ASSESSMENT OF INSECTICIDAL EFFECTIVENESS OF SELECTED CRUDE PLANT EXTRACTS ON THE TOMATO LEAF MINER, *Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae)*

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF NATURAL PRODUCTS TECHNOLOGY AND VALUE ADDITION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Effects of *Commiphora swynnertonii, Synadenium glaucescens* and *Allium sativum* ethanolic extracts on the tomato leaf miner, *Tuta absoluta* Meyrick were evaluated under laboratory and screen house conditions. The threes life stages (eggs, second instar larvae and adults) of the tomato leaf miner were involved in the experiment. All the life stages of tomato leaf miner were treated topically with the plants extracts at (2%, 4% and 8%). The mean percentage mortality of eggs, second instar larvae and adults were recorded daily for 5 days. Results indicated that, each plant extract caused significant mortality to larvae and adults of *T. absoluta* after 5 days in comparison to the control. In laboratory, using the leaf dipping technique, *Commiphora* showed the highest effects on *T. absoluta* second instar larvae while *Synadenium* extract exhibited the least effect. The *Commiphora* ethanolic extract was the only one that showed potential to be used as a control agent against eggs as it led to 0% egg hatchability. *Synadenium* resulted in no mortality of *T. absoluta* eggs in this trial as there was 96.6% hatchability. Leaf dipping against second instar and adult of *T. absoluta* proved to be the most effective for all plants extracts at 30-100%. *Commiphora* resulted in the highest second instar larval and adults' mortality of 100%. In the screen house *Commiphora* showed the high reduction of infestation for Tanya and Cal J varieties. Treatment with this plants extracts resulted in the highest fruit yield and the lowest yield loss compared to all the plant extracts. *Commiphora swynnertonii* extract is recommended into integrated pest management strategies for the control of *T. absoluta*.

DECLARATION

I, Rehema Esther Matendo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other Institution.

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The above declaration is confirmed by;

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Dr. H.E. Nonga Date
(Supervisor)

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Prof. A.P. Maerere Date
(Supervisor)

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DEDICATION

This work is dedicated to my parents Etienne Matendo Bugale and Celine Ngezirabona Njabuka

&

My husband Joseph Rwizibuka, my lovely children Josué, Gad and Graciella, my sisters and brothers, cousins and friends, nieces and nephew.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CI	Confidence Interval
CV	Coefficient of Variation
FAO	Food and Agriculture Organization
INTRA-ACP	Intra-African Caribbean and Pacific
LD 50	Lethal Doses 50
MAFSC	Ministry of Agriculture, Food Security and Cooperatives
MS	Means of Squares
NC	Negative Control
PC	Positive Control
SE	Standard error
SS	Sum of Squares
SUA	Sokoine University of Agriculture
UEA	Université Evangélique en Afrique
USDA	United States Department of Agriculture

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum* L.) is one of the world's major vegetables. It is an excellent source of many nutrients and its secondary metabolites like folate, potassium, vitamins C and vitamin E, flavonoids, β-carotene and lycopene are essential for human health (Tehniat *et al*., 2014). In the world, tomato is grown on more than 5 million ha with a production nearly 129 million tons per an (Srinivasan, 2010). In Tanzania, tomato production is higher than other vegetable crops with a total annual production estimated to be more than 235 000 tons (FAO, 2012).

In Tanzania, Mbeya, Iringa, Tanga, Kilimanjaro, Arusha and Morogoro are the major producing regions (MAFSC, 2003). Survey results in Morogoro Region, indicate that under current management practices, tomato yields vary from 2.2 to 16.5 t/ha (Maerere *et al*., 2006) which is below the world average of 27.5 t/ha (FAO, 2012). Among the constraints that prevent farmers from achieving potential yield are abiotic stresses like salinity, drought, excessive heat, declining soil fertility, poor crop management, lack of well-adapted and high yielding varieties and excessive pests and diseases (Ojiewo *et al*., 2010).

The tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest that originates from South America and devastates tomato plants (Miranda *et al*., 1998). Since its introduction in Spain in 2006, it has invaded most of the countries in Europe, Mediterranean, Middle East, Northern, Western and Eastern Africa, and India in South Asia (Deusneux *et al*., 2011). In Northern Africa, it was reported in

Tunisia in 2008, North of the Sahel in 2008 (NAPPO, 2012) Western Africa in 2010, Sudan and Ethiopia in 2012, Nairobi in 2013, Tanzania and India in 2014 (Muniappan, 2014).

There are no official records of this pest in Tanzania; but the main concern is that it can cause serious yield losses of 50 to 100% (Maneno *et al.*, 2015). Its control is a challenge as its rapidly develop resistance towards conventional insecticides (Moreno, 2011). The presence of *T. absoluta* in Tanzania also affects the export trade as the productivity is reduced and the local price of tomato soars. In 2015, tomato prices in the country rose by 375 percent in just a month, pushing the commodity beyond the reach of majority poor folks due to scarcity of the product resulting from the tomato leaf miner outbreak (Global Plant Protection News, 2015). Thus, quarantine of the pest is important to limit the propagation of the pest to nearby countries where it hasn't yet invaded.

1.2 Problem and Justification

Tomato is grown for the purpose of food as an important nutritious vegetable crop and source of income for many families (FAO, 2012). However, *T. absoluta* is one of the key insect pests of the tomato crop that can reduce productivity up to 100% (Derbalah *et al.*, 2012). *T. absoluta* was reported for the first time in Tanzania in 2014 and was first identified in August 2014 in Ngabobo village, Ngarenanyuki, King'ori in the Arumeru district. The pest was identified in a major tomato cultivation area, with serious consequences to the agricultural economy in Tanzania.

Although chemical control has been the main method used against *T. absoluta* in South America, the low efficiency of the active ingredients against the insect was reported since the 1990s (Campos *et al.*, 2014). Furthermore the use of synthetic pesticides poses a threat to the environment and health of tomato consumers (Mtui *et al*., 2015) but also increases production costs of up to 20 percent (Reis *et al*., 2005).

The environmental problems caused by overuse of pesticides have been a matter of concern for both scientists and public in recent years (Opender *et al*., 2008; Yusuf *et al*., 2011). About 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually. The high toxicity and non-biodegradable properties of pesticides and the residues in soil, water resources and crops affect public health (Agarwal and Walia, 2003). Thus, the need to search for the biodegradable and environmental friendly pesticides and develop techniques that can be used to reduce pesticide use while maintaining crop yields.

Survey results in Northern Tanzania showed that the use of pesticide to control pests is high, with over 40 different formulations (Actelliv 50 EC, Mamba, Cobox, Blue Copper, Round Up, Sumithion, Tilt…), probably because farmers assume that the only solution to pest problems is to spray more frequently and using different types of pesticides (Ngowi *et al*., 2007). The high dependence on pesticides by tomato's farmers is an indication that they are not aware of other pest management strategies that are effective, inexpensive and yet friendly to the environment. Extracts of many higher plants are reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory and field tests. Natural products isolated from plants may be alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Yusuf *et al*., 2011). Plant products have several uses in insect control (Moreira *et al.*, 2004; Moreno *et al.*, 2011). These products have also been studied for acute toxicity, antifeedant, or repellent, and fumigant effects, as well as inhibiting reproduction of many pest species (Ben *et al.*, 2010). Some tropical plants extracts have been used for pest control and have shown high insecticidal activity. They could therefore be used to control *Tuta absoluta* (Moreno *et al.*, 2011). Plant extracts can also increase the capability of activating defense responses of plants. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to plant-based pesticides owing to the complex mixtures of constituents that characterize many of plant (Koul *et al.*, 2008).

In this study, extracts from plants namely *Synadenium glaucescens, Commiphora swynnertonii* and *Allium sativum* were tested. These plants have been used in Tanzania traditional medicine (Bakari *et al*, 2011; Mabiki *et al.*, 2013). Previous findings show that these plants have a broad spectrum activity against common pathogen in human and animals (Bakari *et al*, 2011; Mabiki *et al.*, 2013). The exudates of *C. swynnertonii* have been used traditionally to cleanse bladder and kill insects such as ticks, lice, bed bugs and mange mite (Mabiki *et al.*, 2013). Garlic oil which is an oviposition deterrent and has been found to be highly toxic to eggs of *P. xylostella*. (Lanzotii, 2006; Kaonekane *et al.*, 2007). Stem branches and buds of *S. glaucescens* have insecticidal and repellant properties against aphids, grasshoppers and mosquitoes (Grainge and Ashmed, 1988).

1.3 Objectives

1.3.1 Overall Objective

Development of alternative method of plants extracts to use of synthetics pesticides in controlling *T. absoluta* in tomatoes in Morogoro, Tanzania.

1.3.2 Specific Objectives

i. To determine the *in vitro* effectiveness of *Commiphora swynnertonii*, *Synadenium glaucescens* and *Allium sativum* plants extracts against *T. absoluta* at three different stages (egg, larvae and adults).

ii. To assess the *in vitro* effective concentration of the plant extracts against *T. absoluta* on egg, larvae and adults.

iii. To determine the effectiveness of the plants extracts on the tomato leaf miner in the screen house.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tomato Production

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and has been used as food by the inhabitants of Central and South Americas since prehistoric times (Choudhoury, 1967). It originated from the tropics of Central and South America, extending from Mexico, Ecuador, through Chile. Tomato was transported to Europe, and improved further before reaching the United State of America and Asia (Mahmud *et al.*, 2009). It is now the most widely grown vegetable crop in the world, giving grower's income, expanding export potential and improving the supply of vitamins and minerals in human nutrition (Rajkumar, 2007).

Tomato is grown throughout the tropical and the temperate region of the world. It is noted as one of the most adaptable cultivated plant that requires a wide range of climctic conditions for seed germination, seedling growth, flavour and fruit quality. The crop grows well in fertile, well drained soils with pH of about 6 and an average monthly temperature of 21°C to 23°C. Commercially it may be grown at a temperature ranging from 18°C to 27°C (Ssekyewa, 2006). In most tropical countries, average yield ranges between two to 10 tons of fruit/ha. China is the world's top tomato grower, accounting for more than one-quarter of the world's tomato hectarages. Egypt and India together account for more than one-fifth of world total tomato production. Asia and Africa account for about 79 percent of the global tomato area with about 65 percent of world output (Srinivasan, 2010). In Tanzania tomato is produced in large quantities in Kilimanjaro (Hai, Moshi and Rombo), Arusha (Arumeru), Morogoro (Mvomero), Iringa (Kilolo), Tanga (Lushoto), Mbeya (Mbeya Rural) and Singida Region (MAFSC, 2003).

Tomato plants are of two types of growth habits, namely indeterminate and determinate. Plants with an indeterminate growth habit have main stem which extend regularly while issuing a cluster of flowers. On average, leaves are produced at an interval of every three leaves for indeterminate, while in the determinate its one to two leaves after each inflorescence. The determinate types have main stems that stop growing and produce a terminal inflorescence after having issued two to six trusses (Raemaekers, 2005). Although some cultivars have 30 or more flowers per clusters usually 4-12 flowers develop up to maturity. Self-pollination is commonly observed. Fruits of most cultivars are globoid while some are elongated. Ripe fruits colours are usually solid red, pink and orange or yellow (Rubatzky, 1996). There are three main types of tomato based on fruit size: the cherry type with small fruit of 10 to 20 g and 20 to 30 fruits; the standard type with medium-sized fruits of 80 to 120 g with five to six fruits per truss and the beefsteak type with fruits of up to 250 g with one to three fruits per truss (Mike and Hilmi, 2009).

2.2 Tomato Leaf Miner

2.2.1 Classification and Distribution of *Tuta absoluta*

Tuta absoluta (Meyrick) with common name Tomato leaf miner, tomato borer, South American tomato moth and South American tomato pinworm (NAPPO, 2012) belongs to the family *Gelechiidae* which includes nearly 400 genera and about 4000 species of small moths represented nearly all over the world. The most important insects at this family, the pin boll worm *Pectinophora gossypiella* (*Saunders)* or *Platyedra gossypiella (Saund.)* is the widest spread and one of the most destructive cotton pests in the world. The potato tuber moth *Phthorimace operculella* is the most widely spread pest of stored potato (Richards and Davies, 1983).

T. absoluta originated from South America., where it was reported since the early 1980s in Argentina, Brazil and Bolivia (Easty, 2000). From there it rapidly invaded many European and Mediterranean countries. It was first recorded in Eastern Spain in late 2006 (Urbaneja *et al*., 2007), then Morocco, Algeria, France, Greece, Malta, Egypt and other countries (Mohammed and Lobna, 2012). *Tuta absoluta* is one of the most important insect pest of tomato which pose serious threat to tomato production across the Mediterranean. This pest is crossing borders rapidly, devastating tomato production substantially. Several countries are currently considered infested with *T. absoluta* as shown on Fig. 1.

Figure 1: *Tuta absoluta* distribution

Source: www.tutaabsoluta.com site visited September 2016.

