Sudan University of Science and Technology

College of Graduate Studies

Effect of Neem Seeds Ethanolic Extracts on Mortality of Tomato leaf-miner *Tuta absoluta* (Lepidoptera: Gelechiidae)

الاثر القاتل للمستخلصات الكحوليه لبذور النيم على حافرة انفاق الاوراق فى الطماطم

(Lepidoptera: Gelechiidae) (*Tuta absoluta*)

A thesis submitted in partial fulfillment of the requirements for the M. Sc. degree in plant protection

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الآيـــة

قال تعالي:

...وقُ الْمَاءَ إِلَى الأَرْضِ الْجُرُزِ فَنُخْرِجُ بِهِ زَرْعًا
...هُ أَنْعَامُهُمْ وَأَنفُسُهُمْ ۖ أَفَلَا يُبْصِرُونَ (27)

صدق الله العظيم

سورة السجدة الآية (27)

DEDICATION

To my father and mother whom I love too much

To my brother and sisters whom I wish to them a beautiful life

To all my family

To all my teachers and friends with

love

&

respect

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All thanks are due to Almighty Allah (SWT) who gave me health and strength, and helped me tremendously to produce this work.

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CONTENTS

الآيـــة I

DEDICATION II

ACKNOWLEDGEMENTS.. III

CONTENTS.. IV

List of Tables IX

List of Figures X

List of Plates XI

Abstract... XII

ملخص البحث.. XIII

CHAPTER ONE 1

INTRODUCTION.. 1

CHAPTER TWO 4

LITERATURE REVIEW 4

2.1 Tomato 4

2.1.1 Classification.. 4

2.1.2 Description... 4

2.1.3 Distribution 5

2.1.4 Importance of tomato 6

2.1.5 Nutrient value content of tomato 6

2.1.6 Medical uses of tomato.. 7

2.1.7 Tomato Diseases 7
2.1.8 Tomato insects 7
2.1 Tomato leaf miner 8
2.2.1 Classification 8
2.2.2 Taxonomic history and synonyms 8
2.2.3 Ecological Range 10
2.2.4 Life cycle 11
2.2.4.1 Adults 11
2.2.4.2 Eggs 12
2.2.4.3 Larvae 12
2.2.4.4 Pupae 14
2.2.5 Developmental Rates and Day Degrees 14
2.2.6 Behavior 15
2.2.7 Dispersal 15
2.2.8 Damage 15
2.2.8.1 Damage in leaves 16
2.2.8.2 Damage in shoots 16
2.2.8.3 Damage in flower and fruit 16
2.2.9 Economic impact 17
2.2.10 Control procedures 17
2.2.11 Treatment options 18
2.2.12 Eradication 18
2.2.13 Cultural control 19
2.2.13.1Sanitation 19

2.2.13.2 Management of tomato leaves miner in nurseries and Greenhouse Tomato Production 19

2.2.13.3 Management of tomato leaves miner in field Grown Tomatoes 20

2.2.13.4 Vegetable packing stations 20

2.2.14 Managing Insecticide Resistance 21

2.2.15 Procedure 22

2.2.16 Insecticides 22

2.2.17 *Bacillus thuringiensis* 23

2.2.18 Azadirachtin 23

2.2.19 Population Monitoring 24

2.2.19.1 Population-baited traps 24

2.2.19.2 Mass-Trapping 24

2.2.19.3 Pan traps 25

2.2.19.5 Light traps 25

2.2.20 Biological control 26

2.2.20.1 Egg parasitoids 26

2.2.20.2 Larval Parasitoids 27

2.2.21 Predators 27

2.2.22 Microorganisms 28

2.2.23 Mating disruption 28

2.2.24 Integrated Pest Management 28

2.3 Neem 30

2.3.1 Taxonomy 30

2.3.2 Origin 30
2.3.3 Morphology 30
2.3.4 Distribution 31
2.3.5 Ecology 31
2.3.6 Active ingredients 31
2.3.7 Chemical Compounds of Neem tree 32
2.3.8 Mode of action 32
2.3.9 Uses of Neem in pest and disease control 32
2.4 Spinosad 34
2.4.1The Spinosad story 34
2.4.2 Symptomology 35
2.4.3 Mode of action 35
2.4.4 Registrations 36
2.4.5 Reduced risk classification 36
2.4.6 Ecotoxicology 37
2.4.7 Environmental fate 37
2.4.7.1 Fate in soil 37
2.4.7.2 Fate in water 37
2.4.7.3 Fate in plants 37
CHAPTER THREE 38
MATERIASLS AND METHODS 38
3.1 Field survey 38
3.2 Laboratory studies 38
3.3 Insect culture 38

3.4 Preparation of neem ethanolic extract 39

3.5 Extraction method 39

3.6 Bioassay procedure 39

3.7 Statistical Analysis 40

CHAPTER FOUR 43

4. RESULTS 43

4.1 Field survey 43

4.2 laboratory study 43

4.2.1 Effect of neem extracts on mortality of third larval instars of *T. absoluta*. 43

4.2.2 Propit analysis 44

CHAPTER FIVE 51

DISCUSSION 51

CONCLUSION AND RECOMMENDATIONS 53

REFERENCES 54

List of Tables

Table (1): Occurrence of *Tuta absoluta* in different areas during different months 45

Table 2 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 24hrs) 46

Table 3 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 48hrs) 47

Table 4 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 72hrs) 48

Table 5 : LC_{50} values of seeds ethanolic extracts of neem against third larval instars of the tomato leaf miner. 50

List of Figures

Fig. 1 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of *Tuta absoluta*. 49

List of Plates

Plate 1: life cycle of *Tuta absoluta* 13

Plate 2: Soxhlet Extractor Apparatus 41

Plate 3: Soxhlet and Rotary Evaporator 41

Plate 4 : Rearing cages. 42

Plate 5: Experiment Design 42

Abstract

The survey data showed that there was significant difference between mean population of *Tuta absoluta* from the three areas Elselate, Abu Halima and Wad Elnaaeem in Khartoum State during July, August and September (2013). The population in July and August was significantly higher than September. The population in Elselat and Abu Halima was significantly higher than Wad Elnaaeem.

Laboratory experiments were conducted at College of Agricultural Studies of Sudan University of Science and Technology to evaluate the lethal effect of neem seeds *Azadirachta indica* ethanolic extracts on the third larval instars of tomato leafminer *Tuta absoluta*. Five concentrations of neem seeds ethanolic extract (15%, 7.5%, 3.75%, 1.8% and 0.9) were used in this study.

The highest concentration used in this study (15%) gave the higher mortality percentage of (93.3) after 72h of exposure that significantly different compared with other concentrations. However, it was not significantly different from the recommended dose of Spinosad.

The probit analysis showed that the LC_{50} values for seeds ethanolic extracts of neem were 16.3 %, 9.7% and 3.2% after 24, 48 and 72 hrs respectively.

ملخص البحث

اوضحت الدراسه الاولى عن المسح لحافرات انفاق الطماطم ان هناك فرقا معنويا فى متوسط اعداد الحشره فى الثلاث مناطق السليت وابوحليمه وود النعيم فى ولايه الخرطوم خلال يوليو ,اغسطس وسبتمبر. اظهرت النتائج ان اعداد الحشرات فى يوليو واغسطس اكثر من سبتمبر فى كل المناطق. كما اوضحت الدراسه ان اعداد الحشرات فى مناطق السليت وابوحليمه اعلى معنويا من ود النعيم.

