 ± 0.30a
NGB00698	2.1 ± 0.36ab	3.3 ± 0.27a	5.6 ± 0.11a
NGB00711	3.4 ± 0.1bc	5.9 ± 0.17ab	8.1 ± 0.19cd
NGB00714	4.6 ± 0.15bcd	9.0 ± 0.30c	11.3 ± 0.24df
NGB00715	3.2 ± 0.19ace	7.5 ± 0.25bcd	13.6 ± 0.48egh
NGB00716	3.6 ± 0.33acef	7.2 ± 0.34bcd	13.7 ± 0.36egh
NGB00717	5.1 ± 0.17cf	8.3 ± 0.33cd	11.0 ± 0.21df
NGB00719	4.1 ± 0.16cef	7.1 ± 0.20bcdf	12.7 ± 0.6ef
NGB00720	5.8 ± 0.39de	10.4 ± 0.42cg	14.7 ± 0.44egh
NGB00721	4.1 ± 0.26cef	8.8 ± 0.43cdfg	13.1 ± 0.28degh
NGB00724	3.5 ± 0.56bcef	7.0 ± 0.45bdf	13.0 ± 0.34degh
NGB00725	4.3 ± 0.42cef	7.9 ± 0.58cdf	15.9 ± 0.66fh
NGB00726	3.7 ± 0.31acef	7.8 ± 0.33bcdf	13.9 ± 0.47egh
NGB00729	2.2 ± 0.29acef	5.1 ± 0.33abfh	9.7 ± 0.27cd
NGB00734	3.9 ± 0.6acef	7.2 ± 0.58bcdf	13.8 ± 0.48efgh
NGB00735	5.9 ± 0.11d	9.2 ± 0.24cdgi	13.3 ± 0.44degh
NGB00737	3.0 ± 0.27acef	8.2 ± 0.32cdfi	13.7 ± 0.14egh
NGB00741	4.2 ± 0.38cef	8.2 ± 0.62cdfi	13.7 ± 0.37egh
NGB00746	3.6 ± 0.41acef	8.4 ± 0.36cdfi	12.4 ± 0.42defg
NGB00749	2.1 ± 0.16acef	6.9 ± 0.17bdfh	12.7 ± 0.51defgh

n=4. Means followed by the same letter(s) in the same column is not significantly different from one another (p>0.05)

hours, and 24 hours interval. There was significant difference in the damage done by the larval on the different accessions. At 8 hours after infestation the highest damage was on accession NGB00735 (5.9 %). Other notable damages at 8 hours include accessions NGB00720 (5.8%), NGB0071 (5.1%). The least damage at this timeframe was seen on accessions NGB00749 (2.1%), NGB0000698 (2.1%) and NGB00696 (2.2%).

At 16 hours after infestation, damages had increased to 10.4% in NGB00720, while it was 9.2% in NGB00735 and 9.0% in NGB00714. Damages, below 5%, were recorded in accessions NGB00696 (4.3%), and NGB00698 (3.3%). Damages in other accessions exceeded 5%. After 24 hours of infestation, damage was highest on NGB00725 (15.9 ± 0.66), followed by NGB00720 (14.7 ± 0.44). Thirteen accessions recorded damages in the range of 13.0% to 13.8%, while the remaining five accessions had the following data.

Table 4.24 shows the leaf damages by third instar assessed at different timeframe (8 hours, 16 hours and 24 hours). At 8 hours frame, damage was highest on NGB00724 (12.2%), NGB00720 (12.1%) and NGB00719 (12.0%), while those with less than 5% were accessions NGB00734 (3.7%), NGB00729 (4.0%) and NGB00737 (4.0%) and NGB00735 (4.9%). (28.4 ± 0.75) respectively. At 16 hours after introduction of the third instar, accession NGB00729 (11.6 ± 0.19) had the lowest leaf damage by tomato leaf miner. The highest damages were recorded on accessions NGB00698 (18.7%), NGB00724 (18.7%), and NGB00724 (18.7%). After 24 hours, the lowest value of leaf damage was recorded on accession (NGB00696) with 20.2 ± 0.67. Whilst the highest value was recorded for accession NGB00721 (28.4%). There was significant difference in the values at 5% level of significance.

4.11.3. Pre-adult developmental period of *Tuta absoluta* on different tomato accessions (Antixenosis bioassays)

The developmental period on the pre-adult stages were observed and measured for eggs, the four larval stages and pupal stage. The results are presented in Table 4.25. The developmental period for the eggs on the accessions ranged from 3.1 to 5.2 days. The longest period was on NGB00714 (5.2 ± 0.02), while the fastest time of development was on accession NGB00726 (3.1 ± 0.01). There were significant differences in the developmental period across the accessions. For the first instar stage, the longest period was on accession NGB00716 at 3.8 ± 0.04 days, followed by accession NGB00721 at 3.7 ± 0.02 days. The fastest development was on accession NGB00696 at 2.9 days. The development on other accessions significantly differs