Since the introduction of *T. absoluta* in Northern Africa and North Sahel in 2008, it invaded Western Africa in 2010, Sudan and Ethiopia in 2012 (NAPPO, 2012) and Tanzania in 2014 (Muniappan, 2014). Fig. 2 shows the predicted spread of *T. absoluta* in East and Central Africa.

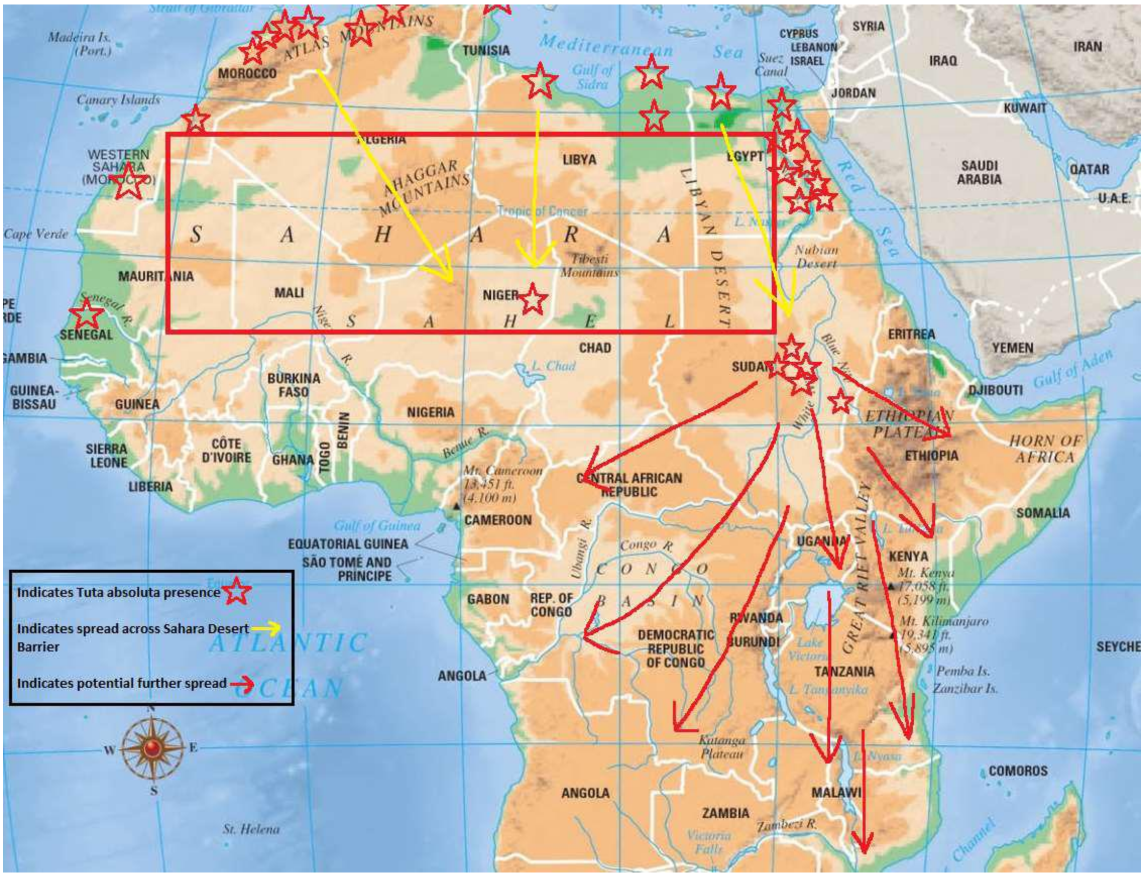


Figure 2: Predicted spread of *T. absoluta* in East and Central Africa

Source: www.tutaabsoluta.com site visited on September 2016

2.2.2 Life cycle

Tuta absoluta is a micro Lepidoptera moth with high reproductive potential, capable of up to 12 generations per year under optimal conditions. Its life cycle (Fig. 3) comprises four development stages: egg, larva, pupa and adult. The life cycle is completed within 24 days at 27°C. Eggs are small, cylindrical, creamy white to yellow-orange, and 0.35 mm long. Females usually lay eggs on the underside of leaves or stems and to a lesser extent on fruits. Egg hatch occurs in four to six days (NAPPO, 2012).

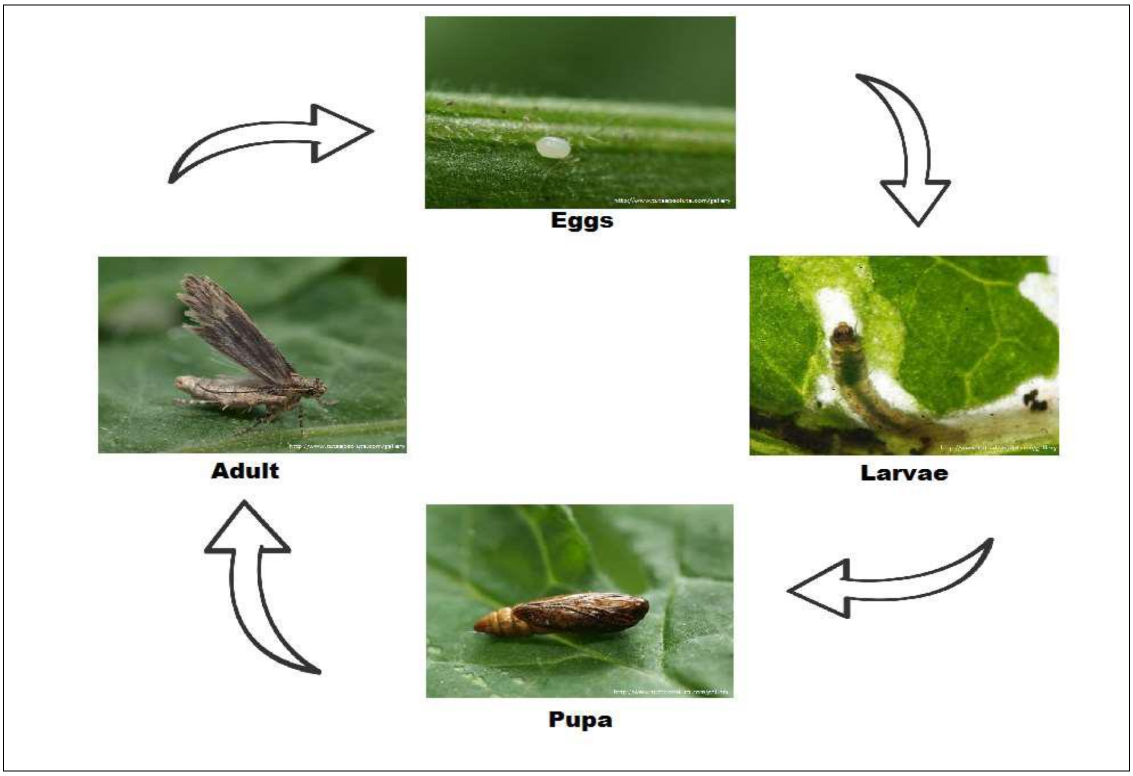


Figure 3: Life cycle of *T. absoluta*

Source: www.tuta absoluta.com site visited September 2016.

Larvae are cream coloured with a characteristic dark head and lateral spot that extends from the ocellus until the posterior margin. Larvae lack a typical dorsal plate in the prothorax. Instead they have a dark oblique band that does not cover the dorsal midline. The larval has four larval instars before transforming into the pupae stage. No diapause for the larval instars if food is available. As they grow older, they became greenish to light pink in the second to fourth instars (by feeding on leaves) and measure between 1-8 mm. The larval period is the most damaging period to plants and is completed within 12-15 days. The feeding behaviour results in irregular mines on the leaf surface. Older (3^{rd}- 4th instars) larvae can feed on all part of tomato plant. Fully-grown larvae usually drop to the ground on a silk thread and pupate in the soil. Pupation may also occur on leaves or in the calyx.

Pupae are cylindrical in shape and greenish when just formed, becoming darker in colour as they near adult emergence. They are often coated with a white silky bud. Pupae have been found outside the mine, the soil, as well as beneath posts and under greenhouse benches (Derbalah *et al.*, 2012). Adult are 5-7 mm long with wing span of 8-10 mm. The most important identifying characters are the filiform antennae, silverfish-gray scales and black spots present on the anterior wings. The adult leaf miners are small, yellow and black coloured moths. The biological cycle of this moth depends on temperature (Table 1). Low temperatures are a limiting factor for its survival, although it can overwinter as eggs, pupae or adults, depending on environmental conditions.

Table 1: Average length of the life cycle of *T. absoluta* at different temperatures

Pest stage	**Duration (Days)**		
	14°C	20°C	27°C
Egg	14.1	7.8	5.13
Larvae	38.1	19.8	12.2
Pupa	24.2	12.1	6.5
Total Egg-Adult	76.4	39.7	23.8

Source: Easty (2000).

There are indications that the moths can spread several kilometres by flying or drifting with the wind and can survive in fairly harsh conditions. This suggests that the insect spread may occur naturally or be facilitated through agricultural trade (NAPPO, 2012). Intensive spreading and dissemination of tomato leaf miner should be correlated with fruit importation and commercialization (Potting, 2009). One of the possible pathways for a long distance dissemination of *T. absoluta* could be through packaging materials (boxes) coming from infested countries (EPPO, 2010).

Since the initial detection, tomato leaf miner has become the most serious pest causing severe damage on tomato in invaded areas (Germain *et al.*, 2009). This moth was first identified in Tanzania in 2014 at Ngabobo, Ngarenanyuki, King'ori Villages in the Arumeru district. Fig. 4 shows its presence at Horticulture Unit of Sokoine University of Agriculture in Morogoro Region. Conspicuous economic losses and rapid spread along the areas of traditional tomato production make this pest the most serious threat to tomato production. Cost-benefit analysis showed that existence of a new and such a severe pest in tomato crops significantly increased costs of pest management, primarily related to the increased use of insecticides, which is causing a heavy disruption of existing Integrated Pest Management (IPM) systems (Lietti *et al*., 2005).

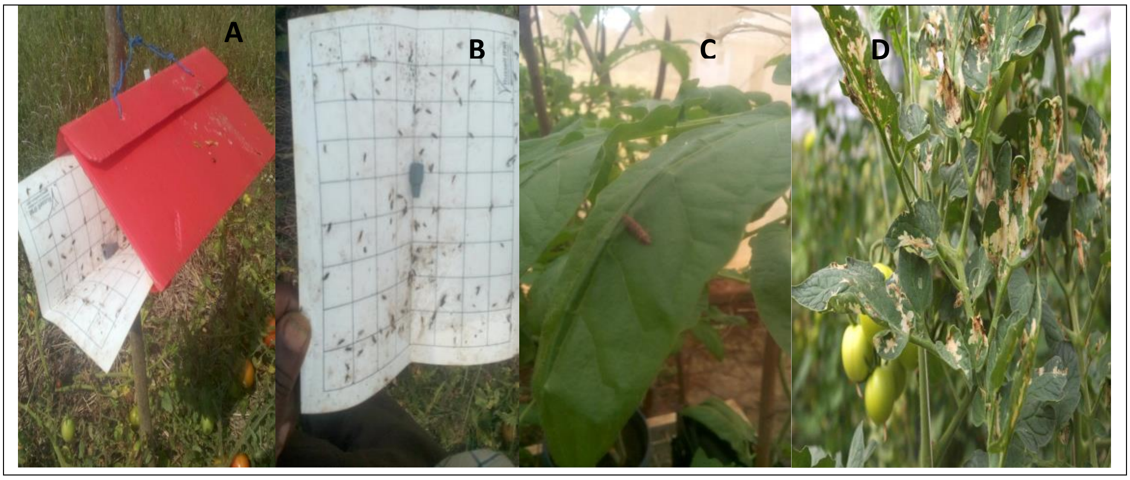


Figure 4: Presence of *T. absoluta* at Horticulture Unit at Sokoine University of Agriculture A and **B**: Mass trapping of *T. absoluta* at Horticulture Unit at SUA, **C**: Presence of adult of *T. absoluta* on tomato leaf in the screen House at Horticulture Unit at SUA, **D**: Damage caused by *T. absoluta* in the screen house.

2.2.3 Damage caused by *T. absoluta*

The larva feeds voraciously upon tomato plants, producing large galleries in leaves, burrowing in stalks, and consuming apical buds and green and ripe fruits. This can lead to cosmetic damage, leaves drying out or even early defoliation. It is capable of causing a yield loss of 100% (Derbalah *et al*., 2012). Female adults cause feeding marks where they feed. Indirect damage occurs when fungi or bacteria enter the feeding areas. Yield and fruit quality are both significantly impacted by direct feeding of the leaf miner as well as secondary pathogens entering host plants through wounds made by the pest. Larvae penetrate the fruit, leaves, or stems of host plants, creating conspicuous mines and galleries and also allowing for invasion by secondary pathogens which may lead to fruit rot (Gonzalez *et al.*, 2011).