اجريت التجربه الثانيه تحت ظروف المعمل بكليه الدراسات الزراعيه بجامعه السودان للعلوم والتكنولوجيا , لتقييم الاثر القاتل للمستخلص الايثانولى لبذور النيم *Azadirachta indica* ضد الطور اليرقى الثالث لحافرة اوراق الطماطم *Tuta absoluta*. تم استخدام خمسه تراكيز فى هذه الدراسه (15%,7.5%,3.75%,1.8و0.9).

اوضحت النتايج المتحصل عليها ان كل التركيزات المختبره للمستخلص الايثانولى لبذور النيم باستثناء التركيزين (0.9% و1.8%) اعطت نسبة موت عاليه مقارنه بالشاهد بعد 24 ساعه للتجربه. اعطى اعلى تركيز (15%) نسبه موت عاليه % 93 بعد 72 ساعه من المعامله بدون فرق معنوي بالمقارنه مع الجرعه الموصى بها من الاسباينوساد (1مل/لتر).

اظهرت النتائج المتحصل عليها ان التركيزات النصفيه القاتله للمستخلصات الايثانوليه لبذور النيم 16.3%,9.7% و 3.2% بعد 48,24 و72 ساعه على التوالى .

CHAPTER ONE

INTRODUCTION

Tomato *Lycopersicon esculentum* Mill is a vegetable crop of large importance throughout the world. Tomatoes are grown both under plastic covered greenhouses and in open field.

The commercial production in the Sudan is faced by many problems that wory producer such as the attack by pests and diseases. The losses due to pests attack were estimated at 25% of vegetable production (Elhassan,1994). The area utilized for vegetable production in sudan in the center of AlGezira and Managil states, the basins of the Roseries and Sennar dam and on the banks of the White and Blue Nile in the state of khartoum (Elshofie,1980).

Tomato is a major vegetable crop in the Sudan. It comes in the second order to onion. It is consumed fresh or marketed for cooking, and for processing as whole juice, or puree (Ahmed, 2006). The increased market demand for fresh and processed tomatoes has encouraged many farmers in the irrigated schemes and small growers along the banks of the Nile to increase their production and extend the season. Tomato consumption is increasing steadily in the various regions of the country. It is becoming an extremely rewarding cash crop to the farmer because of the high prices it fetches especially in certain periods of the year. However, its production in the Sudan is traditionally low compared with yields in the USA and Europe (GTZ,1987). The production area of tomato in the Sudan is unknown, it is estimated as more than 100,000 feddans. Wheraes more than 20,000 feddans are grown in AlGezira region and also in a great area in AlRahad scheme, river banks, and valleys (Ahmed, 2006). Recently, certain disease and pest problems have inflicted heavy losses of tomato production. The deadly tomato leaf curl virus sometimes leads to complete failure of the crop. The parasitic plant locally called "haluk" an Orobanche species, is a new a major problem in the Northern region (GTZ,1987). White fly is the most serious insect pest attacking tomato in the Sudan (Ahmed, 2006). The tomato leaf miner ,*Tuta absoluta* meyrick*,(*Lepidoptera:Gelechiidae*)* is a serious pest of both outdoor and greenhouse tomatoes. The insect deposits egg usually on the underside of leaves, stems and to a lesser extent on fruits and leaves on which they feed and develop creating mines and galleries. On leaves, larvae feed only on mesophyl leaving the epidermis intact (OEPP,2005). Tomato plants may be attacked at only developmental stage, from seedlings to mature stage.

Originating from South America, *T. absoluta* has been reported since the early 1980s from Argentina, Brazil and Bolivia (Estay,2000); the insect rapidly invaded many European and Mediterranean countries. It was first recorded from eastern Spain in late 2006 (Urbaneja,2007), then Morocco, Algeria, France, Greece, Malta, Egypt and other countries .

Chemical control using synthetic insecticides is the primary method to manage the pest, but it has serious drawbacks, including reduced profits from high insecticide costs, destruction of natural enemy populations and inset resis (Campbell *et al*.,1991), For example, resistance development has been reported against abamectin, carta, methamidophos and permethrin in Brazil (Siqueira *et al* 2000) and against deltamethrin and abamectin in Argentina (Lietti *et al*.,2005). Thus, in order to avoid selection of resistant biotypes, a careful management with frequent changes of active ingredients is desirable. Furthermore, modern integrated pest management recommends effective pesticides that have low mammalian toxicity, low persistence in the environment and high degree of selectivity. Since insecticide control currently remains an indispensable tool, the goal is to minimize the amount and impact of pesticides through the diversification of active ingredient used.

Higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenoids, and phenols and another secondary metabolite are produced by higher plants (Siddig , 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantage over synthetic pesticides in being more environmentally friendly, to be accepted by the majority of the farmers, governmental organizations and decision makers (Kelany, 2001). Stoll (2000) demonstrated that the use of plant extracts to control destructive insect was not new .Rotenone, Nicotine and Pyrethrin have been used for a considerable time both in small scale subsistence and also commercial agriculture.

This research was designed to fulfill the following objectives:

To determin the abundance of *Tuta absoluta* through quantitive survey in Khartoum State.

To evaluate the efficacy of neem seed ethanol extract in comparison with Spinosad against tomato leaf miner.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tomato

2.1.1 Classification

Kingdom: Plantae

Subkingdum :Tracheobionia.

Division: Magnoliopsida.

Sub class: Asteridae.

Order: Solanales.

Family: Solaneceae.

Genus: Lycopersicon.

Species: *Esculentum* Mill

2.1.2 Description

Tomato *(lycopersion esculentum*) is a tender warm season perennial cultivated as an annual (Peirce,1987 ,GZT,1987;Decteau, 2000 and Ahmed, 2006). It is an annual shrubby member of the solanaceae. In a protected environment, tomato is a short-lived herbaceous perennial. Determinate tomato hight,3-4 ft, and indeterminate,7-15ft,spread in 24-36 and most of the roots are found in 8 in. But some fibrous spreading roots extend 4-8 ft deep (Decteau.2000).Tomato leaves are compound, alternate, from 10-30cm in long, and 10-15 in diameter (Tindall,1986). The leaves are 10-15 cm (3.9-9.8 in) long, old pinnate, with 5-9 leaflets on petioles (Acquaah,2002). Tomato is characterized by glandular hairs (trichomes) that emit a strong aroma when broken.

Tomato flowers are relatively small and consist of a five lobed corolla and calyx. The staminal cone represent a fusion of five ansther around the ovary. Style and stigma, ensures a high level of self-pollination and homozygosis. Pollination is not a function of insects activity but occurs as flowers vibrate from wind currents .The fruit botanically a berry has two or more cavities (locules) containing seeds imbedded in a gelatinous matrix that softens as fruit reach mature size and seeds are fully developed. Small fruited cultivars generally have only two locules. However, those with large fruit have many (Peirce,1987). Ripe fruit colors may be red , pink, yellow or orange, a function of several independent genes controlling either flesh or skin color (Peirce,1987,and Decteau,2000). In addition to these basic fruit colors other genetic factors modify flesh color intensity (crimson or high pigment) or chlorophyll expression in unripe fruit , ripe color is a function of two carotenoid pigments, lycopne(Peirce 1987).Shapes may vary from globe or round to slightly flattened, or spear like (Decteau,2000).