Table 4.24: Leaf damage assessment of third larval stage on different accessions

Accession	8hrs (Mean ± SE)	16hrs (Mean ± SE)	24hrs (Mean ± SE)
NGB00696	8.9 ± 0.44a	13.2 ± 0.35a	20.2 ± 0.67a
NGB00698	9.6 ± 0.19ab	18.7 ± 0.45b	27.0 ± 1.50b
NGB00711	11.2 ± 0.47bc	16.6 ± 0.47bc	23.0 ± 0.35abc
NGB00714	11.2 ± 0.27bc	18.7 ± 0.44bd	23.6 ± 0.46abcd
NGB00715	11.2 ± 0.4bc	16.2 ± 0.42ab	22.4 ± 0.63acd
NGB00716	10.1 ± 0.32bc	13.1 ± 0.32a	22.9 ± 1.58acd
NGB00717	10.5 ± 0.25abc	17.6 ± 0.21bd	25.0 ± 0.17bcd
NGB00719	12.0 ± 0.98c	17.3 ± 0.53bd	25.5 ± 1.06bcd
NGB00720	12.1 ± 0.17cd	17.9 ± 0.66bd	24.6 ± 0.81bcd
NGB00721	11.3 ± 0.34bcd	17.0 ± 0.4bde	28.4 ± 0.75bd
NGB00724	12.2 ± 0.29cd	18.7 ± 0.63bd	24.6 ± 0.67bcf
NGB00725	9.6 ± 0.33abc	17.5 ± 0.25bd	26.4 ± 1.08bcdf
NGB00726	6.6 ± 0.40	16.5 ± 0.26abd	26.0 ± 0.35bcdfg
NGB00729	4.0 ± 0.27e	11.6 ± 0.19acf	23.7 ± 0.66abcdfg
NGB00734	3.7 ± 0.19e	15.7 ± 1.93abdg	23.5 ± 0.43abcdg
NGB00735	4.9 ± 0.34e	13.9 ± 0.54acefg	22.2 ± 0.48acdg
NGB00737	4.0 ± 0.30e	13.3 ± 0.5acefg	22.1 ± 0.81acd
NGB00741	8.2 ± 0.57abf	13.6 ± 0.57acfg	24.8 ± 0.27bcdfg
NGB00746	9.0 ±0.44abcf	13.1 ± 0.37afg	25.4 ± 0.49bcdfg
NGB00749	9.1 ±0.77abcf	13.8 ± 1.01acefg	23.9 ± 0.75abcdg

n=4. Means followed by the same letter(s) in the same column is not significantly different from one another (p>0.05)

Table 4.25: Pre-adult development period, in days, on twenty tomato accessions in the screen house

Accession	Egg (Mean ± SE)	1st stage (Mean ± SE)	2nd stage (Mean ± SE)	3rd stage (Mean ± SE)	4th stage (Mean ± SE)
NGB00696	4.1 ± 0.02abd	2.9 ± 0.21a	2.6 ± 0.18a	2.5 ± 0.11f	2.72± 0.184a
NGB00698	4.2 ± 0.08ab	3.4 ± 0.04b	2.5 ± 0.03a	2.8 ± 0.05ef	3.217± 0.055b
NGB00711	4.1 ± 0.00ab	3.4 ± 0.02b	3.4 ± 0.03bc	3.6 ± 0.08b	2.543± 0.003ac
NGB00714	5.2 ± 0.02	3.4 ± 0.04b	3.3 ± 0.02bc	3.5 ± 0.02b	2.375± 0.002c
NGB00715	3.6 ± 0.06c	3.3 ± 0.03bc	3.3 ± 0.02bcd	3.2 ± 0.01d	3.283± 0.017bd
NGB00716	4.3 ± 0.06a	3.8 ± 0.04d	3.3 ± 0.00bcd	2.8 ± 0.10e	3.433±0.032bde
NGB00717	4.1 ± 0.00ab	3.6 ± 0.00bd	3.3 ± 0.01bcd	3.1 ± 0.00de	3.102± 0.007bd
NGB00719	3.9 ± 0.02bd	3.2 ± 0.01ab	3.4 ± 0.01bc	3.2 ± 0.02cd	3.185± 0.002bd
NGB00720	4.0 ± 0.00abd	3.5 ± 0.01bd	3.4 ± 0.01bc	3.3 ± 0.01bc	3.542± 0.003e
NGB00721	3.9 ± 0.03bd	3.7 ± 0.02d	3.4 ± 0.02bc	3.6 ± 0.00a	3.46± 0.006de
NGB00724	4.1 ± 0.12ab	3.4 ± 0.01b	3.4 ± 0.00bc	3.4 ± 0.01b	3.443±0.005bde
NGB00725	4.0 ± 0.00abd	3.7 ± 0.13cd	3.4 ± 0.01bc	3.5 ± 0.02b	3.058± 0.01bd
NGB00726	3.1 ± 0.01	3.3 ± 0.01b	3.2 ± 0.01cde	3.0 ± 0.02e	3.213±0.003bde
NGB00729	4.0 ± 0.03abd	3.3 ± 0.01b	3.3 ± 0.02bcd	2.8 ± 0.02ef	2.462± 0.008c
NGB00734	3.9 ± 0.01bd	3.9 ± 0.01d	3.6 ± 0.01b	3.1 ± 0.01d	3.413±0.007bde
NGB00735	3.9 ± 0.01bd	3.2 ± 0.00ab	3.2 ± 0.00cde	3.2 ± 0.01d	3.16± 0.004bd
NGB00737	4.5 ± 0.01	3.3 ± 0.01b	3.0 ± 0.01e	3.0 ± 0.00de	3.19± 0.005bd
NGB00741	4.1 ± 0.01abd	3.5 ± 0.02b	3.2 ± 0.01cde	3.4 ± 0.01b	3.412± 0.008bd
NGB00746	4.1 ± 0.01abd	3.2 ± 0.00b	3.0 ± 0.01e	3.1 ± 0.02d	3.81± 0.006
NGB00749	4.2 ± 0.02abd	3.5 ± 0.00bd	3.3 ± 0.00bcd	3.8 ± 0.00	3.558± 0.004e