2.2.4 Control measure of *T. absoluta*

2.2.4.1 Good agricultural practices

Good agricultural practices includes cultural practices, rotation with non-solanaceous crops, ploughing, adequate fertilization, irrigation, destruction of infested plants and post harvest plant debris. If at any time of the growing cycle it is detected or fruit stalks are seen damaged by larvae of *T. absoluta*, there will be an overhaul of the whole plot. To prevent the pest from completing its life cycle and continue to spread we have to remove the infected plants. Wild host plants should also be removed to prevent the further build up of a potential population (USDA, 2011).

2.2.4.2 Chemical and biological control

Some insecticides (indoxacarb, imidacloprid) and non-traditional methods (culture filtrate of *Bacillus thuringiensis* (Bt), *Artemisia cina (*Berg) *extract*, clove oil and nanosilica) were evaluated against *T. absoluta* in tomato under greenhouse conditions (Kaoud, 2014). Synthetic pesticides are currently the most effective means of pest control. However, the unceasing and indiscriminate uses of these substances have not only caused adverse effects on mammals' health, but have also affected many other non-target organisms (Bughio and Wilkins, 2004). Furthermore, pests such as *T. absoluta* with a high reproductive capacity and very short generation have an increased risk of developing resistance (Suinaga *et al.*, 1999; Lietti *et al.*, 2005). Some populations of *T. absoluta* have developed resistance to organophosphate and pyrethroid pesticides. Newer compound and bacteria such as spinosadimidacloprid and *Bacillus thuringiensis* (Bt) (Lietti, 2005) have demonstrated some efficacy in controlling European outbreaks of this moth. Experiments have revealed some promising agents of biological pest control for this moth, including *Nabis pseudoferus* (Hem: Nabidae) a species of damsel bug (Molla *et al.*, 2011).

2.2.4.3 Other control methods

Mass trapping: Mass trapping is a technique that involves placing a higher number of traps in the crop field in various strategic positions to remove a sufficiently high proportion of male insects from the pest population. It is widely used in conjunction with other control measures to achieve acceptable level of damage and to reduce the reliance on insecticide treatments. Mass trapping is a potential option for open field production. However, and for practical reasons, application in protected agriculture has a higher chance of success (USDA, 2011).

The light traps: It has been used to control *T. absoluta* in greenhouse tomato production in Italy as installing traps at a height of 1 meter or less from the ground and at rate of one trapper 50 to 100 m. Russel IPM, developed a light trap for *T.*

absoluta that is capable of capturing thousands of male insects in addition to a substantial number of females per night. The trap named Fibrolite –TUA uses a combination of sex pheromone and a specific light. Also there laboratory studies on the effect of gamma radiation on life stages of *T. absoluta* have been conducted in Brazil. The lethal dose for eggs was found to be 100 Gy, for preventing larvae from completing development was 200 Gy and the dose that prevented adult emergence from pupae was 300 Gy .Overall, a dose of 300 Gy was found to be lethal to all stage of *T. absoluta*. The dose that resulted in sterile adults when pupae were treated with gamma radiation was 200 Gy (Arthur, 2002).

2.3 Plants Extracts as Pesticides

Bio pesticides that include plant extracts, pheromones, plant hormones, natural plant derived regulators and enzymes as the active ingredients are used primarily as preventive measures. Thus they may not be effective as quickly as some synthetic chemical pesticides. However, bio pesticides are generally less toxic to the user and non-target organisms, making them desirable and sustainable tools for pest management (Harry *et al.*, 2014).

Many plants have essential oils or extracts that show broad spectra of activities against insect pests and plant pathogenic fungi ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities (Ghanim and Abdel, 2014). These plants also have a long tradition of use in the protection of stored products (Opender *et al*., 2008). Plants are composed of chemical substances of which some are not directly beneficial for their growth and development. These secondary compounds have usually been regarded as part of the plants' defence against plant-feeding insects and other herbivores (Rosenthal and Janzen, 1979). The pesticidal properties of many plants have been known for a long time and natural pesticides based on plant extracts such as rotenone, nicotine and pyrethrum have consequently been used in pest control during the earlier half of this century. However, after the Second World War, they lost their importance due to the introduction of synthetic organic chemicals (Harry *et al.*, 2014). The synthetics chemicals are concentrated products with a high knock-down effect on pest organisms. The chemicals can be produced in large quantities relatively cheaply and hence rapidly substituted most other pesticides in the 1950s.

Generally, farmers use synthetic chemical pesticides to abate pest incidence in the absence of alternatives. Their use has been an important part of pest management for many years; however with known disadvantages and risk; pesticide residues on food possess carcinogenic risk. This predicament results in the search for less hazardous alternatives to conventional synthetic insecticides. Among the recent efforts is the exploitation of natural products from plants that contain toxic metabolites or the use of microbial antagonists to control diseases (Leticia *et al.,* 2014).

The concept of “Green Pesticides” refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. They are safe and more compatible with the environmental components than synthetic pesticides (Isman and Machial, 2006). Controlling plant pests with plant extracts as components in integrated pest management strategy has been tested by many scientists since 1990s.

Nadia *et al*. (2014) reported that *Azadirachta indica (A. Juss)* and *Jatropha curcas* L. (Euphorbiaceae) extracts exhibited insecticidal activities on egg and larvae of tomato leaf miner under laboratory conditions. Ghanim and Abdel (2014) studied the effects of basil, geranium, chinaberry, onion and garlic aqueous extracts against the tomato leaf miner *Tuta absoluta* Meyrick in laboratory and greenhouse Chinaberry, geranium, onion and garlic showed the highest effects on *T. absoluta* second instar larvae. In greenhouse, chinaberry exhibited the highest effects on *T. absoluta* population. Some medicinal plants *Commiphora swynnertonii* (Burrt.) and *Synadenium glaucescens* (Pax) have been used as antibacterial, anti-inflammatory and anti-viral in Tanzania traditional medicine (Bakari *et al*., 2011; Mabiki *et al.*, 2013) In vitro studies of these plants have shown also effect against ticks, mites, fleas, aphids, grasshoppers and mosquitos (Grainge and Ashmed, 1988; Sambuta and Masola, 2006; Kaonekane *et al.*, 2012). On the other hand the chemical present in garlic has demonstrated insecticidal properties (Lanzotii, 2006).

2.3.1 *Commiphora Swynnertonii* (Burrt.) *(Oltemwai* in Masai language*)*

Commiphora swynertonii belonging to Burseraceae family is widely distributed in Africa and Asia and it is among the plants species commonly used in Tanzania for treatment of dysentery, while its sap is applied on animals for controlling of ticks, fleas and tsetse flies (Minja, 1999). In Asia, *Commiphora* species have been used as antibacterial, anti inflammatory, anti-cancer and antiviral agent (Praskeva *et al*., 2008). Although several scientific studies have been carried out to assess and validate the medicinal properties of *Commiphora* species (Aliyu *et al*., 2007; Musa, 2008 and Parskeva *et al*., 2008), only scanty information is available in the literature. *Commiphora swynnertonii* has been researched extensively with regard to its applications in ethnomedicine. Shen *et al*. (2007) reviewed traditional uses, phytochemistry and pharmacology of the plant. Resinous exudates of the bark of the genus *Commiphora* have been proved to have anti-inflammatory, antimicrobial and anti-cancer activities. The chemistry of the genus *Commiphora* has also been extensively studied and more than 300 compound have been isolated and identified (Hanus *et al.*, 2005; Su *et al.*, 2012), including flavonoids, terpenoids, steroids and phytosterols. Others constituents include carbohydrates and aliphatics chain derivates. Leaves, roots, barks and exudates tapped from incisionned bark of the tree are used for various medicinal applications both in human and animals (Willbroard *et al.*, 2014).

2.3.2 *Allium Sativum* (Garlic)

Garlic (*Allium sativum*) is a plant classified originally in the family *Alliceae* with edible bulb. *Allium* is the largest and most important genus of the *Alliceae* family consisting of 450 known species widely distributed in the Northern hemisphere (Muammed *et al.*, 2014). Studies have revealed that steroid saponins and sapogenins, chemicals also present in garlic such as β chlorogenins demonstrate antifungal, anti-bacterial, antitumor, anti-inflammatory, anti-thrombotic and insecticidal properties (Matsuura, 2001; Lanzotii, 2006). Garlic is a major source of sulphur containing compounds (alkyl sulfides) with different numbers of sulfur atom (i.e. mono-, di-, and trisulfide). Volatile compounds such as allicin, diallyl disulphide, diallyl trisulphide (a major constituent of garlic oil), dithiins, and ajoene originate from different metabolic pathways by tissue damage through cutting, crushing or chewing. These compounds provide to garlic its characteristic odour and flavour as well as its biological properties (Villamiel *et al.*, 2010).

The protein content (lectins being the most abundant) in garlic was found to be higher than that in other vegetables such as bean and pea (Cemeroglu and Acar, 1986). Garlic contains carbohydrates, fibre as well as fat. Garlic is known to contain high levels of potassium (21 g/kg), phosphorous (6 g/kg), magnesium (1 g/kg), sodium (532.78 mg/kg), calcium (363.61 mg/kg), iron (52.91 mg/kg), zinc (1.1 mg/kg) and iodine. In addition, to garlic contains minerals such as selenium and germanium. The amount of these minerals present in the garlic bulb like all phytochemicals is greatly influenced by the environment in which it is grown (Bandyopadhyay *et al.,* 2001). Despite the fact that the above mentioned compounds contribute in part to garlic's bioactivity, evidence from several investigations suggests that the biological function of garlic is mainly attributed to the presence of high content of organo- sulphur compounds which work synergistically with other compounds such as organo-selenium compounds. (Augustin and Mathew, 1974; Wargovich *et al.*, 1988).

2.3.3 *Synadenium glaucescens (Milk bush, Mvunjakongwa* in Swahili*, Liyugi* in *Bena* language*)*

Members of the Euphorbiaceae family are widely utilized for different purposes in the world (Mwine and Damme, 2011). Finger euphorbia is planted near coconut trees to prevent attack by Rhinocerous beetle (*Oryctes* spp.) in Kenya and similar practice occurred with another Euphorbiaceae *Pedilanthus cucullatus* in the Coastal regions of Tanzania before the Second World War. The genus *Synadenium* is indigenous to East Africa (Dev and Koul, 1997). The species *Synadenium glaucescens* (milk bush, *Mvunjakongwa* in Swahili, *Liyugi* in *Bena* language) are found growing in several regions in Tanzania where indigenous people have been using them for treatment of both animal and human illness. The plant is now used in Handeni to protect tree seedlings from termites. The milk bush, is a succulent shrub or tree of several meters high which occurs in grassland and savanna woodland throughout tropical Africa including Madagascar as well as in the Arabian Peninsula, India and the Far East. It is easy to propagate by stem cuttings and is often planted in hedges around villages in Eastern Africa (Polhill, 1988). The plant is also used as a fish poison. The plant sap, latex, can be applied as a seed dressing against the plant parasitic nematodes *Tylenchorhynchus brassicae* and *Rotylenchus reniformis* to protect vegetables. Leaves, seeds and roots are soaked in water and the solution is sprayed to protect vegetables from caterpillars and seedlings from termites (Mabiki *et al*., 2013).

The aqueous extract of the leaves and stems of *S. glaucescens* is reported to have a positive reaction for tannins, triterpenoids and coumarins while the methanol extract has steroids, triterpenes and anthocyanins its petroleum ether extract contains carotenoids, steroids, triterpenoids, volatile oils and glucosides (Rukunga *et al.*, 1990; Neuwinger, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the Experiment

The experiments were conducted at Sokoine University of Agriculture (SUA), Morogoro, Tanzania located at 6.822 latitude South and 37.661 of longitude East. The experiments were conducted in laboratory and screen house from April 2016 to August 2016. The laboratory work of preparation of plants extracts was done at the Department of Veterinary Medicine and Public Health, SUA while rearing and monitoring of *T. absoluta* was conducted at the Horticulture Unit.

3.2 Experimental Material

Seeds of tomato varieties ‘Tanya VF’ and “Cal J” were obtained from an agro-dealer in Morogoro Municipality. Roots of *S. glaucescens* were collected from Gairo district in Morogoro Region, while bulbs of *A. sativum* were purchased from the Mawenzi market in Morogoro Municipality. Extract of *C. swynnertonii* was obtained from Natural Product Laboratory in the Department of Veterinary Medicine and Public Health.

3.3 Experimental Design and Treatments

The design of the laboratory experiments consisted of a 3x3 factorial (plants extracts from 3 plants species x 3 plants extract concentrations) arranged in a completely randomised block design with three replications. In the screen house, the experiments were also arranged in a completely randomised block design of a 3x2 factorial (plants extracts from 3 plants species x 2 varieties of tomato Cal J and Tanya) with four replications. In both experiments, a synthetic insecticide BELT®SC (480

Flubendiamide) was used as the positive control; whereas sterile distilled water (SDW) containing 0.1% Tween® 20 was used as negative control. The insecticide was used as per the manufacturer's recommendations.