2.1.3 Distribution

The written literature of tomato began in the 1500s when Spanish and Portuguese explorers found these plants first in Mexico and then along the west coast of South America, mainly peru, and then along on the Galapagos Islands. Tomato is native to Peru-Equador region of South America, evolving from the cherry from (Lycopersicon esculentum var cerasiform).The first historical mention of tomato by Malthiolusin (1544), placed in Italy. However, it was first introduced to Spain by explorers returning from south and Central America. The plant received little notice in Spain (Peirce,1987). Based on the world food and Agricultural Organization(FAO) in 1994,tomato fruit-for fresh market and processing worldwide was approximately 2.8 million hectares (Benton,1999).Tomato is one of the most important vegetable crops in the Sudan(GTZ,1987).The production area of tomato in the Sudan is unknown ,it is estimated at more than 100.000 feddans. Whereas more than 20.000 feddans are grown in Al Gezira region and also grown in a wide area in Al Rahad Scheme , rivers banks, and valleys in small separate areas.

2.1.4 Importance of tomato

Tomato is grown for its, commercially important fruits. Its grown for fresh market in the field or greenhouse and for processing as whole pack, juice, or puree.(Decteau,2000). Tomato contain significant amounts of vitamins A and C , although levels of both in fruit from shaded plants as in those in strong sunlight , the carotenoides are effected by temperature(light intensity), but vitamin A(β-Carotene) is relatively stable (Peirce,1987). Besides being eaten fresh, tomatoes can be boiled, stewed, fried, juiced, or pickled and used in soups, salads, and sauces. Tomatoes are an important in salsa along with onions, garlic , peppers , cilantro, cumin and lime juice. Cherry tomato (cultivar for fresh market) producing many small fruits , cherry tomatoes are often served as appetizer , are generally considered the best suited for cooking in it sauces and ketchup due to their lower water contained (Decteau,2000).

2.1.5 Nutrient value content of tomato

Tomato is considered as an important source for some vitamins and mineral salts, such as; vitamin C , vitamin B , and Riboflavin , which are considered necessary for growing , and safety of skin. The external part of fruit contains high level of vitamin C (Ahmed, 2006).

2.1.6 Medical uses of tomato

Tomato is known for its medical value, it is important in protection from cancer , it use in constipation , disorder in liver and kidney function. However, it is important in protection and cure due to containing anti-oxygenate substance such like , vitamin C , glutathione , or β-carotene, as well as it rich in lycopine which result in activation of human body and mind moreover (Ahmed, 2006).

2.1.7 Tomato Diseases

Several bacterial diseases (caused by fungi and viruses) affect tomato. These in clue:fusarium wilt , verticillum wilt, bacterial wilt, bacterial spots, early blights , anthracnose, powdery mildew and nematodes.

2.1.8 Tomato insects

Several insects infest tomatoes. Some of those are white fly (*Bemisia tabaci*), Flea Beetle (*Epitricx cucumeries)*, Colorado potato beetle (*Leptinotarsa decimlineata)* the green peach aphids (*Myzus persicae*), potato aphid (*Macrosiphum solanifolliare*), leaf hoppers (*Circulifor tenellus*) , tomato horn worm (*Poptoparce quinquemaculata)* , tomato fruit worm (*Heliothis armigera)*, leaf miner (*Liriomyza pusilla)*, corn borer (*pyransta nubilalis)*, spider mite(*Tetranychus bimuculatns)*, common stem borer (*papaipema nebris*) , fruit fly (*Dorsphila melanogaster*) and stink bugs (Cantelo *et al* ,1974 ;Peirce,1987; Decteau, 2000; Snyder *et al*, 2005 and Ahmed, 2006).

Recently tomato leaf miner *T.absoluta* became a pest of great economic importance in tomato in Sudan. The pest has spread from Latin America, to Middle East, North Africa then Sudan in 2010.

2.1 Tomato leaf miner

2.2.1 Classification

Phylum: Arthropoda

Class: Insecta

Order: Lepidoptera

Sub order: Glossata

Super family: Gelechioidea

Family: Gelechiidae

Sub family: Gelechiinae

Tribe: Gnorimoschemini

Genus: *Tuta*

S.N.: *Tuta absoluta*

C. N.: tomato leaf miner

2.2.2 Taxonomic history and synonyms

The generic assignment of *T. absoluta* has been questioned. Povolny (1964) split the species among many genera, several of which he described himself and many of which he later synonymized .Other gelechiid taxonomists have questioned the validity of many of these genera. Three of povolny's genera that included North American species , including *Tuta* , were recently synonymized in the checklist of North American gelechiids by Lee, *et al*.(2009). However, the publication did not list *absoluta* as a new combination because *absoluta* does not occur in North America (Brown 2010).

Meyrick(1917) described *Phthorimaea absoluta* from a single adult male collected in Huancayo, Peru (at 10,650 ft (3,246m)) .

Clarke (1965) transferred *absoluta* to *Gnorimoschema* as a new combination and reported it from the Juan Fernandez Islands off the coast of Chile (apparently introduced). Clarke also mentioned specimens reared from potato and tomato from Chile,Peru, and Venezuela.

Povolny (1964) described *Scrobipapula* in 1964, and transferred *absoluta* to this genus .Becker (1984) included *absoluta* in *Scrobipalpula* in Heppner's atlas of Neotropical Lepidoptera, but did not list it as a new combination. Povolny (1987) described *Scrobipalpuloides* as a new genus and transferred *absoluta* to this genus as a new combination. Povolny (1994) later transferred *absoluta* from *Scrobipalpula* to *Tuta* as a new combination.

The genus *Tuta* and its type species, *atriplicella,* were made available by Kieffer and Jorgensen in 1910. Meyrick (1925) subsequently placed the genus *Tuta* in synonym with *Gnorimoschema.* Hodges considered *T.atriplicella* to be congeneric with the type species of *Phthorimaea, P.operculella,* and thus these two genera were synonymized in Hodges and Becker (1990). However, they did not mention *absoluta* as a new combination in this work. Povolny (1993) reinstated *Tuta* as a valid genus without giving any morphological basis for doing so. The two genera were again synonymized by Lee *et al.* (2009) based on similarity of male genitalia of *Tuta atriplicella,* the type species, and *Phthorimaea operculella.* However , a recent study of gnorimoschemini of Europe (Huemer and Karsholt,2010) has adopted a conservative approach to recognizing *Tuta* as a valid genus. Thus, *Tuta* is recognized here as the valid genus for the species *absoluta.*

A synonymy that only includes the various named combinations of *absoluta* would be as follows:

Phthorimaea absoluta (Meyrick, 1917)

Scrobipalpula absoluta (Clarke, 1965)

Scrobipalpuloides absoluta (Povolny, 1987)

Tuta absoluta (Povolny, 1994)

2.2.3 Ecological Range

The tomato leaf miner is not known to occur in the United States. However, Garcia and Espul (1982) erroneously reported that this pest had spread from the United States (California) in to Central and South America. A closely related Gelechiid species, the tomato pinworm, *Keiferia lycopersicella* (Walshingham), occupies the ecological niche of the tomato leaf miner on tomatoes in the United States (CABI, 2011).

The Tomato leaf miner is a neotropical oligophagous pest of solanaceous crop (Lietti *et al* ,2005). It is native to South America (Urbaneja *et al.,* 2007). A recent reference suggested that the tomato leaf miner is distributed throughout the South American continent with the exception of the Andean Region at altitudes higher than 1,000m (Viggiani *et al* .,2009). However, it is worth noting that the type specimen for *Tuta absoluta* was collected from the Andean region of Peru at an altitude of 10,650 ft (Meyrick,1917*)*.

South America- Argentina (Giganti *et al*.,1993; Pastrana,2004); Brazil (Pastrana, 2004);Colombia (Colomo and Berta, 2006);Ecuador (Povolny,1994); Panama (USDA,2011; Russell IPM,2009a); Paraguay(EPPO,2011a); Uruguay (Pastrana,2004); Venezuela (Fernandez and Montagne,1990a).