n=6. Means followed by the same letter(s) in the same column is not significantly different from one another (p>0.05) Tukey's Studentized Test)

from each other. For the second instar stage, development of the larvae was retarded on accession NGB00734 at 3.6 ± 0.02 days, while it was fastest on accession on NGB00698 at 2.5 ± 0.03 days. The developmental period was around 3 days in most of the accessions, while on accessions NGB00696, NGB00737, NGB00746 the developmental period was 2.6 ± 0.18, 3.0 ± 0.01 and 3.0 ± 0.01 respectively, these, however, were not significantly different from many of the other accessions. Table 4.24 shows the pre-developmental period on the different accessions. The third instar stage was delayed on accession NGB00749 at 3.8 ± 0.00, the fastest development period was on accession NGB00696 at 2.5 ± 0.11. Also, NGB00698 (2.8 ±0.05), NGB00716 (2.8 ± 0.10) and NGB00729 (2.8 ± 0.02) encourage the fast development of third instar larval stage. Fourth instar larval stage was short on accessions NGB00714 (2.4 ± 0.00 days) and MGB00729 at 2.5 ± 0.01 days. The development was less than three (3) days on accessions NGB00696 (2.7 ± 0.18), and NGB00711 (2.5 ± 0.00 days), while it lasted a longer duration on accessions NGB00720 (3.5 ± 0.00 day) and NGB00721 (3.5 ± 0.01 days). There were significant differences among some of the accessions, such as NGB00696 and NGB00749, while other accessions were interwoven such as NGB00735, NGB00737 and NGB00741. The ANOVA showed that there was a significant difference at 5% level of significance, on the developmental biology of *Tuta absoluta* at egg, and larval stages but not on the pupae stage.

4.11.4. Resistance to *Tuta absoluta*: Performance of different tomato accessions under screen house conditions

Table 4.26 shows the result of the harvest from the screen house. All the accessions flowered, but there was a high rate of dropping. Highest yield was on accessions on NGB00734 (15kg/plot), followed by NGB00716 (9.8kg/plot), NGB00737 (6.7kg/plot), and NGB00749 (6.6kg/plot). Accessions NGB00721 (2.3kg/plot), NGB00726 (1.9kg/plot), NGB00717 (1.8kg/plot), NGB00715 (1.3kg/plot), NGB00720 (1.0kg/plot), NGB00724 (1.8 kg/plot), and NGB00729 (1.3 kg/plot) produced less than 3kg/plot tomatoes. The remaining accessions (NGB00714, NGB00719, and NGB00735) had yields less than 2 kg/plot. There was no harvest from accessions NGB00696, NGB00711 and NGB00718. These included the highest yielding accessions on the open field such as NGB00718 and NGB00711. Hence, yield loss (%) was 100% in those three accessions. Yield loss was lowest in NGB00734 (64.5%), followed by accessions NGB00716 (75.1%), NGB00735 (87.6%), and NGB00737 (89.8%). In the remaining accessions the yield loss exceeded ninety percentages (90%).

Table 4.26: Yield of tomato under infestation in screen house

Accession	Harvest (kg/plot)		Yield loss (kg)	Yield loss (%)
	Control	Infested		
NGB00696	34.5	0	34.5	100
NGB00711	158.3	0	158.3	100
NGB00714	28.5	0.7	27.8	97.5
NGB00715	49.9	1.3	48.6	97.4
NGB00716	39.4	9.8	29.6	75.1
NGB00717	57.3	1.8	55.5	96.9
NGB00718	188.6	0	188.6	100
NGB00719	75.4	0.3	75.1	99.6
NGB00720	58.0	1.0	57.0	98.3
NGB00721	55.5	2.3	53.2	95.9
NGB00724	56.6	1.8	54.8	96.8
NGB00725	30.0	1.2	28.8	96.0
NGB00726	29.5	1.9	27.6	93.6
NGB00729	35.4	1.3	34.1	96.3
NGB00734	42.2	15.0	27.2	64.5
NGB00735	48.2	0.6	42.2	87.6
NGB00737	65.7	6.7	59.0	89.8
NGB00741	72.0	0.2	71.8	99.7
NGB00746	37.0	0.5	37.0	100
NGB00749	65.6	6.6	59.0	90.1

CHAPTER FIVE

DISCUSSION

Agriculture is germane in addressing most of the Sustainable Development Goals (SDGs), and horticultural crops play key role in achieving some of these goals. However, horticultural crops production is usually fretted with lot of constraints-biotic and abiotic factors. Insect infestation is one of the concerns in tomato production globally, especially leaf miners. In Nigeria, tomato production is usually during the dry season up north using irrigation system, and during the raining season down south hence there are different array of insect pests that attack tomato at these different seasons of planting.