3.4 Tomato Plant Establishment and Management

3.4.1 Nursery Establishment

Seeds of the selected tomato varieties (Tanya and Cal J) were sown in a primary nursery bed on 30 April 2016 onto compost medium. After watering, they were put in a germination chamber for three days to enhance germination and thereafter transferred to a nursery shed. Seeds germinated within six days and were watered regularly to keep the medium adequately moist.

3.4.2 Plant management

The seedlings were transplanted at four weeks from seed sowing, when they had developed four to five true leaves. Seedlings were transplanted into polytots (one seedling per polytot) of 20 cm diameter containing 4 Kg of a mixture of forest soil, sand and compost at the ratio of 3:1:1. The polytots were kept on the bench in the screen house to avoid contact with the ground. Application of fertilizer was done three times during the experiment at the rate of four gram of NPK (15:9:20 + microelements) as per the manufacturer's recommendations. The first application was done at one week, the second after two weeks from transplanting, while the last application was at flowering period. One month after transplanting, the plants were staked using poles.

3.5 Collection of Plant Material, Extraction and Formulation

Plant parts that were used in the experiment included; root bark of *S. glaucensens*, resin of *C. swynnertonii* and bulb of *A. sativum*. The plant materials were obtained as mentioned in section 3.2. Plant materials were packed into polyethylene bags, transported to the laboratory within 24 h after collection. Plant materials were cleaned of debris using running tap water. The bark materials were first peeled from root stumps and chopped into small pieces before sun drying. For *A. Sativum*, preparation of powder was carried out according to method described by Mahmood (2009). After cleaning, the bulbs were aseptically cut into small pieces with a knife and then dried under the shade for 7 days at 32 - 35ºC. The semi-dried pieces were then blended using pestle and mortar, and left to dry under shade at room temperature for further 7 days. The materials were thereafter finely powdered with the help of a mixer-grinder and then stored in airtight bags in a cool dry room until when they were used. The resin was just kept in airtight bottle in the refrigerator until when it was used.

Solvent extraction was carried out according to the method described by Parekh and Chanda (2007) with some modification. Each elite plant powder (resin) was separately extracted in ethanol (99.8%). Exactly 100 g of ground plant materials were soaked in 500 ml of ethanol in a conical flask plugged with aluminium foil and kept for 72 h in a dark place at room temperature. The extracts, were filtered with Whatman filter paper 1 and the filtrate were concentrated using a rotary evaporator until all the solvent was cleared and then were stored at 4°C in airtight bottles until use. Serial dilution method was used to prepare the working solutions at the three different concentrations of 2%, 4% and 8% v/v. Figure 5 shows the different steps of extraction.

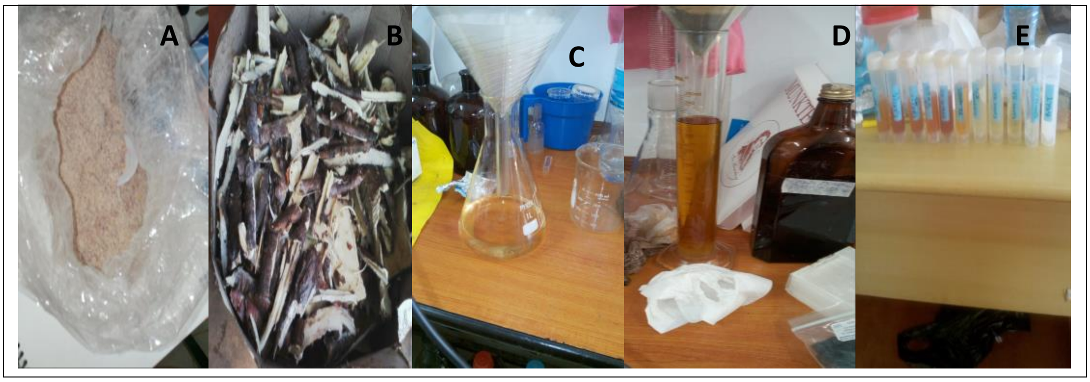


Figure 5: Preparation and formulation of different plants extracts

A: Garlic powder, **B**: roots barks of *S. glaucescens*, **C**: Filtration of *Garlic* extract **D**: Filtration of *Synadenium* extracts **E**: Preparation of test tubes with a solution of 2%, 4% and 8% of *C. swynnertonii*, *A. sativum* and *S. glaucescens*

3.6 Rearing of the Insects

Three life stages of *T. absoluta* were tested; eggs, second instar larvae and adults. The three life stage were used in assumption that if the extract had the ability to kill the egg the pest will not end his lifecycle, thus the reduction of the population of the pest can be warranted. Cohorts at the desired life-stages were obtained using the methods describe by Cuthbertson *et al*. (2007). *Tuta absoluta* adults for rearing were obtained from tomato leaves of a crop grown in the screen house at the Crop Museum at SUA. Leaves showing symptoms of leaf miner as described by USDA (2011) were collected. The leaves with typical symptoms were placed in polyethylene bags and taken to the laboratory. Leaves were sampled randomly from different infected plants within the screen house. A colony of *T. absoluta* was established from larvae and pupae collected from infected tomato. The crop in the screen house had not been sprayed with any pesticide during the season. The insect which emerged were reared and maintained in a small container (25x15x15 cm) with an anti-thrips mesh on top as shown on Fig 6. The containers were maintained filled to one fifth with sterile soil in an insectarium at 24±2°C. Leaves of tomato varieties 'Tanya and Cal J' were used to feed the insect during the larval stage and a piece of cotton wool saturated in 10% honey during the adult stage.

Figure 6: Rearing of *T. absoluta* from infested leaves collected at Crop Museum (SUA). A: Infected leaves by *Tuta absoluta* **B**: Mass rearing of *T. absoluta* in the laboratory, **C**: Cage with 5 weeks seedlings in presence of *Tuta absoluta* adults to allow egg laying

3.7 Bioassay of *T. absoluta* under Laboratory and Screen house Conditions

3.7.1 Bioassay under Laboratory Conditions

In this study three sets of experiments involving the *T. absoluta* second larvae instars, egg and the adult moth were conducted. The dipping method as describe by Cuthbertson *et al.* (2009) was used as follows:

3.7.1.1 Second Larval Instars of *Tuta absoluta*

Bioassay was performed with second instars larvae (L2) of *T. absoluta* using 2%, 4% and 8% concentrations of each plant extract. The second instars larvae (25 larvae) were carefully extracted out from their mines by using zero brush and then transferred on newly uninfected tomato leaves in a Petri-dish (15 cm in diameter). The uninfected tomato leaves were soaked in extract solution for 10 sec then air dried before being introduced to the larvae. The Petri-dishes were lined with filter paper to protect the larvae from excessive humidity. To maintain turgor of the petiole of each leaves, these were wrapped in humid cotton wool. Petri-dishes were maintained at conditions of the insectariums at 24±2°C. The positive and negative control treatments as described in section 3.3 were also included. Each treatment was replicated three times. The numbers of live and dead larvae of each treatment as well as control were recorded after 1,2,3,4 and 5 days of treatment.

3.7.1.2 Eggs treatment

When adults emerged, moths were provided with 10% honey solution and allowed to mate for one day in a 60 x 60 x 75 cm rearing cages. Plants having four to six true leaves (5 weeks seedlings) were transferred to the rearing cages and maintained there for two to three days to allow egg laying. Leaflets of the tomato's plants were examined under binocular microscope and *T. absoluta* eggs were counted, extracted and placed into Petri dishes (15 cm diameter) containing tomato leaf treated by the dipping method. The status of treated eggs was recorded as live (hatched larvae) or dead (unhatched). The egg bioassay was repeated three times.

3.7.1.3 Adult Treatment

In this experiment, uninfected leaves were dipped in different treatments and then allowed to air dry. Twenty five adult *T. absoluta* were placed in boxes (5x10x10 cm) provided with a piece of cotton wool saturated with 1% honey to which a particular concentration of a treatment has been added. After 1, 2, 3, 4 and 5 days of exposure, *T. absoluta* adults were observed in each treatment to assess mortality. Figure 7 shows the different stages of larvae, egg and adult moth leaf dipping bioassays.

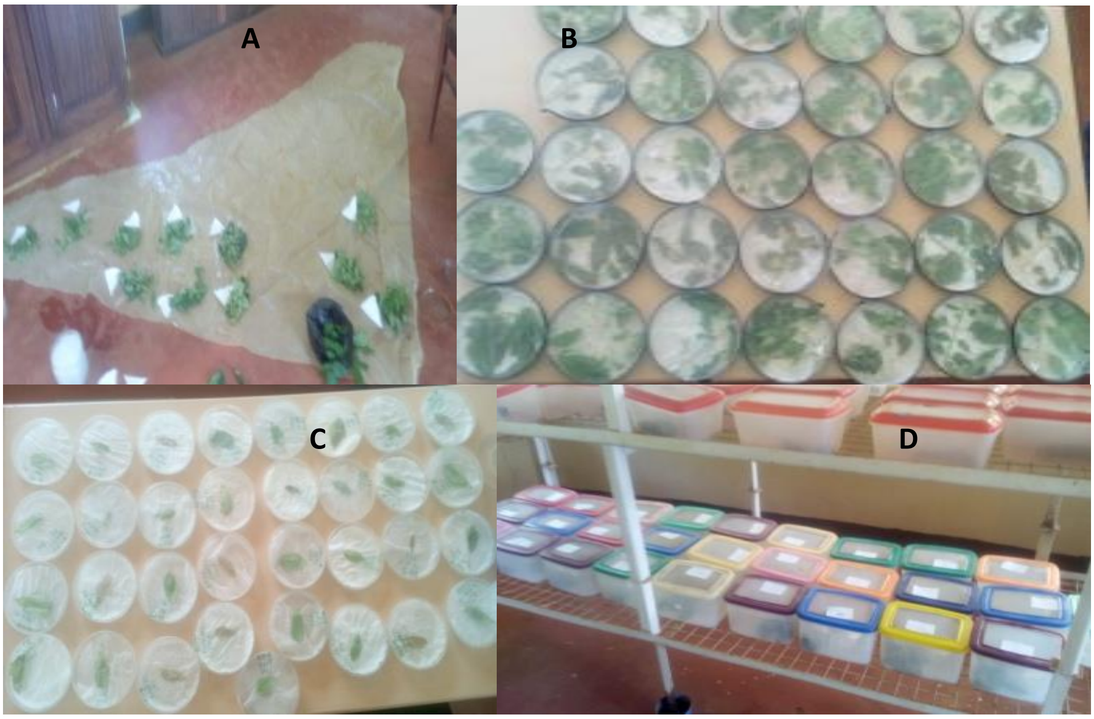


Figure 7: Treatment of *T. absoluta* at different stage using leaf dipping method

A: Drying of leaves after being treated by dipping method**, B**: Bioassay on Larvae,

C: Bioassay on egg, **D**: Bioassay on adults.

3.7.2 Bioassay under screen house conditions

To evaluate the effect of the treatment on *T. absoluta* population, naturally infested tomato plant of the two varieties (Tanya and Cal J) were grown in a screen house at the Crop Museum (SUA). Plants of the two varieties Tanya and Cal J were planted in separate rows were used. The screen house had not been sprayed with any pesticide. Pre treatment larval count was carried out by sampling 40 leaves randomly taken from each plot as 4 replicates of 10 each. The three different plants extracts were applied at 4%. Treatments were applied by spraying the extract solution on the plant to run-off, using a hand held sprayer. To minimise cross infestation the plants were sprayed in the morning since *T. absoluta* is a nocturnal insect. The positive control treatment were sprayed with BELT ®SC 480 at the concentration of 0.2 ml/l, while the negative control were sprayed with 0.1% Tween® 20 in sterilized distilled water.

Larval counts were performed after 0, 1, 2, 3 and 7 days of treatment, 40 tomato leaves (10 × 4 replicates) were randomly taken from each treatment. On each day, these 40 leaves were taken from the different treatments and brought to the laboratory for examination under a binocular microscope (Leica MZ 12.5). The number of larvae of *T. absoluta* present on the leaves was counted and recorded. In screen house, for both varieties the incidence was equal, thus destructive sampling was used to calculate the reduction of the infestation within the treatment. The percentages of infestation reduction were calculated according to Henderson and Tilton's equation (1955) as follows:

Reduction % = (1- (a/b x c/d)

Whereas;

a = Population in treatment after spraying

b = Population in treatment before spraying

c = Population in check untreated (control) before spraying

d = Population in check untreated after spraying

Reduction percentages were calculated after 1, 2, 3 and 7 days after treatment for the larvae.

3.8 Data Collection

3.8.1 Yield components

The yield of the tomato plants of the two varieties was assessed in term of number of fruits per plants, fruit size, marketable and none marketable fruits. Number of fruits per plant was assessed by counting the fruits of all trusses for the different treatments. Fruits size was measured by weighing the fruits from each treatment using an electronic balance.