Europe- Albania (EPPO,2009k); Bulgaria (EPPO,2010a); France (EPPO,2009a); Germany (EPPO,2010c) ; Greece and Guernsey (EPPO,2010h); Hungary (EPPO,2009f); Italy (EPPO, 2009b, 2009f, 2009m, 2009q); Kosovo (EPPO,2010g); Malta (EPPO,2009g); Netherlands (EPPO,2009c); Portugal (EPPO,2009l); Slovenia (Knapic and Marolt,2009); Spin (Torres-Gregorio et al., 2009;Urbaneja et al., 2007).

Africa- Algeria (EPPO,2008b); Egypt (Russell IPM, 2009a); Libya (Russell IPM, 2009b); Tunisia (EPPO, 2009d) ; Morocco (EPPO,2008c); Sudan (Russell IPM, 2009a).

2.2.4 Life cycle

Tuta absoluta is a holometabolous insect with a high rate of reproduction. It may complete 12 generation per year depending on environmental condition (EPPO,2005). At a constant temperature of 25^{o}C and 75 percent R.H. In the laboratory , *Tuta absoluta* completes a generation in 28.7 days (Vargas, 1970) .Given the field conditions in the Africa Valley in Chile, *T.absoluta* could complete seven to eight generation per year (Vargas, 1970).

Since this pest can infest hosts grown in protected situations (such as greenhouses) its rapid reproductive rate should be kept in mind. The species can overwinter in the egg, pupal, or adult stage (EPPO, 2005). No information is available on whether this species is capable of diapauses.

2.2.4.1 Adults

The sex ratio in field-collected population in Venezuela was 1 male to 1.33 females (Fernandez and Montagne, 1990a). Adult males live longer than females. In the laboratory, mated males lived 26.47-+7.89 days while virgin males lived 36.17+-6.55days. Mated females lived 23.24+-5.89 days while virgin females lived 27.81+-10.78days (Fernandez and Montagne, 1990a).

Both genders mate multiple times. The first mating usually occurs the day after adults emerge. Mating occurs at dawn (Vargas, 1970). Studies in Chile revealed that the greatest number of males were captured in pheromone traps during the period from 7 to 11 a.m., suggesting that this is the time when males are searching for calling females (Miranda-Ibarra,1999).

The average preoviposition period for females was 2.4+-0.61 days (Fernandez and Montagne, 1990a). Female fecundity can range between 60 to 120 eggs (Torres *et al.,* 2001) . Oviposition studies in laboratories showed that females can lay egg for more than 20 days ; however, 72.3 percent of the eggs were deposited during the first 5 days and 90 percent in the first 10 days (Fernandez and Montagne, 1990a).

2.2.4.2 Eggs

Eggs are laid singly (rarely in batches) on all above-ground parts of the host plant (plate 1).

2.2.4.3 Larvae

Larvae complete four instars that are well-defined and are of different size and color (Estay, 2000), but variation in the number of instars is well-documented within many species of Lepidoptera. After hatching, larvae enter the plant tissue and begin feeding, thus creating mines.

In tomato, young larvae can mine leaves, stems, shoots, flowers, and developing fruit; later instars can attack mature fruit (Vargas,1970). larval mines increase in length and width as the larva develops and feeds. In cases of severe attack, all leaf tissue is consumed leaving behind a skeletonized leaf and large amounts of frass larvae spin silken shelters in leaves or tie leaves together (Vargas, 1970).

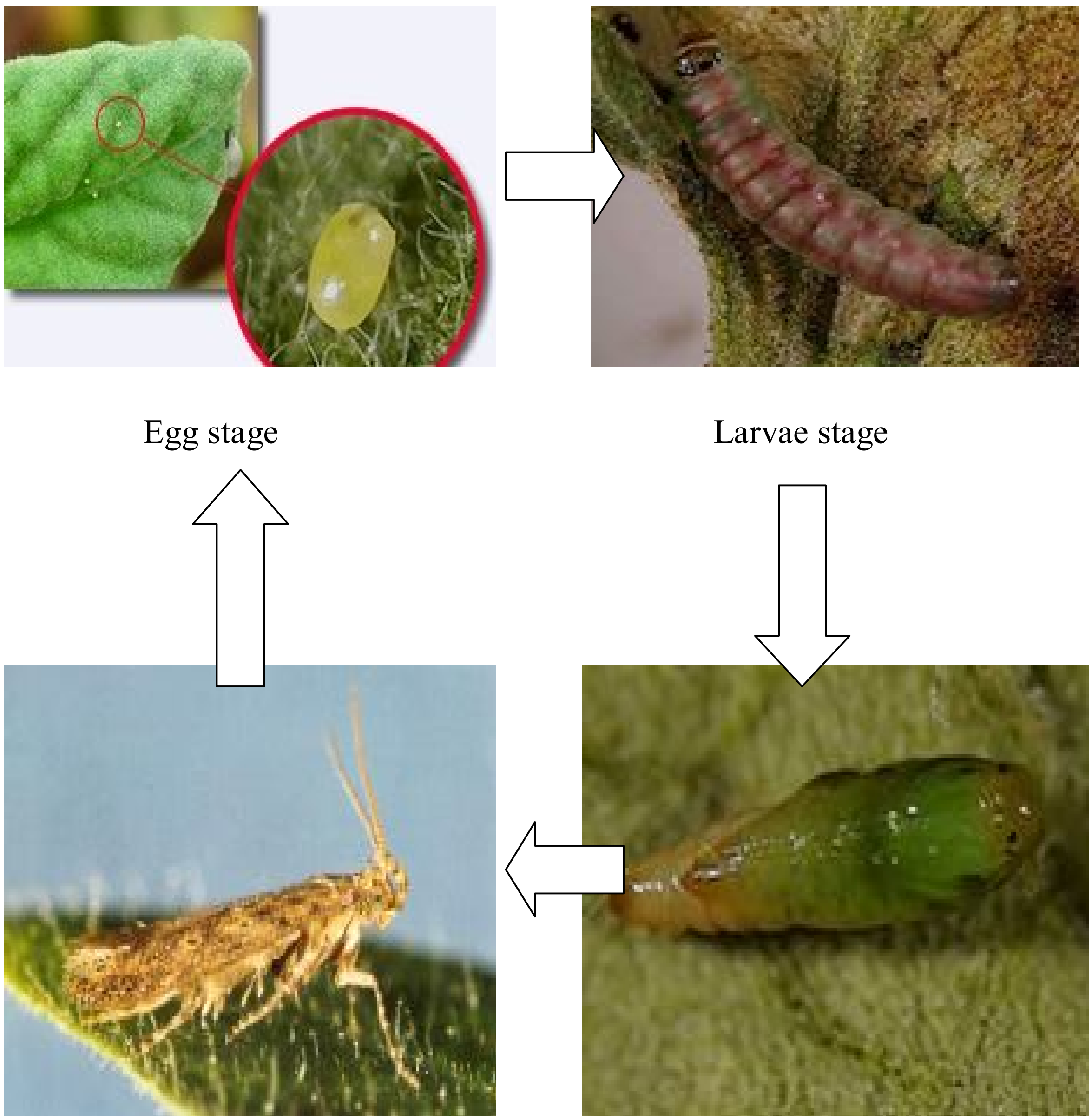

Plate 1: life cycle of *Tuta absoluta*

2.2.4.4 Pupae

Mature larvae purge themselves of food and build a silken cocoon where larva transforms into a pupa.