In this research, we set up a town-gown interaction to have a better understanding of *Tuta absoluta* infestation on tomato production in Southwest Nigeria. The findings showed that majority of the farmers in Southwest, Nigeria are males. The modal age range was 40 – 59, there is a need to encourage younger generation to get involved in agriculture, putting to use their technological know-how to increase productivity. Majority of the respondents (88.7%) were married, while just 7.3% were single. The import of this is that many cannot afford to take risks as there are dependents looking up to them for sustenance, unlike the single that are not yet saddled with the responsibility of parenting. The singles can take calculated risks to increase productivity through different means like obtaining more landed properties, investing in hydroponics, experimenting with new varieties and hybrids, among other options available.

A demographical study of these states showed that males play a major role in tomato farming (79.1%), while the womenfolk contribute 20.9% to farming of tomatoes. This agrees with the report of Banjo, *et al.,* (2003) that 75% male in Ogun, Oyo, and Lagos States participated in horticultural crops production. Okunlola and Ofuya (2010) reported 60% male participation in Ondo State. However, more women are involved in the marketing aspect of the production. A survey of the major markets in the Southwest showed that 100% of Yoruba people that sell tomatoes are female folks. This helps in achieving one of the SDGs, which is to achieve gender equality and empower all women and girls.

For most of the respondents, farming is the only source of income for them, this agrees with Umeh *et. al.,* (2002) who reported that majority of tomato farmers derived more than 50% of their income from farming only. The importance of this is that any infestation or attack on their farm would affect their source of livelihood. This was the situation in the North in 2016, where tomato plantations were lost to *Tuta absoluta* infestation; many farmers became despondent and committed suicide. However, this is helpful for researchers, because it makes the farmers willing and ready to participate in research that will benefit their farming production and increase productivity.

Education plays a major role in farming enterprise in various ways. In this work, more than 65% of the respondents have secondary school education, 5.3% had no formal education, while 11.3% had tertiary education. Okunlola and Ofuya (2010) reported that 44% of their respondents had secondary school education, while 6% had tertiary education. This makes it easy for extension workers to communicate with farmers on pest infestation prevention measures, and management practices to prevent loss on the farm. Increase in agricultural productivity has been attributed to education, this research thus corroborates the works of Alene and Manyong (2007) which opined that schooling enhances agricultural productivity in a rapidly changing technological or economic environment.

Education helps farmers make informed decisions as per the activities on the farms to prevent, or control pest's infestation. Hence, the high percentage of educated farmers in Southwest would be expected to respond more quickly to changes than their counterparts in the Northern Nigeria. This work showed that the level of education is reflected in the responses of the farmers to the questions posed to them. Many of them knew about biopesticides, the importance of parasitoids and predators on the field, and intercropping with right combination of crops as control measures on the farm. However, less than 40% knew about the inefficiency of chemical pesticide in controlling *T. absoluta,* majority believed that chemical pesticide is efficient in controlling the pest. This can be attributed to the fact that *T. absoluta* is not a major pest on the open field, thus the assumption by many that chemical pesticide is efficient in controlling it. The use of chemical pesticides poses environmental risks to the fauna in the ecosystem and potential bioaccumulation of these toxic substances in higher animals like man. Hence, its use should be discouraged, or minimal, if compulsory.

It is, however, praiseworthy to note that majority of the farmers are educated as this would increase farmers' adoption of new technologies and then increase the ability of the farmers to reduce insect pest infestation through adoption of new varieties, efficient practice of Integrated Pest Management (IPM), judicious use of pesticide and fertilisers among many others. Furthermore, being educated makes it easy to disseminate information and knowledge of the pest to the farmers.

Cropping system plays a major in the fauna ecosystem of a farm, sole cropping encourages pest infestation at a large scale, while mixed cropping encourages a gamut of predators and parasitoids on the field, thus reducing the effect of pests on the field. Interestingly, more than fifty percent of respondents sampled practiced mixed cropping system. This could also have contributed to the absence of *T. absoluta* on the field. The absence of *Tuta absoluta* on tomato field in the Southwest could be associated with the fact that majority of the farmers engaged in mixed cropping, which helps increase the insect diversity on the field. Increase in species diversity balances the predator-prey interaction in an agro-ecosystem. The findings of the survey are in alignment with what Degri and Samaila (2014) reported that there is a decrease in insect pests of tomato when intercropped with maize in the North, and Okunlola (2009) reported decrease in insect pests on leaf vegetables when intercropped in Southwest, Nigeria. The finding showed that in Southwest, Nigeria majority of farmers practiced mixed cropping system which is different from what is practiced in the north as reported by Umeh *et. al.* (2002), where more than 50% farmers practiced monocropping in Bauchi, Kaduna, and Plateau States, while 90% practiced monocropping in Kano State.

Furthermore, the absence of *T. absoluta* on the open field can be associated to the rainfall pattern during planting season, which exceeded 218 mm (8.6 inch) during 2019 and 2020 raining seasons. Chen *et al.,* (2019) reported in their work on diamondback moth (*Plutella xylostella*), that rainfall reduced rate of development of diamondback moth by limiting it to pupation without adult emergence. Farmers plant during the raining season in the Southwest unlike their counterparts in the north who plant during dry season while depending on irrigation to water the plants.

Many of the respondents have more than 20 years of farming experience, thus they can easily identify most of the common insect pest that affects their tomato plants. They can also separate emerging or new pest from the regular pest they usually experience. This makes it easy to alert extension workers, who can provide the needed information for management of such new pest that they may notice.