After harvesting, all tomato fruits were sorted and graded into marketable and none marketable fruits and weighted separately on a balance. Marketable fruit yield constituted of only fresh, non-damaged fruits suitable for selling. While none marketable fruit yield was made up of fruits damaged by pests. The total fruit yield was the sum of weights of marketable and none marketable fruits yields. Yield loss was calculated using the formula:

$$\text{Yield losses} = \frac{\text{None marketable}}{\text{Marketable + None marketable}} \text{x100}$$

3.8.2 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using GENSTAT 14^{th} Edition, at a difference declared significance of 5% level. In the laboratory the experiment was done in a triplicate, and two-way ANOVA was used with concentration (3 level) and plants extract (3 level) as source of variation. In the screen house, the experiment was done in quadruplicate using two-way ANOVA with plants extract and varieties as factors. Descriptive statistics (mean, standard error of mean and coefficient of variation) were generated using GENSTAT procedures. Multiple means comparison was done using Turkey GENSTAT tool at 5% level of significance. The determination of the Lethal Dose 50 of each plant extract was done by probit regression dose-response analysis using MedCalc software version 17.6.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Treatment under Laboratory Conditions

4.1.1 Effect of Botanical Extracts and Control Treatments on Eggs of *T. absoluta*:

Tuta absoluta eggs were treated in the laboratory to evaluate the effect of selected plant extracts on their hatchability. In vitro egg hatchability was significantly ($p<0.05$) different among the plant extracts and the controls at days 3, 4 and 5 (Fig. 8). *Commiphora* extract was the most effective during the three days of treatment. Among the plant extracts *Synadenium* had the lowest inhibition effect on *T. absoluta* egg hatchability. Its effect was comparable to that of the negative control (NC) at days 3, 4 and 5. The results showed 0% egg hatchability after 5 days of application of *Commiphora* extracts, compared to the NC (98.4%). There was no significant difference between *synadenium* extract (94.4%) and the NC (98.4%) after 5 days of treatment. All the plant extract in the study were not significantly different after the 1st and 2nd day. During the all period all plant extract had an effect on egg hatchability when compared to NC (Fig. 8). The interaction concentration-plants extract was not significantly different on egg hatchability.

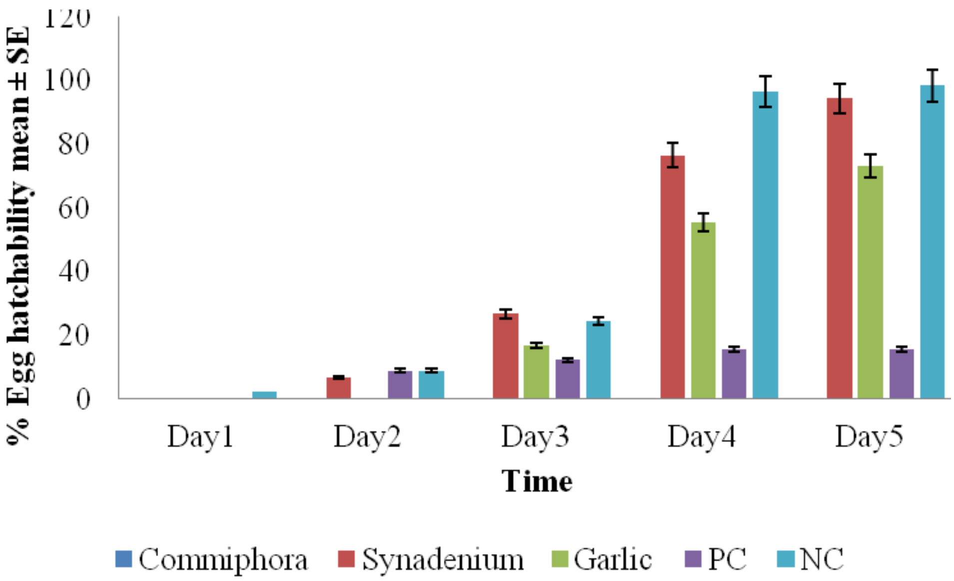
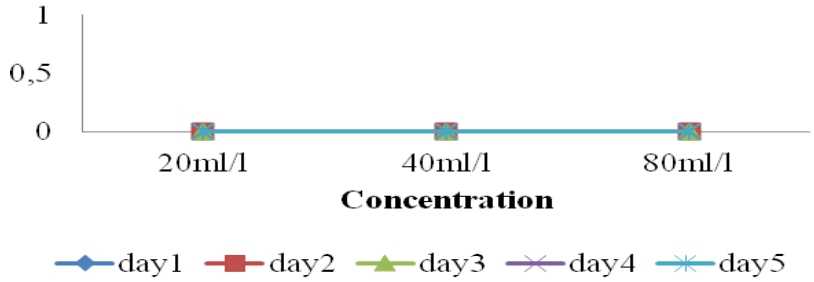
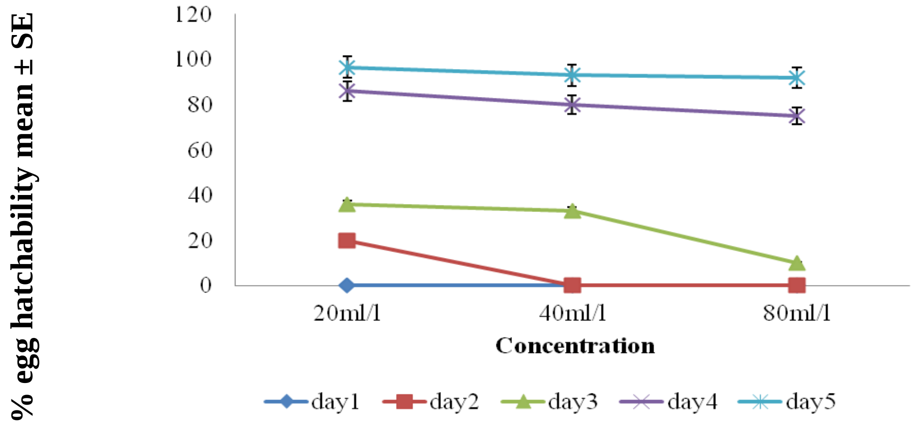


Figure 8: Effect of plants extracts on egg hatchability in percent

Fig. 9 presents the dose-response of each plant extract at different concentrations on the egg hatchability of *T. absoluta*. The curve showed that with *commiphora* extract at different concentration and different day no egg hatchability occurred. For *garlic* on the 5th day the results showed 100% hatchability was recorded at lower concentrations. No difference between concentrations was recorded from first day to the second day. *synadenium* showed high hatchability of egg at days 4 and 5.



(a)



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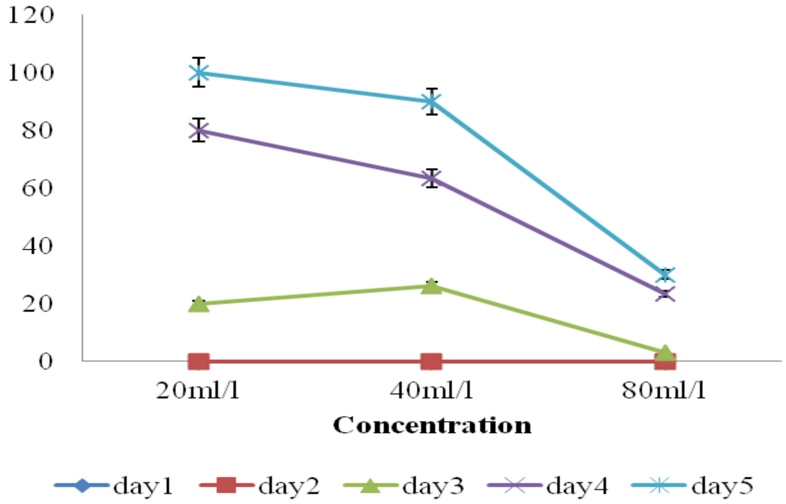


(c)

(a)*Commiphora* (b) *Synadenium* (c) Garlic

Figure 9: Dose-response curve of each plant extract on egg hatchability

4.1.2 Effect of botanical extracts on Larvae of *T. absoluta*

Combined data analysis for plant extracts revealed significant differences ($p<0.05$) on larvae mortality of *T. absoluta* (Fig. 10). Larval mortality of 57.7% was obtained with *Commiphora* after 24h .The mortality rate increased rapidly thereafter and reached 100% by the 5th day, higher than the mortality recorded in positive control (PC) (82.2%). *Synadenium* induced the lowest larval mortality but it was still higher compared to the NC (15%) after the 5th day.

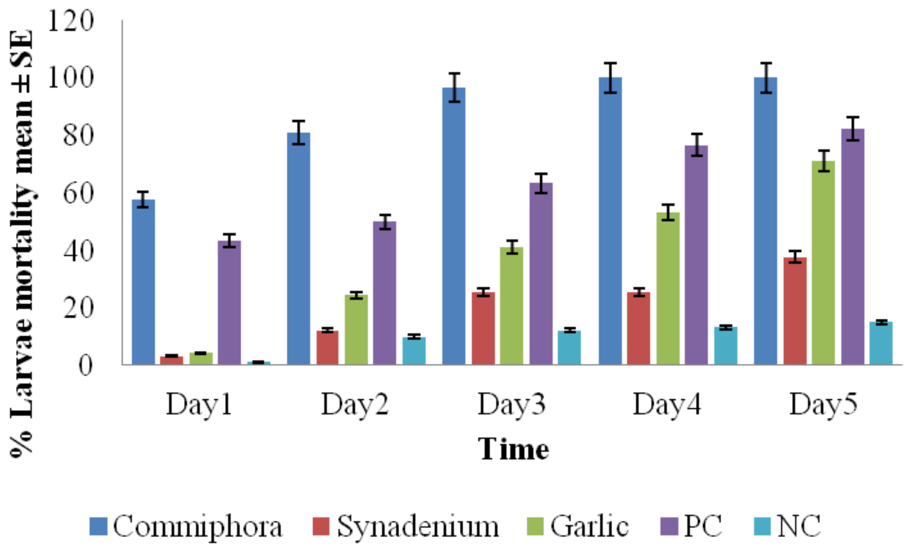


Figure 10: Effect of plants extract on the mortality of larvae of *T. absoluta*

Results on the interaction between plant extract and concentration are presented in Fig. 11. The different concentration of *garlic* extract were significantly different on the 3rd and 4th day while for other plant extract there was no significant differences between concentrations. By the 5th day, larval mortality, ranged between 59% - 76.6% was recorded in concentrations of *garlic* extract.