2.2.5 Developmental Rates and Day Degrees

The main lower developmental baseline temperature for *T. absoluta* is 8.14^{o}C for adult. (6.9^{o}C for eggs, 7.6^{o}C for larvae and 9.2^{o}C for pupe respectively). Using the mean baseline temperature of 8.14^{o}C, *T. absoluta* requires 459.6 degree days to complete its development.

A degree day is a measurement of heat units over time, calculated from daily maximum and minimum temperatures. Degree days are based on the rate of an insect's development at temperatures between upper and lower limits for development. The minimum temperature at which insect first start to develop is called the lower developmental threshold, or baseline temperature (Murray, 2008).Degree day requirements to complete egg, larval and pupal development are 103.8,238.5 and 117.3, respectively (Barrientos *et al*., 1998).

Laboratory studies in Chile showed that the development of *T. absoluta* from egg to adult requires 76.3 days at 14^{o}C (57^{o}F), 39.8 days at 19.7^{o}C (67^{o}F), and 23.8 days at 27.1^{o}C (81^{o}F).

Barrientos *et al.,(* 1998). reported that the egg stage of *T. absoluta* lasted 4.4 to 5.8 days at a temperature of 24.6^{o}C and a relative humidity of 76.17 percent; larval development was completed in 11 to 15 days at 24.09^{o}C and 70.64 percent R .H.; and males emerged in 7 to 8 days and females in 6 to 8 days at 26.3^{o}C and 72.3 percent R.H. the sex ratio was 3 males to 4 females, or 1:1.33 male : female.

2.2.6 Behavior

Adult *T. absoluta* are most active at dusk and dawn, and rest among leaves of the host plant during the day (Fernandez and Montagne, 1990a; Viggiani *et al.,* 2009). Mating usually occurs the day after adults emerge, usually at dawn.

Studies in Chile revealed that the greatest number of males was captured in pheromone traps during the period from 7 to 11 am, suggesting that this is the time when males are searching for calling females (Miranda-Ibarra, 1999).

Hickel *et al*. (1991) studied the mating behavior of *T. absoluta* in the laboratory and determined the sequence of male mating behavior. It can be divided into two phases: long-range female location and short-range courtship.

2.2.7 Dispersal

Tuta absoluta caterpillars have observed walking on leaves outside of their mines. This behavior might be related to the temperature inside the mine, the depletion of feed, and/or the accumulation of fecal material (Torres *et el.,* 2001). When outside of the mines, larvae move quickly and spin silken threads to move around safely (Fernandez and Montagne, 1990a). Mature larvae will sometimes exit the plant and move to the soil before pupating (Torres *et al*., 2001).

Adults have well-developed wings that allow them to disperse, but no information concerning their ability to fly.

2.2.8 Damage

Larvae of *T. absoluta* mine the leaves, flowers, shoots, and fruit of tomato as the leaves and tubers of potato (Pastrana, 2004). After hatching , larvae penetrate apical buds, flowers, new fruit, leaves, or stems. Conspicuous irregular mines and galleries as well as dark frass make infestations relatively easy to spot. Fruits can be attacked soon after they have formed, and the galleries made by the larvae can be colonized by pathogens that cause fruit rot. The damage caused by this pest is severe, especially in young plant. When potato plants have completed the vegetative cycle, larvae of *T. absoluta* mine the tubers underneath the epidermis. Larval feeding can cause tubers to rot (Pastrana, 1967).

2.2.8.1 Damage in leaves

After hatching, larvae mine the leaf tissue. The serpentine-shaped mines increase in length and width as the larva develops and feeds. In some cases, especially at the beginning of the infestation, the mines can be mistaken for those caused by leaf miners in the family Agromyzidae. In cases of severe attack, the larva consumes all the leaf tissue and leaves behind a skeletonized leaf and copious amounts of frass. It is common for larvae from the second to fourth instar to spin silken shelters in leaves or tie leaves together (Vargas, 1970).

2.2.8.2 Damage in shoots

Larvae are capable of penetration and mining tender shoots, usually gaining entry through the apical end or at angle formed between the petioles and the leaves (Vargas, 1970). Larvae can also pull together new shoots using silk produced by specialized salivary glands.

2.2.8.3 Damage in flower and fruit

Larvae of *T. absoluta* can destroy the developing fruit by mining its flesh. Infested fruit will usually fall to the ground. Larvae can attack the flower, but the most severe damage is found in developing (early instars) or maturing fruit (later instars). The larva usually enters the fruit under the calyx and tunnels the flesh, leaving galleries clogged with frass, that cause the fruit to drop or to rot on the vine. Larvae can also enter the fruit through the terminal end or through other fruit parts that are in contact with leaves, other fruits, or stems.

2.2.9 Economic impact

The tomato leaf miner *T. absoluta* is a pest of great economic importance with high reproduction capabilities and short generation life cycle which makes it at risk of developing resistance. The pest has spread from Latin America, where there is already resistance to a range of MOA groups, to Europe, the Middle East and North Africa. Using the *T. absoluta* poster IRAC is taking pro-active steps to advise on key management strategies to evaluate and reduce the spread of resistance.

In Latin America, *T. absoluta* is considered as a key pest of tomato both in the field and under protected conditions. Both yield and fruit quality can be significantly reduced by the direct feeding of the pest and the secondary pathogens which may then enter through the wounds made by the pest. Severely attacked tomato fruits lose their commercial value. 50–100% losses have been reported on tomato (mainly under low rainfall). According to, CIP (1996) considers that *T. absoluta* is one of the major pests of potato foliage, occurring in warm zones of low altitudes (below 1000 m).

2.2.10 Control procedures

Because of its biology and behavior, *T. absoluta* is a challenging pest to control. *T. absoluta* produces several broods each year. (Pastrana, 1967), after emergence, larvae can either tie leaves or young shoots to create a shelter to feed. Alternatively they immediately penetrate the young fruit, leaves, buds, or stems where they feed and develop.

Pupation occurs inside galleries, in dried material, or in soil. Effective chemical control is difficult because *T. absoluta* feeds internally, and because produces many offspring and the developsment pesticide resistance. For these reasons, a combination of control methods has been used in South America and Europe for containment or eradiation of the tomato leaf miner

Control methods include mass-trapping of adults, well-timed pesticide applications, and a variety of cultural controls such as removal and destruction of infested plants, scheduled host-free periods, and removal of wild hosts in the vicinity of places of production. Integrated pest management (IPM) is being developed in several South American countries where *T. absoluta* is a serious pest of tomato. Biological control method are being investigated in most countries where *Tuta absoluta* is present.

A successful IPM program included chemical, biological and cultural techniques, were applied to reduce pest populations.

2.2.11 Treatment options

All treatments listed in the guidelines should only be used as a reference to assist in the regulatory decision making process. It is the National Program Manager,s responsibility to verify that treatments are appropriate and legal for use. Upon detection and when a chemical treatment is selected, the National Program Manager should consult with PPQ,s FIFRA Coordinator to ensure that the chemical is approved by EPA for use in the United States prior to application.

Treatments can include any combination of the following options:

- Sanitation and other cultural control methods.
- Application of insecticides.

2.2.12 Eradication

Eradication is the first action to consider with the introduction of a new pest. Eradication may be feasible in some conditions, but if it fails then other strategies will be considered. Eradication may be feasible when the following conditions exist: pest population is confined to a small area, detection occurs soon after the introduction, or pest population density is low.

If an infestation of *Tuta absoluta* is discovered that meets the above named conditions, eradication will be attempted. Measures will include but may not be limited to removal and destruction of all infested plant material, removal of host material within 2 miles (3.2km) of the find, and treatment of the soil and surrounding vegetation with an approved pesticide after removal of the infested plants.