The general perception of the respondents was that *T.* absoluta was not a problem in the open field, and in case of infestation, majority of the respondents (69.5%) supported the implementation of immediate control measure to nib it at the bud. Borisade et al., (2017) reported that 90% of their respondents held same views. However, our respondents opted for the use of botanical (88.5%) and chemical pesticides (87.7%) because they are readily available when compared to biopesticides and resistant varieties.

The farmers reported different insect pests of importance to them, and this varies with each state surveyed. In Oyo State, three insects were reported and observed to be of economic importance to the farmers which were Aphids, *Helicoverpa armigera* and grasshoppers. Farmers in Ekiti State reported that *Helicoverpa armigera*, and grasshoppers are the major insects of economic importance to them, while farmers in Ogun State complained of locust as the insect pest of concern to them, although *Helicoverpa armigera* was also reported as an important pest. The importance of this is that each state must be treated separately when it comes to insect pest management practices, since they are faced with different insect pests of concern. All the farmers believed that *Tuta absoluta* is an important pest but not a threat in open field.

Although, there were no presence of *Tuta absoluta* in the open fields surveyed in the various states, it is important to say that *Tuta absoluta* is present in some screen houses. The samples used for this research work were obtained from an infested screen house farm in Agege, Lagos. Furthermore, there was reported incidence of *Tuta absoluta* in two screen houses in Ibadan, Oyo State and Abeokuta, Ogun State; the invasive led to discontinuation of tomato production in those two screen houses. From the samples obtained from Lagos, the morphological and molecular characterization of the pest confirmed the identity of the insect to be *Tuta absoluta*. Thus, *Tuta absoluta* is in Southwest, Nigeria.

The samples were reared to obtain baseline information on the biology and ecology of the pest needed for its management. Although, several researchers have recorded varying developmental periods for *T. absoluta* based on different environment and treatments. This research provided information on the life cycle of *T. absoluta* recorded on sweet tomato variety, and this was the same with what was reported by Muniappan, (2013) having four main stages; egg stage, four larval stages, pupal, and then adult moths which are small (7.0 mm).

A survey for occurrence and abundance of *Tuta absoluta* on tomato plant in Ibadan (open field) was negative. There was no occurrence of *Tuta absoluta* infestation on the tomato in the open field. This could be attributed to the fact that farming is usually done during raining season in Southwest, Nigeria. Rainfall has been reported to have adverse effect on the development of insects. It affects insect pests such as *Bemisia tabaci* (whitefly), *Plutella xylostella* (diamondback moth), and *Frankliniella occidentalis* (thrips) (Palumbo, 2019). *Tuta absoluta* being a microlepidopteran like *Plutella xylostella* might not be able to withstand the impact of the heavy rainfall associated with rainforest. Bacteria wilting were the major problem observed during the field trial.

Postharvest losses were due to attack by birds and shrews, which fed on the fruits and cut the stems of the plants. Yield was highest on accession NGB00718 (26.1 tonnes/ha), while the lowest yield was on accession NGB00714 (2.4 tonnes/ha). The harvest would have been much than that if not for the attacks by birds and shrews. There were accessions with large fruit weight that still yielded low harvest such as NGB00716 with 20.5g/fruit yet the average harvest was 4.9 tonnes/ha which was lower than NGB00741 (2g/fruit) at 7.9 tonnes/ha. Although, NGB00741 had more fruits per truss than NGB00716, but that should not lead to the high yield disparity as seen during the experiments. Similar yield disparity was noticed among other accession; NGB00714 (10g/fruit) produced the lowest yield (2.4 tonnes/ha) while NGB00725 (2g/fruit) yielded 3.2 tonnes/ha. Thus, small fruits can yield large harvest just like accessions with large fruit weight too.

The survey for *Tuta absoluta* in the open field in selected states in Southwest was also negative. However, farmers reported that *Helicoverpa armigera* (cotton bollworm), *Zonocerus variegates* (grasshopper), and *Bemissia tabacci* (whitefly) were insect of economic importance in tomato production. The distribution of these insect pests was scattered across the states. The main insect pest reported in Oyo state was *Helicoverpa armigera*. This can be attributed to difference in agroecology differences of the ADPs surveyed in each state.

However, the presence of *Tuta absoluta* is confirmed in screen houses in Oyo and Ogun states, unfortunately the screen houses have closed operations due to the impact of infestation. This corroborates the works of Borisade *et al.* (2017), Sanda *et al.* (2018) and Aigbedion-Atalor *et al.* (2019), who all reported the presence of *Tuta absoluta* in Southwest, Nigeria. However, the distribution range was not clear.

The molecular characterization and damage characteristics on tomato leaves confirmed the moth to be *Tuta absoluta*; this shows that *Tuta absoluta* is present in Southwest, Nigeria. It is, however, not a major pest of concern on the open field from the field survey conducted during this research. Tomato plants in screen houses are susceptible to infestation; there are undocumented reports of infestation in screen houses in Ibadan, and Abeokuta. This was confirmed by the preliminary survey carried out during this research work.