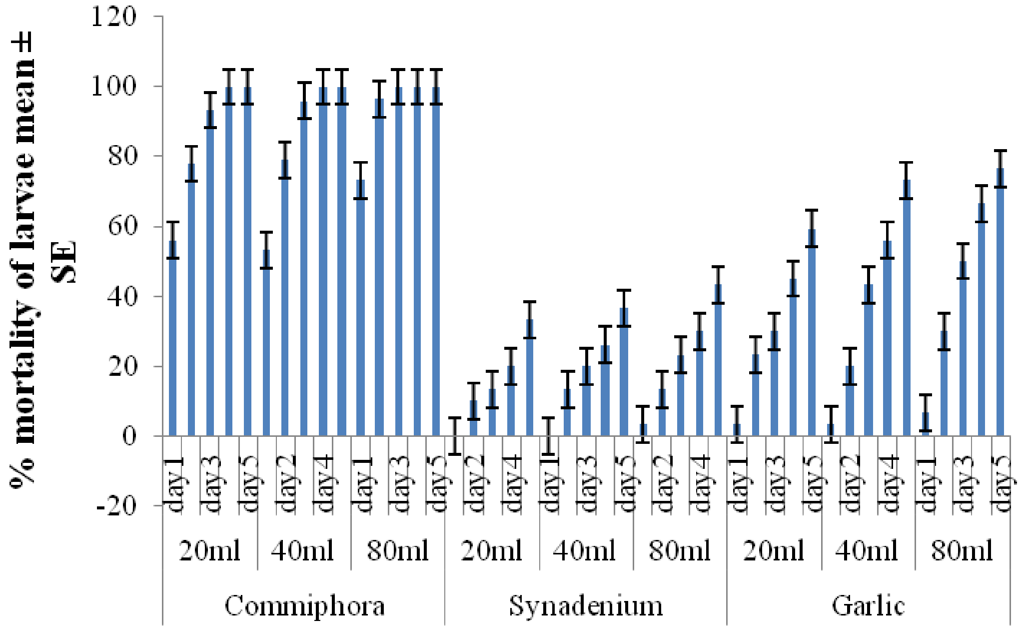
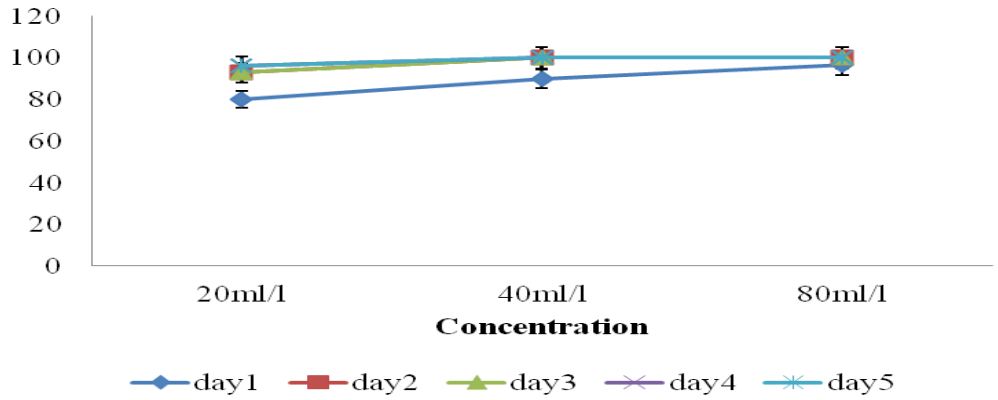
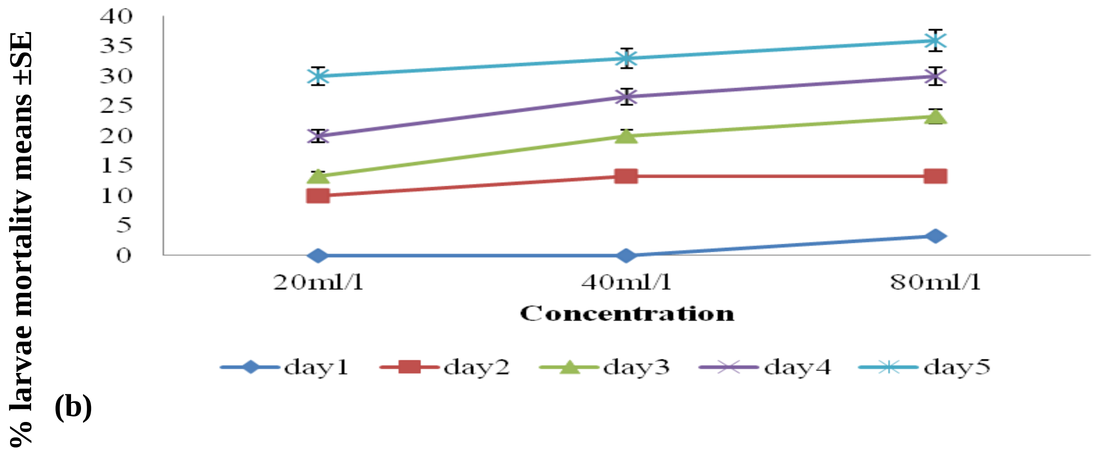


Figure 11: Effect of the interaction between concentration and different plant extracts on larvae mortality

Fig. 12 presents the dose-response curve of each plant extract at different concentration on larvae mortality of *T. absoluta*. The curve showed that with all the plants extracts in the study at different concentration and different day the larvae mortality increased. On the 5th day 100% mortality of larvae was obtained by *commiphora* at the concentration of 40ml/l and 80ml/l and 96% larvae mortality was obtained at the concentration of 20ml/l. On the other hand on the 5th day with *Synadenium* (b) and *garlic* (c) the larvae mortality ranged between 30-36 and 52-68 respectively.



(a)



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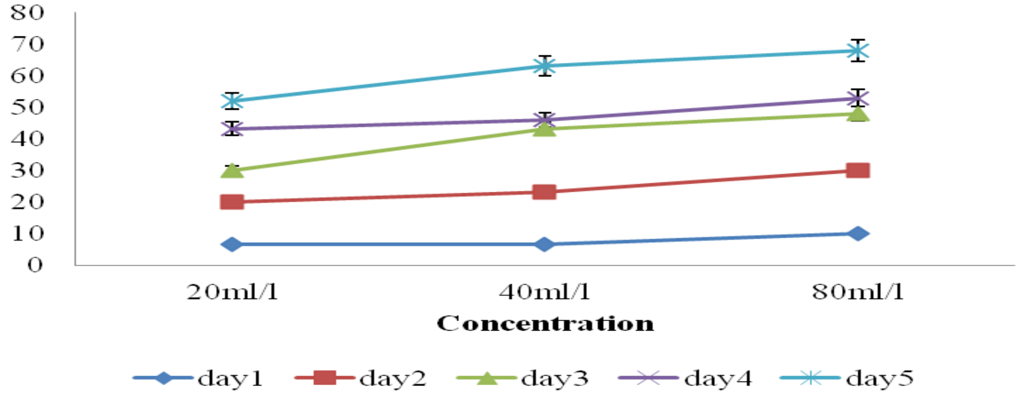


(c)

(a)*Commiphora* (b) *Synadenium* (c) Garlic

Figure 12: Dose-response curve of each plant extract on larvae mortality

4.1.3 Effect of botanical extracts on the mortality of adults of *T. absoluta*:

Results on the effects of the treatments on the adult stage of *T. absoluta* under laboratory conditions are presented in Fig. 13. The results showed that mortality of 10 % occurred in the NC group within the test durations. Adult's mortality level under the laboratory conditions was significantly different among the treatments. *Commiphora* extracts caused highest mortality of *T. absoluta* adults. The mortality caused ranged from 75.5 on day 1 to about 94% on day 5. *Synadenium* extract caused the 2nd highest mortality, while garlic extracts and the conventional insecticides caused comparable mortality of about 63 to 64%. This was significantly higher than the mortality caused by the NC.

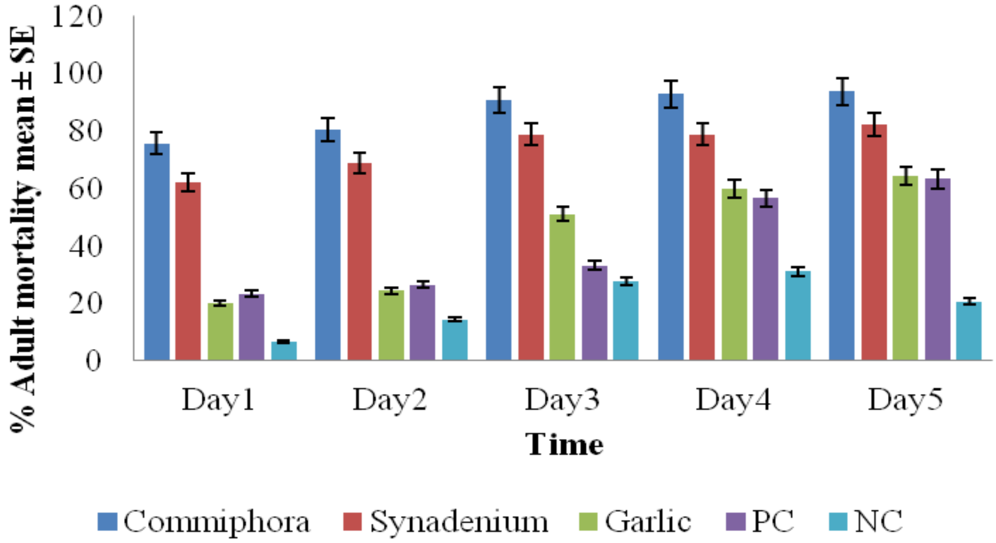
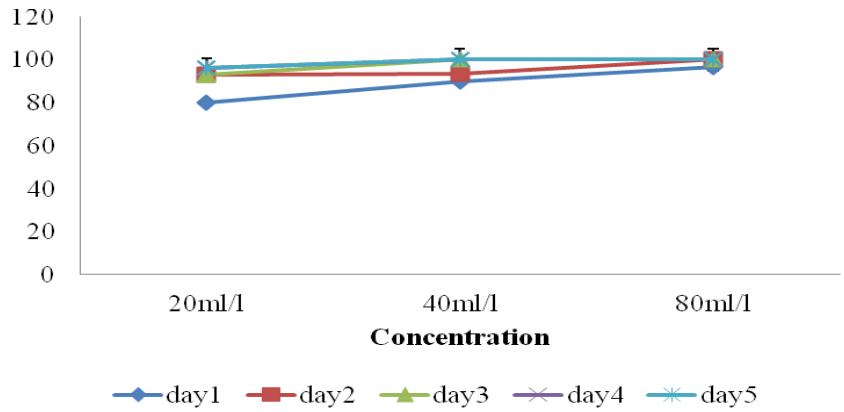
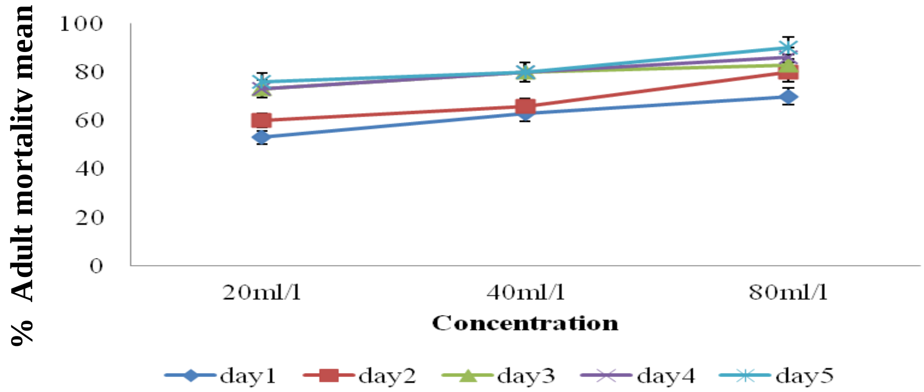


Figure 13: Effect of plant extracts on adults of *T. absoluta*' mortality

Results of the dose-response of each plant extract at different concentration on adult mortality's of *T. absoluta* are presented in Fig. 14. The three different plants extract show their effectiveness in controlling *T. absoluta* at the adult stage.



(a)



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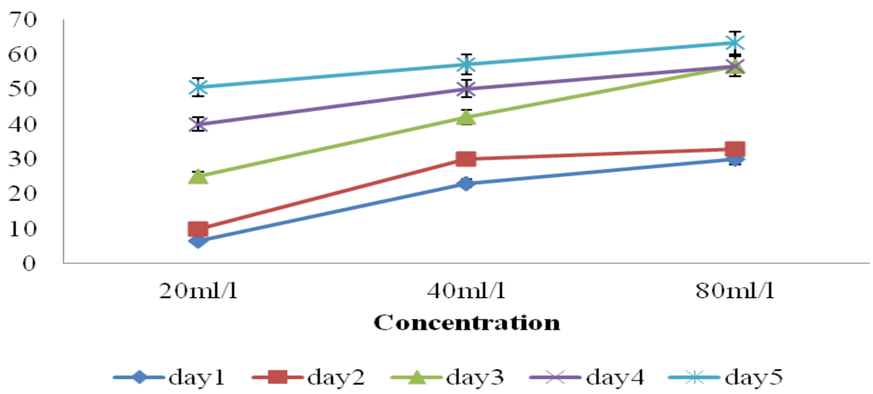

(c)

(a)*Commiphora* (b) *Synadenium* (c) Garlic

Figure 14: Dose-response curve of each plant extract on adults mortality

4.1.4 Lethal dose 50 of plant extracts

The lethal concentration 50 of plant extract at different lifestage are presented in Fig. 15, 16 and 17. At egg stage, almost all the egg treated by *synadenium* and the control hatched after 5 days. Results showed that a concentration of 5,934 ml/l is needed at the 1st day for *synadenium* to kill 50% of the population (Fig. 15) then the concentration decrease a little bit with time. For *garlic* a concentration of 1,811ml/l was needed . At larvae stage, at day 1 a LD 50 of 7,717ml/l was needed for *Synadenium* but for *commiphora* only a concentration of 0.104ml/l was needed to kill 50% of the population and for *Garlic* 5,020 ml/l was needed (Fig. 16). On the adult stage for *commiphora* the LD50 was of 0.13ml/l on the 5th day and of 12.1ml and 12.8ml/l for *synadenium* and *garlic* respectively (Fig. 17).