2.2.13 Cultural control

2.2.13.1Sanitation

Population of *Tuta absoluta* can carry over on infested plant left in greenhouses or fields after harvest or can arrive at these sites via the movement of infested plants. Production nurseries, tomato production sites, and tomato packing sites should follow strict sanitation guidelines to prevent the arrival and spread of *Tuta absoluta.*

2.2.13.2 Management of tomato leaves miner in nurseries and Greenhouse Tomato Production

Installing double self-closing doors and covering windows and other opening with 1,6 mm (or smaller) insect mesh can prevent entry or exit of *T. absoluta* adult in greenhouse (InfoAgro Systems, 2009). Pots, carts and greenhouse tools should be inspected and thoroughly cleaned before moving them to other areas.

Inside the greenhouse, plants should be routinely examined: leaves and stems should be checked for evidence of egg, mines, frass, and other damage. The underside of fruit calyces and the fruit itself should be checked for the presence of small heaps of frass that indicate larval entry holes (Mallia, 2009). Infested plants or plant parts should be removed, especially at the beginning of cultivation, and residues should disposed of carefully, ensuring that they are stored in sealed containers until they are sent to a wastes management facility (InfoAgro Systems,2009).

Solanaceous weeds in the vicinity of infested greenhouses should be removed and destroyed, to prevent the build-up of a potential population reservoir (Koppert,2009). Greenhouse workers should check their clothing before moving to other greenhouses in order to avoid the presence of eggs, larvae, and resting adults of *T. absoluta.*

2.2.13.3 Management of tomato leaves miner in field Grown Tomatoes

Destruction and incorporation of crop residues after harvest effectively interrupts the life cycle of *Tuta absoluta* by killing the immature stages present in the plant material. Mechanical harvesting and tilling equipment should be cleaned using high pressure washing or steam after use in infested fields. Clean and inspect all harvesting containers, field boxes, carts, etc., should be done before moving them to other areas.

Solanaceous weeds in the vicinity of infested areas should be removed and destroyed to prevent build-up of a potential population reservoir (Koppert,2009). If at any time during the growing cycle *T. absoluta* is detected, the whole plot should be removed and securely destroyed to interrupt the lest (by plowing, burning, etc.) interrupt the pest life cycle and spread (Russell IPM, 2009b).

2.2.13.4 Vegetable packing stations

The United Kingdom has published best practices guidelines for managing *Tuta absoluta* at tomato packing sites since these could provide a pathway for movement of the pest to the open environment (FERA, 2009). strict waste management procedures are employed so that no plant waste is left uncovered and exposed. Larvae, pupae and adults might hide in plastic or cardboard packing materials in tomato grading areas.

To mitigate the risk, packing sites are encouraged to regularly examine and clean grading containers and/or use plastic bags in grading containers and replace the bags daily. Tomato crates that are returned to suppliers should be cleaned to prevent introduction of *T. absoluta* to growing sites.

Alternatively, non-returnable tomato packing boxes can be used and should be assembled and stored in an area of the packing station away from infested crates (Sixsmith, 2010). Inspection measures at packing stations call for examination of fruit with calyces for evidence of larval mining.

In particular, vine (or truss) tomatoes should have stems and calyxes examined, and this should be a priority over the examination of fruit. At all times, windows and other openings should be kept covered with 1.6 mm (or smaller) mesh to prevent the entry or exit of moths (InfoAgro Systems, 2009).

Tomatoes awaiting packaging should also be protected with insect-proof mesh or plastic tarpaulin. As mentioned before, solanaceous weeds should be removed and destroyed, to prevent the build-up of a potential population reservoir.

2.2.14 Managing Insecticide Resistance

The non-judicious application of insecticides can lead to the development of resistance. In Bolivia and Chile, *T. absoluta* was reported to be resistance to organophosphates in the early-to mid-1980s. More recently, laboratory studies of resistance in field strains of *T. absoluta* in Argentina revealed reduced efficacy of deltamethrin and abamectin (Lietti *et al.,* 2005). Resistance to cartap, abamectin, permethrin and methamidophos (Siqueira *et al*., 2000), and acephete and deltamethrin (Branco *et al.,* 2001), has been reported in Brazil.

The newer insecticide classes have provided good activity against the tomato leaf miner (IRAC, 2009a). However, the modes of action, usually provides a sustainable and effective approach to managing insecticide resistance (IRAC, 2009c).

2.2.15 Procedure

One of the key steps in resistance management is to minimize the continuous use of pesticides with the same MOA classification system makes it easy for farmers and farm advisors to understand which pesticides share the same MOA without having to know the biochemical basis. The MOA classification thus provides growers, advisors, extension staff, consultants and crop protection professionals with a simple guide to the selection of insecticides for use in an effective and sustainable insecticide resistance management strategy.

2.2.16 Insecticides

Historically, *Tuta absoluta* has been controlled with chemicals.

Organophosphates and pyrethroids were used during the 1970s and 1980s until new products introduced in the 1990s (such as abamectin, spinosad, tebufonzide, and chlorfenpyr) became available (Lietti *et al.,* 2005). At least 12 classes of insecticides control *T.absoluta (*IRAC, 2009b).

Control failures with organophosphates and pyrethroids in South America (Salazar and Araya, 2001) prompted research on the resistance status of *Tuta absoluta* (Siqueire *et al*., 2000; Lietti *et al.,*2005); however, newer classes of insecticides are providing good control of this pest (IRAC, 2009a).

This section describes pesticides used in current outbreaks and also mentions insecticide used to control *Keiferia lycopersicella* (tomato pinworm), a gelechiid moth that occupies the ecological niche of *T. absoluta* in the United States.

Indoxacarb, Spinosad, Imidacloprid, Deltamethrin, and *Bacillus thuringinsis* var. *kurstaki,* were applied for the control of larvae infestations in Spain (FERA, 2009; Russell IPM, 2009b). chlorpyrifos and pyrethrins were used in Italy (Garzia *et al.,* 2009a). abamectin, indoxacarb, spinosad ,imidacloprid, thiacloprid, lufenuron, and *Bacillus thuringiensis (*Bt), were recommended for outbreaks in Malta (Mallia, 2009). Indoxacarb and Bt were recommended for use in france (FREDON-Corse, 2009a). In Brazil, abamectin, cartap, chlorfenapyr, phenthoate, methamidophos, spinosad, and indoxacarb, were recommended for use in the south, southeastern, and savannah tomato-growing regions, while chlorfenapyr, phenthoate, and spinosad were recommended for use in the northeastern region (IRAC, 2007). In Argentina, Bt and triflumuron were recommended for control of *Tuta absoluta* larvae as part of an IPM program that also included parasitoids (Riquelme, 2006).

2.2.17 *Bacillus thuringiensis*

Bacillus thurnigiensis var. kurstaki (Btk) is a Lepidoptera- specific microbial that, when ingested, disrupts the midgut membranes. For larval control, neutral solutions of Btk should be applied to crops once per week at the end of the end of the day (FREDON-Corse, 2009a). The leaf epidermis presents a significant barrier to control with chemical or microbial insecticides (Salvo and Valladares, 2007). As such, Btk may not be effective once *T.absoluta* larvae enter plant parts (Sixsmith, 2009). Btk is registered for use on tomatoes in United States.2.2.18 Azadirachtin

Azadirachtin is the key insecticidal ingredient found in neem tree (*Azadirachta indica* A.Juss.) oil. It is structurally similar to ecdysones, insect hormones that control metamorphosis. It is thought to as ecdysone blocker interfering with the insects ability to molt (EXTOXNET, 1995). Azadiractin is effective on larvae (all insects) and pupae. After ingestion, insects stop feeding; however, death may not occur for several days.