The molecular characterization shows that the sequences of cytochrome oxidase subunit I (COI) gene from *Tuta absoluta* shares same root with those from USA (MT021750), Egypt (KY129667), Kenya (KU565497), Togo (MN759250), Nigeria (MK189159), and South Africa (KY212128). This presupposes that the strain might have migrated from USA to Africa through Egypt from where it must have moved down to East Africa before getting to West Africa and finally in South Africa, this seems probable as Mansour *et al.* (2018) reported the same pattern in their review. However, it is a strain that is different from sequences from Spain and Brazil. The implication of this is that the biology might be different and hence management practices should be localized to address our local strain. It also shows that *T. absoluta* is highly invasive and constantly expanding its distribution across the globe. Its ability to achieve this can be associated with its high reproductive rate, wide developmental thermal regime covering winter, summer, and autumn, and absence of proper surveillance and monitoring being an invasive pest.

Furthermore, we can attribute this wide distribution to inter- and intra-continental trades and lack of effective sanitary and phytosanitary measures at the borders. For instance, the 2016 outbreak in the northern Nigeria was attributed to the importation of new varieties by a major farmer then. Another reason that could be adduced to this increasing distribution across the globe is the resistant nature of this pest to conventional pesticides. Moreover, allowing visitors, who are not properly kitted or sanitized, into the screen houses could also induce infestations.

A thorough understanding of the reproductive and developmental biology of an insect pest helps in effective control of such pest. Hence, the study showed that the biology of *Tuta absoluta* on sweet tomato variety takes 20.23 days from egg laid to adult emergence at 27.3 ± 2.17 ºC, and RH 75.3 ± 1.6%. This is shorter to what was reported by Borisade *et al.* (2017), who reported a developmental period of 23.8 days at temperature of 27 °C; this difference can be attributed to the change in temperature which modulates the developmental period of the pest. Cuthbertson *et al.* (2013) reported development from egg to adult at 37 days at 19 °C, 23 days at 25 °C. Munniapan (2013) reported a developmental period of 83 days at 14 °C. Duarte *et al.* (2015) reported the effects of temperature on the developmental period of *T. absoluta*, pupal viability and sex ratio. Thus, temperature plays an important role in the development of *T. absoluta*. However, the difference could also be because of varieties used, the secondary metabolites in varies from variety to variety, and would have the development of insect pest on it in some ways. In they work, Duarte *et al.* (2015) reported difference in pre-oviposition, oviposition, and post oviposition of adult moths reared on cultivars Vyta and Bravo. The duration of *Tuta absoluta* development was, thus, affected by environmental factors and variety. Also, the morphological traits of the emerging adults and sex ratio of emerged adults could be affected. The resistance studies from this work further showed that the development was affected by the different accessions; however, it did not stop the damages on the plants. Hence, there is a need for further investigation of varieties that could affect the development of *T. absoluta*.

The morphometrics showed that the egg was 0.44 ± 0.001 in length, 0.22 ± 0.002 in width and the development was for 4.1 ± 0.02 days. The morphometrics were not too divergent from what other researchers (Munniapan, 2013; Cuthbertson *et al.*, 2013; Mutamiswa *et al.*, 2017) reported in their various works. The larval stage lasted for 12 days, and the most susceptible time for use of insecticide is when the first instar larval stage is making attempt to penetrate the leaf surface. The time observed (25 minutes) in this study for the tunnelling by the first instar larva was shorter to what was reported by Cuthbertson et al. (2013), which was 82 minutes before the larval fully tunnelled into the leaf.

The damaged leaves have blotch-shaped mines, with black frass at the entrance of the mines. The leaves become useless to the plant as they lose their ability to photosynthesize after the mesophyll has been consumed by the pest. Hence, yield was poor; the fruits produced were infested and damaged by the pest feeding on the fruits from the crown of the fruit. Then secondary infestation sets in leading to fruit decay. This was not different from other researchers' work. Damage characteristics of *T. absoluta* seem to be the same globally. This makes identification easy and helps in the introduction of immediate control measures to forestall severe damage on the farm.

Oviposition was lowest on accession NGB00729 (2.8 ± 0.58) in the choice test, but in no-choice test oviposition was lowest on accession NGB00724 (6.2 ± 0.84). However, the highest oviposition was recorded on accession NGB00746 in both choice and no-choice experiments, this shows that accession NGB00746 was the choice plant for oviposition by *Tuta absoluta*. The reason could be associated to the type of trichomes the accession had, or the leaf volatile compounds it produces. Proffit *et al.* (2011) found that leaf volatile composition had effect on the oviposition of female adults on cv. Aromata when compared to cvs Carmen and Santa Clara. They reported that the absence of some compounds, such as terpenes, made cv Aromata less suitability for oviposition. Oliveria *et al.* (2009) reported that presence of allelochemicals such as 2-tridecanone, acyl sugars and/or zingiberenes influences oviposition; this difference in cultivar contributes to the susceptibility of tomato plants to *Tuta absoluta* infestation. This means that there is a need to quantify the chemical compositions of the twenty accessions used in this research to identify the reason for low oviposition on the accessions.