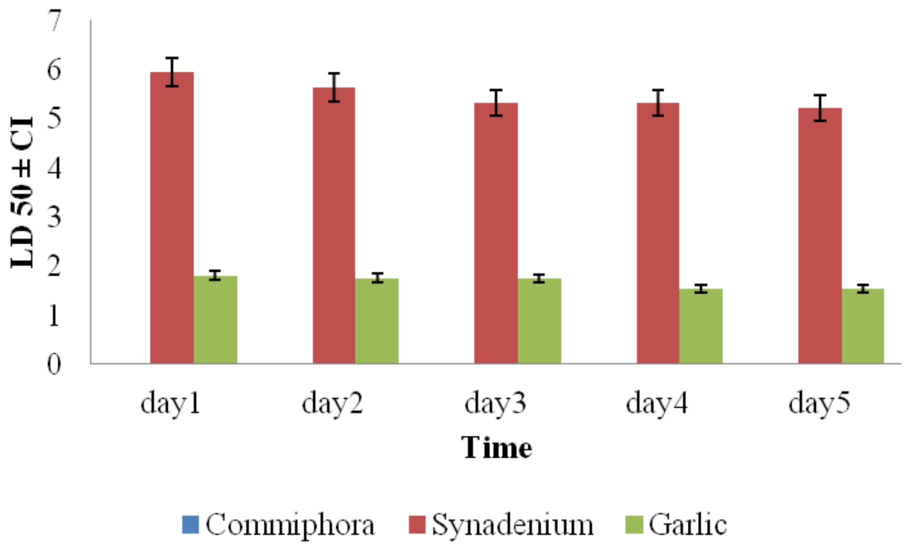

Figure 15: Lethal concentration 50 of plant extracts at egg stage

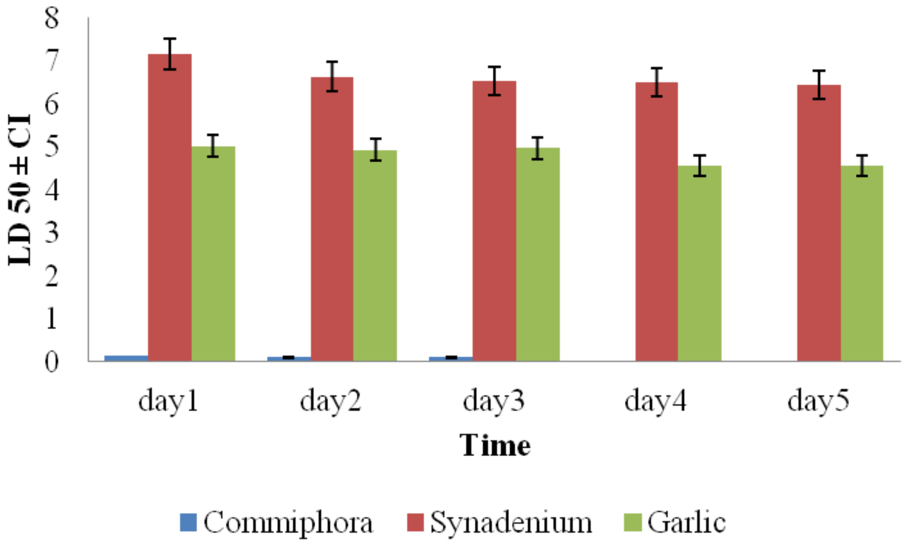

Figure 16: Lethal concentration 50 of plant extract at larvae stage

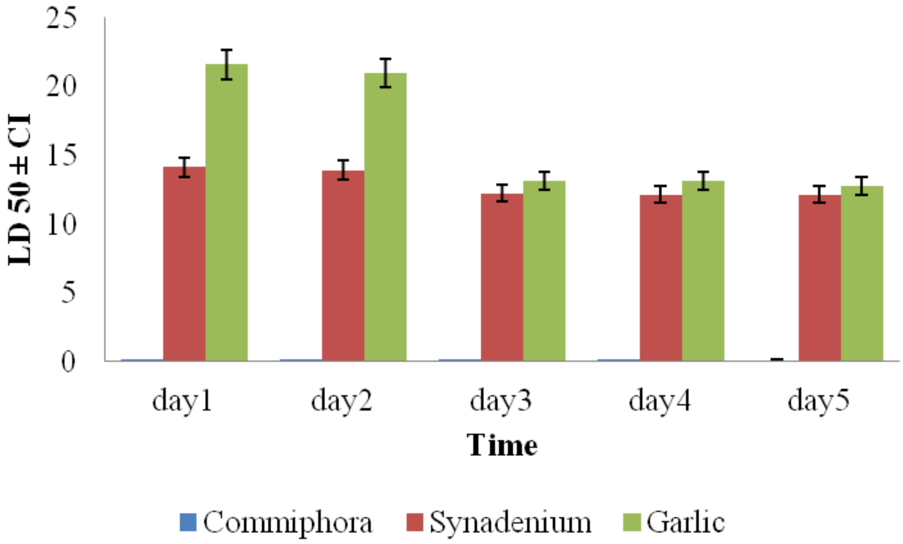

Figure 17: Lethal concentration 50 of plants extract at adults stage

4.2 Effects of the Treatments under Screen House Conditions

4.2.1 Bio-efficacy of Pesticides against Larvae of *T. absoluta* on two tomato varieties

Reduction of the pest in the screen house was calculated as presented in section 3.7.2. Two varieties of tomato namely Cal J and Tanya were evaluated in this study to see the effectiveness of the plant extracts. The results for variety Cal J are presented in Table 2 and for Tanya in Table 3. The results show that reduction percentage of *T. absoluta* infestation on tomato leaves with the different tested plant extracts for the two varieties of tomatoes ranged between 19.3 – 80.1 % for Cal J and 22.7 - 79.1% for Tanya after 7 days from application (Table 2 and Table 3 respectively). There was no significant difference in the reduction of *T. absoluta* ($p>0.05$) between the two varieties, while different plant extracts showed significant differences ($p<0.05$).

Generally, *Commiphora* was more effective at 80.1% for Cal J and 79.1% for Tanya in controlling *T. absoluta* than the PC with 55.4% for Cal J and 54.2% for Tanya. *Commiphora* exhibited the highest effect on *T. absoluta* after three days followed by the PC in both varieties. *Synadenium* showed the lowest reduction (22.7% for Tanya and 19.3 % for Cal J). In general, population of *T. absoluta* was significantly higher in NC treatment than other treatments. Under screen house conditions, the highest effects of the tested plant extracts against *T. absoluta* were obseved after three days of treatment for *Commiphora*, when 87.7% and 89.1% on Cal J and Tanya respectively. The positive control caused reduction of 64% and 63.2% for Cal J and Tanya respectively. After three days for both varieties, plants treated with *Commiphora* extract had at the same time, most of the leaves dried. It seemed that the concentration used in the screen house was high and it killed even the leaves. On the 7th day the effect of the pesticide had decreased. The results are attributed to the fact that *T. absoluta* was more sensitive to some of the tested plant extracts after 2 to 3 days. Thereafter, the effect of the plant extract decreased and pest population increased.

Table 2: Effectiveness of the treatments on larvae of tomato leaf miner (*T. absoluta* Meyrick) on Variety Cal J under screen house conditions

Plants extracts	Pre Spray	Average no of surviving larvae(day)				Reduction percent (days)			
		1	2	3	7	1	2	3	7
Commiphora	32	27	19	12	11	37.59a	65.3a	87.7a	80.1a
Synadenium	29	30	27	32	35	4.6c	28.4d	26.8d	19.03d
Garlic	35	27	28	15	23	24.3b	36.5c	34c	27.08c
PC	40	33	21	13	18	36.50a	55.6b	64b	55.4b
NC	31	34	38	43	47				
P						0.004	0.0003	0.001	0.001
CV						18.2	6.8	11.8	8.8
SE±						7.843	4.419	5.992	4.535

NB: Means followed by the same letter are not significantly different at ($p<0.05$), PC= Positive control, NC= Negative control

Table 3: Effectiveness the treatments on larvae of tomato leaf miner (*T. absoluta* Meyrick) on variety Tanya under screen house conditions

Plants extracts	Pre Spray	Average no of surviving larvae(day)				Reduction percent (day)			
		1	2	3	7	1	2	3	7
Commiphora	25	25	14	9	8	34.6a	65.9a	89.4a	79.1a
Synadenium	31	28	27	29	34	6.3d	23.7d	31.43d	22.7d
Garlic	31	30	24	25	29	20.4c	36.9c	40.79c	23.6c
PC	25	30	17	14	18	31.75b	48.9b	63.2b	54.2b
NC	28	30	35	38	44				
P						0.001	0.0003	0.001	0.0002
CV						13.6	6.5	6	3.5
SE±						2.144	4.592	7.33	3.463

N.B: Means followed by the same letter are not significantly different at ($p<0.05$), PC= Positive control, NC= Negative control

4.2.2 Effect of the treatments on the yield of tomato

4.2.2.1 Yield in number of fruits per plants

Figure 18 presents the results of varieties on the number of fruit of tomato and Fig. 19 shows the effect of the plant extract on the number of tomato fruits. There were no significant differences in number of fruits per plant between the two varieties (Cal J and Tanya). Results show that marketable and none marketable fruit yields significantly differed ($p<0.05$) among plants extracts. *Commiphora* and PC had the highest number of marketable fruits 3.6 and 3.2 respectively. These treatments resulted into lowest number of none marketable fruits (1.9 and 2.6). However, no significant differences were observed in number of marketable fruits between *synadenium, garlic* and NC. Neither there were no significant differences in number of none marketable fruits between PC, *synadenium* and garlic. The NC had the highest number (3.625) of none marketable fruits. The interaction variety and treatment were not significant different on yield parameter.

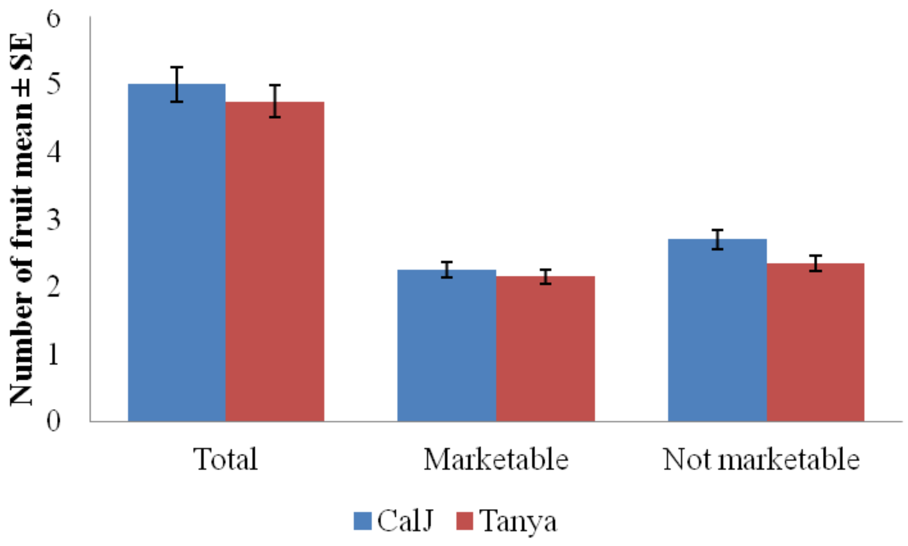

Figure 18: Effect of varieties on number of fruits

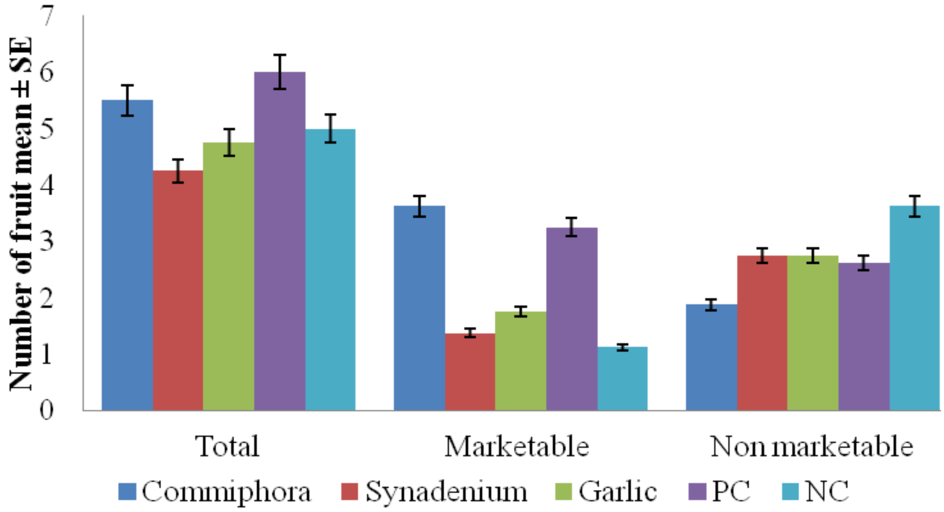

Figure 19: Effect of different plant extract on number of fruit

4.2.2.2 Yield in Fruit Weight

Data recorded on yield are presented in Fig. 20. It was observed that the treatments affected significantly the total yield. *Commiphora* extract gave the highest yield of 401 and 423.5 for Cal J and Tanya respectively. *Commiphora* was followed by PC and garlic. There were no significant differences between the varieties. Negative control and *synadenium* gave the lowest yield for both varieties. There were no significant differences between varieties and treatment on the total yield of the tomatoes.