Azadirachtin has been recommended for use as a preventive spray and for light infestations (<30 adult catches per week) of *T. absoluta* in Spain (Servicio de Sanided Vegetal-Murcia, 2008). In the United States, azadirachtin is registered for use on tomatoes to control tomato pinworm. This product has low toxicity to pollinating bees, butterflies, and parasitic wasps.

2.2.19 Population Monitoring

2.2.19.1 Population-baited traps

Use traps baited with synthetic sex pheromone for monitoring of *T. absoluta* in open fields, greenhouses, and packing sites. Sex pheromone-baited traps will capture only adult males. The sex pheromone of *T. absoluta* has been isolated and identified. The main compounds are (3E, 8Z,11Z)-3,8,11-tetradecatipon-1-yl acetate and (3E,8Z)-3,8-tetradecadien-1-yl acetate in the proportions of 90:10, respectively (Svatos *et al.,*1996).

2.2.19.2 Mass-Trapping

Mass-trapping involves placing a number of pheromone baited traps in strategic positions within a crop. Large numbers of adult males are trapped resulting in an imbalance to the sex ratio which impacts the mating pattern of *Tuta absoluta.* Mass trapping can be used to reduce *T.absoluta* populations and is particularly useful in production of greenhouse tomatoes (Russell IPM, 2009b). For mass trapping, pheromone trap density should be 20 to 25 traps/ha inside greenhouses (30 traps/hafor greenhouses destined for plant propagation) and 40 to 50 traps/ha in open fields (Bolkmans, 2009; FREDON-Corse, 2009a).

2.2.19.3 Pan traps

Pan traps are easier to maintain, and are less sensitive to dust, compared to delta, McPhail, and light traps also have a larger trapping capacity than Delta traps. Rectangular plastic trays that hold 6 to 8 liters of water baited with pheromone lures are recommended for mass trapping (InfoAgro, 2009).

2.2.19.5 Light traps

Light traps can also be used to capture adult males and females of *Tuta absoluta (*Bolkmans, 2009; Rodrigues de Oliveira *et al*., 2008). Light traps have been used to control *T.absoluta in* greenhouse tomato production in Italy as follows: Install traps at a heigh of 1 meter or less from the ground, at a rate of 1trap per 500 to 1000 m^2(Laore Sardegna, 2010). Light traps should be placed near entry doors and used only during sunset and sundown (Bolkmans, 2009). Light traps should not be used in vented greenhouses that lack proper screening in the openings (koppert, 2009).

Russell IPM (2009c) recently developed a light trap for *T. absoluta* that is capable of capturing of male insects in addition to a substantial number of females per night. The light trap, named ferolite-TUA, uses a combination of sex pheromone and a specific light frequency that is highly attractive on *T.absoluta.* the trap has a reported improved effectiveness (over the standard pheromone trap) of 200 to 300 percent.

2.2.20 Biological control

Biological control is being investigated in most countries where *Tuta absoluta* is present.

2.2.20.1 Egg parasitoids

Trichogramma pretiosum, Trichogramma achaeae, and *Trichogrammatoidea bactrae,* parasitize the eggs of *T.absoluta* . In one study, adults of *T.absoluta* were released onto fully developed tomato plants, followed by the release of *Trichogramma pretiosum* 12 hours later. After 24 hours, the level of egg parasitism varied between 1.5 to 28 percent (Faria *et al.,* 2008).

In another study, the optimal number of *Trichogramma pretiosum* that needed to control *T. absoluta* in commercial tomato plantations, was determined to be 16 parasitoids per host egg (Pratissoli *et al*., 2005). In Argentina, inundative releases of *Trichogrammatoidea bactrae* to control *T.absoluta* in greenhouse grown tomatoes gave good results (Botto *et al.,* 2009; Riquelme *et al.,* 2006).

Trichogramma achaeae has been shown to control *Tuta absoluta* in greenhouses in southeastern Spain (Cabello *et al*., 2009a). under laboratory conditions, 100 percent parasitism by *T.achaeae* was reported and of those, 83 percent developed to the blackhead stage. In greenhouse tomatoes there was a 91 percent reduction in damage when 30 *T.achaeae* per plant (75 adults/m^2) were released every 3 to 4 days (Cabello *et al*., 2009a).

2.2.20.2 Larval Parasitoids

Larvae of *Tuta absoluta* spend most of their lifetime inside mines, however, second instars leave their mines during the cooler times of the day making them vulnerable to parasitoids and predation (Torres *et al.,* 2001). Of the larval parasitoids, the braconid *pseudapanteles dingus* is frequently found parasitizing larvae in South America . Studies have shown that female parasitoids attack hosts daily and do not have a preference among larval instars. Parasitism of *T.absoluta* by *p.dignus* can reach up to 46 percent in late tomato crops (Sanchez *et al*., 2009). In Argentina, researchers have tested inoculative releases of *P.dignus* in greenhouses before *T.absoluta* reaches high population levels (Botto, 2011).

2.2.21 Predators

The damsel bug *Nabis pseudoferus* is an effective egg and larval predator of *Tuta absoluta* in Spanish greenhouses (Cabello *et al.,* 2009b). In two semi-field studies, first stage nymphs of *N.pseudoferus* released onto tomato plants (8 to 12 per plant) killed *T. absoluta* eggs, reducing the number of egg by 92 percent and 96 percent. In addition, adults and last instar nymphs of *N.pseudoferus* were also observed preying on larvae of *T .absoluta* , even when these were inside the mines (Cabello *et al*., 2009b). *Nabis pseudoferus* is widely distributed in Europe and is commercially available. The recommended dose for outbreaks is 10 to 15 individuals/m^2.

The mirids *Macrolophus pygmaeus* and *Nesidiocoris tenuis* are endemic to Spain and feed on egg and larvae of *T.absoluta.* In one study, adult *M. pygmaesus and N. tenuis* are available commercially . however, according to a report from the United Kingdom, *Nesidiocoris tenuis* is problematic because it can attack host plants when prey are in short supply. Plant feeding causes brown ring in the vascular tissue and destruction of the plants growing points (Sanchez *et al*.,2008; Sixsmith, 2009).

2.2.22 Microorganisms

Bacillus thuringensis has been recommended for control of *T. absoluta* .More recently, the muscadine fungus *Metarhizium anisopliae (*Metschn) Sorokin has been studied for control of *T.absoluta* (Pires *et al*., 2009). Adult females infected with the fungus did not reduce their oviposition or fecundity; however, infection with *M.anisopliae* resulted in 37 percent female mortality. Eggs exposed to *M.anisopliae* were all infected after 72 hours.

2.2.23 Mating disruption

Michereff-Filho *et al.(*2000) examined the use of mating disruption for *T. absoluta* in small plots of fresh market tomatoes in Brazil. The effectiveness of the technique was assessed through trap captures of mails in disrupted plots, mating frequency in mating tables, as well as plant damage. The highest levels of disruption (60 to 90 percent) were recorded in plots treated with 35 to 50 g/ha of sex pheromone. However, no treatment was capable of significantly reducing the percentage of mined leaflets or bored fruits or the frequency of mating as compared to the control plots. The results may be attributed to the composition of the synthetic pheromone, doses used, high pest population density, and mated female migration to the area treated.