This study shows that different accessions affected the larvae' morphometrics, developmental period and sex ratio of emerged adults of *Tuta absoluta* at ambient temperature and relative humidity. Also, access of adults to water and different concentrations of sugar solution also affected the reproductive biology of *Tuta absoluta*. When fed on water fecundity was lower compared to females fed on 10% sugar concentration, but there was no significant difference in the fecundity of females fed with 5% sugar solution and water. When exposed to multiple mating, females had highest fecundity, the implication is that females would lay more eggs in a screen house and thus damage will be higher than open field. Also, females that have oviposited previously still mated with males and laid more eggs though fewer than the earlier oviposition rate, with lower hatchability. Furthermore, deuterotokous parthenogenesis was observed among the female moths. Although, this is relatively rare in the order of Lepidoptera, yet about 20 species belonging to several families of Lepidoptera, Gelechiidae inclusive, are known to produce asexually (Caparros and Haubruge, 2012). Thus, it is no real surprise to see this phenomenon in *Tuta absoluta*. This means male trapping and mating disruption through sex pheromone, and sterile insect techniques would not be effective in management of infestation. However, mortality of eggs was highest (66.84%) compared to other bioassays. Hatchability of 83.94% was recorded in females fed with 5% sugar concentration; this implies that presence of glucose at a right proportion had positive effect on hatchability of the eggs. Females fed 10% sugar concentration had egg hatchability of 78.34% with 21.66% egg mortality, thus, excess sugar concentration affected hatchability negatively. When fed on water and mated, males' longevity was shortened compared with virgin males and males fed on different sugar concentrations. Mating generally increased the lifespan (longevity) of the female moths compared to unmated moths. This means the females can produce more eggs with increased longevity, coupled with fact that they still mated after oviposition.

The sex ratio varied under the resistance studies carried out. This can be attributed to several factors such as population mating structure, and environmental factors. In this case, the environmental factors would include variety and temperature. Temperature affects sperm viability and thus might favour female population as adaptation for survival by the insect. This might be the reason for the high sex ratio in favour of the female in this work. Since, parthenogenesis is possible, nature helped in preservation of the species by producing more females who can reproduce the next generation of insects. The variety of tomato might have also contributed to this sex ratio as adult emergence was less than 50% in nineteen of the accessions investigated. This might be a survival mechanism by the insect to perpetuate its existence in a harsh environmental condition.

The life table shows that net reproductive rate (R_0) which is higher than one (1), gross reproduction ratio (GRR) and doubling time (DT) indicates fast populational growth rate for *T. absoluta* in the laboratory. The cohort generation time (T_c) and corrected generation time (T) showed that *T. absoluta* can reproduced multiple times in a year under these conditions, and hence it is a multivoltine insect. This confirms Munniapan (2013, 2015) reports that *T. absoluta* can reproduce up to 10 -12 times in a year. At this rate a great devastation would be caused in growing season. This is evident in all the reports *T. absoluta* infestation where 80 % - 100% damage has been reported.

This study showed that *T. absoluta* is preyed on in the screen house by many predators such as ants, spiders, and reptiles. Some of the predators are common fauna seen in and around domestic areas such as *Tapinoma melanocephalum* and *Agama agama*. This could be because of the structure and location of the screen house. From our observation all stages of development were preyed on except the 2nd instar stages. This could be associated to the fact that the development of the 2nd larval stage took place in the leaf where is it almost difficult for predators to access. Eggs were easily scraped off the leaves by ants, such as the *Tapinoma melanocephano* (Fabricius) (black ants), and *Odontomachus clarus* (trap-jaw ants), same with first instar stages; the time of tunnelling into leaves exposes them to predation. Second instars larval were more secured in the leaves than other instar stages. The third and fourth instars larval stages were exposed to predation when exiting a depleted leaf for another leaf. Furthermore, the fourth instars larval stage was also exposed to predation when dropping down to pupate in the ground. Generally, by the time the plant had been totally depleted of all mesophyll; all the instars were faced with this challenge when they all had to drop down from the plants. The predators had a field day feeding on them all.

The ants all belonged to the order Hymenoptera and family Formicidae. The spiders belonging to the order Araneae, and family Salticidae, and reptiles such as tropical house gecko (*Hemidactylus lurcicus),* lizard *(Agama agama)* and skinks *Trachyleplis striata* belong to the order Squamata. These predators were observed preying on different stages of *Tuta absoluta* in the screen house. The reptiles were observed feeding on the adult moths, thus reducing their chances of continuously oviposition. However, rate of success was low. Spiders were also observed preying on the adult moths. Zappala *et al.* (2013) reported more than seventy arthropod species (including members of order Hymenopterans), 20% of which are predators attacking moths across 12 countries, only few parasitoids were recorded. Although, the families recorded were different from what was observed in this research, difference in climatic conditions (Europe, the Middle East, and North Africa) and nature of farming enterprise (open field, protected susceptible crops and wild flora) could be the reasons for this observation. This however shows that the moths are susceptible to a wide range of predators and parasitoids, which can be maximized in Integrated Pest Management this is in correlation with Oke *et al.* (2017) report that state that *Tuta absoluta* is susceptible to large natural enemy pool. These observed natural enemies are different from what other researchers observed on the field. Sankarganesh *et al*. (2017) reported Mirid bug (*N. tenius*) as being the dominant predator on their field experiment.

The study showed that all the accessions were susceptible to attack by the moths, although the moths' development was affected differently by the accessions in a choice and no-choice bioassays. However, the reproductive rate of the pest showed that with just one female moth on a tomato plantation, a great damage can still be caused on the plantation in a relative short time. The sex ratio on "No Choice test" shows that majority had more female than male adult emergence except for two accessions with higher male to female sex ratio NGB00729 (1:0.7) and NGB00735 (1:0.4). Thus, sex ratio, parthenogenicity and increased longevity all combined to favour the invasion of this invasive pest in new areas.