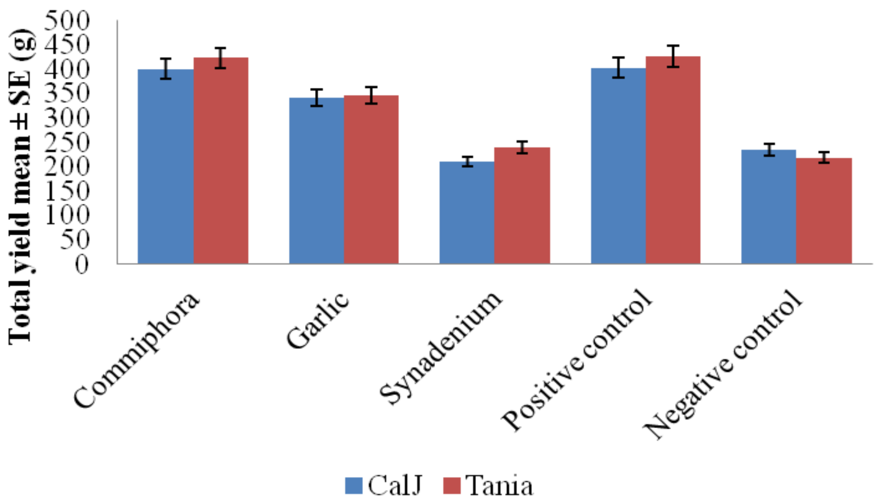

Figure 20: Total yield of Cal J and Tanya in different treatments

4.2.2.3 Yield Loss of Different Varieties of Tomato

Results on tomato fruit weight losses are presented in Fig. 21. The treatments affected significantly the total loss. *Commiphora* extractled to the lowest percent of fruit yield loss with the extend of yield of 35.4 and 32.9 for Cal J and Tanya respectively. The highest yield loss percent of 72.7 and 83.1 for Cal J and Tanya respectively, where recorded from the NC treatment.

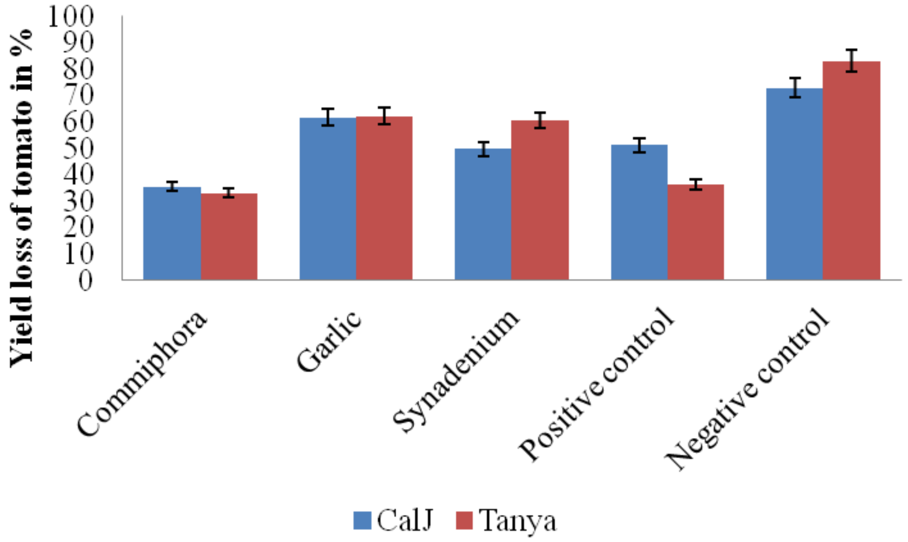

Figure 21: Total yield loss of two varieties of tomato in different treatment

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Plant Extract on the Egg, Larvae and Adults of *T. absoluta* under Laboratory Conditions

This study showed no significant differences on the effects of different concentrations of plants extracts on the life stages of *T. absoluta*. No effect was observed on the eggs after 24 h, of topical application of the three extracts. However, the different concentrations of the three plant extracts caused egg hatchability that ranged between 0-86% within 4 days. On the other hand, most of the eggs hatched normally after 4-5 days of treatment; which indicated absence of the effect of the plants extracts on the egg viability. No hatchability of the egg was recorded with *Commiphora* extract at the different concentration. There are few records on the effects of insecticides or plant extracts on *T. absoluta* eggs. The methods to control egg were through irradiation (Arthur, 2002) and application of biological control using egg parasitoids (Faria *et al*., 2008).

Larval mortality of 57.7% was obtained with *Commiphora* after 24h .The mortality rate increased rapidly thereafter and reached 100% by the 5th day. *Synadenium* induced the lowest larval mortality but it was still higher compared to the NC (15%) after the 5th day.

All plants extracts caused mortality on the adult of *T. absoluta,* garlic extracts and the conventional insecticides caused comparable mortality of about 63 to 64% while the high mortality was recorded by *commiphora* extract.

Many previous studies reported effective larval control of *T. absoluta* with botanical materials. Nadia *et al*. (2014) reported that application of four concentrations of Neem (*Azadirachta indica*) seeds ethanolic extract and Jatropha (*Jatropha curcas*) seeds petroleum ether extract on young larvae of *T. absoluta* resulted in larval mortalities that ranged between 33- 46.7% and 23.5 - 48.5% respectively obtained after 24 h. Also, higher larval mortalities, up to 100%, were obtained with the two extracts after 4 days of treatments. Moreno *et al*. (2011) tested the bioactivity of hexane and ethanol extracts of 23 plants against *T. absoluta* larvae. Their results showed that, hexane extract of *Acomellaoleracea* was the most active against *T. absoluta* larvae. Nilahyane *et al*. (2012) applied extracts of 7 plants against *T. absoluta* larvae; their results showed that, the extracts had varying levels of toxicity for the larvae. The most effective was that of *Thymus vulgaris* (95%), followed by *Ricinus communis* (58%). In a similar laboratory study, Ghanim and Abdel (2014) used 5 plants extract against 2nd instars larvae of *T. absoluta*. Their results showed that, Chinaberry caused the highest effects on *T. absoluta* larvae, followed by geranium, onion and garlic.

Essential constituents of most of the plant extract in this study are primarily lipophilic compounds that act as toxins, feeding deterrents and oviposition deterrents to a wide variety of pests. Insecticidal properties of several plants extract to the housefly, red flour beetle and southern corn root-worm were reported (Rice and Coats, 1994). Although many some plants extracts have insecticidal properties, the degree of toxicity of different compounds to one species differs considerably.

Repeated modes of action of Azadirachtin included induced cessation in feeding (antifeeding) and growth inhibition (McMillian *et al*., 1969; Meisner *et al*., 1981, Raffa, 1987). Studies showed that they reduced moulting and caused deformations in pupae and decreased fecundity of females (Isman and Machial, 2006). The antifeedant effects of azadirachtin are partly due to sensory detection and avoidance by insects (Simmonds and Blaney, 1984).

The insecticidal activity of *Commiphora* exudate parallels other findings. For instance essential oils of *Commiphora* leaves repelled ticks and the repellence activity was associated with presence of sesquiterpenoids (Kaonekane *et al*., 2007). Also bark extracts induced mortality in nymphs and adults of *Rhipicephalus appendiculatus* ticks (Kaonekane *et al.*, 2012). Insecticidal activity in the genus *Commiphora* is not unique to *C. swynnertonii.* There are other species with strong acaricidal activities including *Commiphora Erythraea* (Carrol *et al.,* 1989) and *Commiphora myrrh* (Maradufu, 1982).

The effectiveness of the insecticidal activity of *C. swynertonii* exudates on *T. absoluta* was a function of their concentration as well as the duration of exposure to the exudates. At 8% concentration, more than 90 of adults died by day two. A lower concentration of 2 % did not cause high mortality on day one and day two, but progressively the adults' mortality increased on day three and by day five more than 90% of adults had died. At a higher concentration (4% and 8%) 100% mortality was realized on day three. At a higher concentration the effect was immediate while at a lower concentration there was immediate action but the residual amounts on the adults continue to elicit the effect.

5.2 Bioassay of plants extracts against *T. absoluta* under the Screen House

The present study shows that effect of plant extract decreased with time after treatment. So, the insect populations increased again as a result of the decrease of residual effects. Ghanim and Abdel (2014) studied the effects of basil, geranium, chinaberry, onion and garlic aqueous extracts against *Tuta absoluta* Meyrick and the cotton aphid, *Aphis gossypii* (Glover). The authors reported the highest effect of the plant extracts after four days which also decreased with time. Decreased efficacy of plant extracts over time was previously attributed to activity of insects' secretion system (Sarmamy *et al.*, 2011). Moreira *et al.* (2004) associate differences in plant extracts toxicity with differential susceptibility of the insect.

In this study garlic had moderate effectiveness on the pest. However, Hussein *et al.* (2014) showed a high reduction of *T. absoluta* population after tomato plants were treated with garlic extract. The moderate reduction of *T. absoluta* during this study was due to the extraction method that affects the presence of some volatile oil that confers to garlic its biological properties. Results of this study were however in agreement with those reported by Ghanim and Abdel (2014), who showed effects of garlic on *T. absoluta* second instars larvae under laboratory conditions, but with moderate effects under greenhouse conditions. Also, garlic leaf lectin (ASAL) had detrimental effect on growth and survival of two important homopteran insect pests, *Lypaphis erysimi*, commonly known as aphids and *Dysdercus cingulatus* (red cotton bug) (Bandyopadhyay *et al.*, 2001). Neem, garlic and ginger extracts contain insecticidal properties that are lethal to a wide range of insects (Oparaeke, 2007).

Garlic is a major source of sulphur containing compounds (alkyl sulfides) with different numbers of sulfur atom (i.e. mono-, di-, and trisulfide). Volatile compounds such as allicin, diallyl disulphide, diallyl trisulphide (a major constituent of garlic oil), dithiins, and ajoene originate from different metabolic pathways by tissue damage through cutting, crushing or chewing. These compounds provide to garlic its characteristic odour and flavour as well as its biological and antifeeding properties (Ben *et al.*, 2010). Similar results with other pests were obtained by Panhwar (2002) who reported that good aqueous solution of garlic would effectively control worms, beetles and thrips in cowpea.

5.3 Yield and Yield Losses of Tomato in different Treatments

Commiphora extract induced low level percent of loss with 35.4 and 32.9 for Cal J and Tanya respectively. NC induced the high level of loss with 72.7 and 83.1 for Cal J and Tanya respectively *Commiphora* extract gave highest yield of 401 and 423.5 for Cal J and Tanya respectively. No significant differences on the yield were found between varieties. Negative control and *synadenium* gave the lower yield for both varieties.

Results obtained in this study are similar to those reported by Ahmed *et al.* (2009) on cowpea and Panhwar (2002). These authors showed that, plant extracts increased the yield of vegetables by protecting them from insect pests. According to Gaby (2000), plant extracts application at flowering and pod formation stages reduced the level of infestation of insect pests and increased yield of pea plants.

Tuta absoluta reduced yield and fruit quality grown under green house and open field conditions. Severely attacked tomato fruits lose their commercial value. Losses ranging from 50-100% have been reported on tomato (EPPO, 2005). Damage is directly related to the reduction of plants’ photosynthetic capacity and of production levels in both protected and open field. Indirect damage to the tomato crop can also be caused by secondary infection by pathogens developing on the infested plants and fruits tissues (EPPO, 2012). As larvae are internal feeders it is difficult to achieve an effective control through application of chemical insecticides. Moreover, *Tuta absoluta* can rapidly evolve into strains with reduced susceptibility to insecticides that have been previously effective.

Results obtained by Hussein *et al.* (2014) indicated that controlling *T. absoluta* by essential oil significantly affected fresh weight, shape index, total soluble solids, thickness of pericarp, pH and L ascorbic. Interestingly, the tested plant extracts and essential oils affected the percentage of dry weight of fruits and vegetative growth of tomato plants significantly. Also, the lemon grass and garlic plant extracts and most of the essential aromatic oils (Eucalyptus, Rue, Anise, Basil) increased the total yield of tomato.

Results reported by Tyiagi *et al*. (1990) revealed that when plant growth improved and plant weights also increased with increasing concentration of leaf lemon grass extract and with longer dip duration.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings of this study, it is concluded that:

i. All plant extracts were effective and controlled adult *T. absoluta*

ii. All plants extracts except *Commiphora* had no effect on immature life stages of *T. absoluta*

iii. *Commiphora* extracts were highly effective and controlled *T. absoluta* in screen house

iv. Foliar application reduced *T. absoluta* population, improved quality and yield of tomato

v. This bio-insecticide can be integrated in pest management program and organic farming.

6.2 Recommendations

Based on the finding of the present study, the following are recommended:

i). Isolation and the screening of the bioactive compound against different strain of *T. absoluta*.

ii). Since *Commiphora* exhibited a good activity in *vitro* and in the screen house, it should be subjected in open field application to see its effectiveness

iii). Since only one part of the each plant was used further studies should be carried out in order to study different parts of the same plant and extracted by different solvents to compare their effectiveness.

iv). Molecular characterization of *T. absoluta* insect for understanding distinct strains circulating in Tanzania.

v). Therefore, further studies should be carried out to integrate *commiphora* with other integrated or biological control methods in order to reduce the use of chemicals and, consequently, improve food safety and environment quality.

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