Most of the accessions tolerated the infestation but had low yield. More than sixty percent losses were for all the accessions. There was 100 % loss in accessions NGB00696, NGB00711 and NGB00718. These results corroborated what has been reported by Desneux *et al.* (2010) and Zappala *et al.* (2012) that yield loss was in the range of 80% to 100%.

CHAPTER SIX

Summary and Conclusion

6.1: Summary:

From this research it is observed that women participation in tomato farming is low compared to the males. The major problem, noticed during this research, with tomato production in the Southwest is bacteria wilt. The problem is compounded by rain splashes, these causes a lot of damage to tomato production in the Southwest. Also, noted is the problem of flooding, tomato being a weak stemmed plant cannot withstand the volume of rainfall in the southern part of Nigeria.

The field survey showed that *Tuta absoluta* was not a problem on the open field; however, its presence in Southwest cannot be denied or ignored. Notwithstanding, tomato farmers were able to identified the pest when shown pictures and damage characteristics. More than 88% of the respondents indicated the use of botanicals as management preference for *T. absoluta*. Furthermore, the presence of *Tuta absoluta* was confirmed in screenhouses. Samples obtained from the infested screenhouse were identified at molecular level. The result showed that *Tuta absoluta* is biphyletic (Brazil and Spain), and the variant present in Nigeria is closely related to the Brazilian variant. The bioecology, and natural enemies of the pest were successfully observed and documented for further research purposes.

The complete lifecycle observed for *Tuta absoluta* in Ibadan was 19 to 25 days. The Total developmental period for eggs, larva and pupa was 3-4, 10-13, and 6-8, respectively. The larvae went through four larval instar stages, and the second instar is more difficult to control. The morphometrics showed that female moths are bigger than the male; the longevity of the moth is determined by the sex, with the females living longer than the males. Deuterotokous parthenogenesis was observed.

6.2: Conclusion:

Thus, this work supplies baseline information on the identity and biology of the pest for further research. This information is important for pest's surveillance and control.

6.3: Recommendation:

Female participation should be encouraged to meet up with the Sustainable Development Goals, one of which is to achieve gender equality and equal representation in all fields of endeavours. The fifth goal of the SDGs is to eradicate gender inequality in workplaces, education and wages by empowering all women and girls. Hence, there is the need to encourage female participation in farming, to meet up with this SDGs mandate. To achieve this, government could provide incentives for female farmers, just like female students are encouraged with stipends. Subsistence farming is tasking and demanding, government can invest in mechanized farming, hydroponics, and other advancement in farming technology that are less tasking.

To reduce spoilage by bacteria wilt, raised bedding for tomato planting is suggested for farmers. Furthermore, farmers can plant crawlers which would be properly staked to avoid the problems. Those who can afford it can engage in irrigation during dry season planting or hydroponics. Better still government could encourage farmers to go into large scale farming using screen houses.

There is a need to conduct research on the direct and indirect effect of rainfall on *Tuta absoluta* survival and development on tomato plants. This can include the direct and indirect effect of rain splashes on tomato plants and how it affects development of *Tuta absoluta* on the plant in the open field. This will help to understand the relative absence of *T. absoluta* on the open field in Southwest, Nigeria.

Furthermore, awareness and enlightenment programmes to educate farmers need be conducted to discourage the abuse of chemical pesticides, which have adverse effects on the environment. With the understanding of the biology of *Tuta absoluta* provided in this work, it should be easy to conduct management research to find an effective and efficient means of controlling the pest on tomato field. With the parthenogenesis observed during this research, some methods of control should be discouraged such as sex-pheromone traps, and sterile males' releases. Targeting the eggs and the first instar larval stages before tunnelling into the leaves would be an efficient control method. Once the larval succeeds in tunnelling into the leaves, control becomes difficult. To control the larvae in the leaves, there will be a need for systemic approach; this might involve the use of synthetic chemicals which are dangerous to the environment and human beings. Hence, there is a need for an integrated approach to manage this pest.

The temperature in screen houses, oftentimes, are higher than the surrounding temperature, hence there is a need to study the effect of temperature on adult fecundity, longevity, and sex ratio. These all contribute to the population density of insect pests and could play a role in management techniques.

There is a need for effective monitoring of its spread and conducting pest risk assessment in Southwest. Furthermore, farmers need to be trained especially those with screen houses, on introduction of biological agents, and more coordinated approach by various stakeholders such as extension workers, researchers, and farmers to curb the expansion of the pest.

There is a need to keep testing for resistance in more tomato accessions, in the meanwhile further works should be done with the accessions that exhibited tolerance.

6.4: Contributions to knowledge

1. *Tuta absoluta* is currently not a field pest of tomato but a very serious pest of screenhouse in Southwest, Nigeria.
2. *Tuta absoluta* is biphyletic (Spain and Brazil variants), the variants present in Southwest, Nigeria is the same variant with those from Spain.
3. *Tuta absoluta* has four larval instar stages and completed its entire life cycle within 20 to 23 days at 27.3 ±3 ºC in Ibadan, Nigeria, thus the insect can reproduce many times in a year (multivoltine).
4. High Net Reproductive Rate (R0) of 46.01 and Cohort generation time (Tc) of 23.68 days contributes to its multivoltine nature.

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