2.2.24 Integrated Pest Management

Integrated Pest Management (IPM) programs are being developed in several countries to manage infestation of *T. absoluta.* Most IPM programs include the monitoring of pest population, effective methods of prevention and control, and the use of pesticides when needed. Biological control is also implemented if available.

In Spain, IPM for *T. absoluta* includes the following management tools (Robredo-Junco and Cardenoso-Herrero, 2008):

- Mass-trapping of adults prior to planting.
- Clearing of crop residues from planting soil.
- Application of imidacloprid in irrigation water 8 to 10 days after planting.
- Application of spinosad or indoxacarb when *T.absoluta* is detected.
- Elimination of crop residues immediately after the last fruits have been harvested.

  In Argentina, IPM for *T. absoluta* in greenhouse tomatoes has been tested at Instituto Nacional de Tecnologia Agropecuaria (INTA) over the last 10 years with positive results (Botto, 1999). The strategy includes the following:

  - Monitoring for early detection of adults using pheromone traps and visual inspection of plants, primarily for eggs
  - Inundative releases of *Trichogrammatoidea bactrae,* initiated when the first adults are trapped and/or the first eggs are observed
  - Use of *Bacillus thuringiensis* is in conjunction with (or after) release of egg parasitoids to control larvae
  - Compatible pesticides based on safe pesticide usage if necessary
  - Crop rotation with non-host plants

  Cultural control practices in the greenhouse and surrounding environment (Botto , 1999)

2.3 Neem

2.3.1 Taxonomy

Kingdom: Plantae

Division: Magnoliophyta

Order: Rutales

Suborder: Rutinease

Family: Meliaceae

Genus: Azadirachta

Species: Indica

S.N: *Azadirachta indica* A.juss

E.N: Neem

(Vietmeyer, 1992, and Schmutterer, 2002)

2.3.2 Origin

The Neem is versatile tree of Indian and Burma origin where the ancient healers of that region knew it very well in health (ICIPE, 2002).

2.3.3 Morphology

Neem is a fast growing tree that can reach a height of 15-20m, rarely to 35-40m. Its ever green but under severe drought it shed mostly or nearly all of it leaves. The branches are wide spread, the fairly dens crown is roundish or oval may reach diameter of 15-20m. In old tree standing specimen the trunk is relatively short straight and many reach a diameter of 1.2m. The bark is hard fissured or reddish-brown. The sap wood is grayish white and heart wood reddish when first exposed to the air becoming reddish after exposure. The root system consists of a strong tap root and well developed tateral roots. The alternate, pinnate leaves are medium (Ganguli, 2002).

2.3.4 Distribution

Neem is widely distributed throughout South East Asia and West Africa and part of Central America (Stoll, 2000). Neem was introduced to Sudan in the 20 century. The first tree were planted at Shambat in 1916, today trees are spread in town and villages along the Blue and White Nile, irrigated areas of Central Sudan, Kordofan and Darfur (Schmutterer, 1969).

2.3.5 Ecology

The Neem trees is famous for its drought resistance, normally it thrives in areas with sub-arid to sub humid conditions with an annual rainfall between 400 and 1200 mm. it can also grow in regions with an annual rainfall 400mm. but in such cases it depends largely on the ground water levels. Neem can grow in many different types of soil, but it seems to develop best on well drained, deep sandy soils. It is a tropical and subtropical tree, and exists at annual means temperatures and does not tolerate (Ganguli, 2002).

2.3.6 Active ingredients

The Neem tree produce a compounds of many active ingredients called Azadirachtin and it is tetramer titer penoid compound, which influences the hormonal system, feeding activity reproduction and fling ability of insect. Azadirachtin has low mammalian toxicity. It degrades rapidly in the environment and has low side effects on non-target species and beneficial insects. Seeds of the Neem tree contain the highest concentration of Azadirachin. Salanin inhibits the feeding of insect pests, Nimbin and Nimbidin showed antiviral effects (Ganguli, 2002).

2.3.7 Chemical Compounds of Neem tree

Extract of various parts of the tree were studied by many chemists that isolated many different compounds. Most of the known active compounds belong to the group of titer penoids (Schmutterer, 1990). Azadirachtin and Solanin are the most important constituents of neem seed kernel composition, other active compounds in the seed kernel are Salanin, Salanol, Acetate, Nimbin and Deactly nimbidin (Jacobson, 1989).

2.3.8 Mode of action

Neem acts as insects feeding deterrent and growth regulator, the treated insects usually cannot molt to its next life stage and dies, Azadirachtin is chemically similar to ecdysone responsible for triggering molts. It also acts as repellent when applied to plant and does not produce a quick knock down and kill (Schmutterer, 1990).). Also Neem has some systemic activity in plants, its most effectively growing immature stages and adults are not killed by the growth regulator properties of Azadirachtin, but mating and sexual communication may be disrupted which results in reduced fecundity (Schmutterer, 1990 and Pedigo, 1999).

2.3.9 Uses of Neem in pest and disease control

Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated and is recommended for those who are sensitive to Permethrin. A known insecticide which might be irritants and also the scabies mite has yet to become resistant to Neem, so in persistent cases Neem has been shown to be very effective. Morever, there is also anecdotal evidence of its effectiveness. In treating infestations of head lice in humans, it is also very good for treating worms (soak the branches and leaves in lukewarm water and drink it). In the traditional medicine neem trees originated on the Indian subcontinent. The neem twig is nature's tooth brush to over 500 million people daily in India alone. Herbal medicine is the oldest form of therapy practiced to be mankind and much of the oldest medicinal use of plants seems to have been based on highly developed 'dowsing instinct' (Schmutterer, 2002). Siddig (1993) reported from Sudan that neem seed water extracts at1Kg/1Liter of water repelled foliage pest of potato including *B. tabaci*, *Aphis gossypii* and *J. lybica* and yield increased to 5 ton/ ha. Mohammed (2002) reported that neem seed showed good performance against *A. gossypii*, *B. tabaci*, and *J. lybica* on Okra. Dawood (2001) reported that Neem water extracs at 1Kg/liter water reduced the number of onion thrips by 63.5% under the field condition.

2.4 Spinosad

Spinosad is the first active ingredient proposed for a new class of insect control products, the Naturalytes. Spinosad is derived from the metabolites of the naturally occurring bacteria, *Saccharopolyspora spinsa.* Spinosad has been shown to be highly active on insects including species from the orders Lepidoptera, Diptera, Hymenoptera, Thysanoptera, and a few Coleoptera. Spinosad may be used to control pests in both agricultural and horticultural environments, and also in greenhouses, golf courses, gardens, and around homes.

Spinosad has been developed to provide rapid control of Lepidoptera and other pests with minimum disruption of beneficial insects and other non-target organisms.

Because it is highly effective, only very low use rates are required to achieve efficacy. These attributes permit to many users an opportunity to implement integrated pest management tools for the first time.

Due to its low effective use rate, the safety to the environment, the safety to mammals, and safety to beneficial insects, spinosad was registered under the US EPA,s reduced risk program. Spinosad was also awarded the *Presidential Green Chemistry Challenge Award* during 1999. This award recognizes the unique contribution of spinosad and also highlights Dow AgroSciences commitment to producing safer and more effective products for insect control.

2.4.1The Spinosad story

During the last decades, companies including the Dow Chemical Company and Eli Lilly and Company began to actively look for naturally occurring pest control products. As a result of these efforts, a scientist from the Natural products division of Eli Lilly while vacationing in the Caribbean in 1982 visited an abandoned rum still and collected several soil samples. These samples were returned to the laboratory to determine the presence of biological activity. Three years later the fermentation products from these samples were shown to have insecticidal activity. By 1986 Eli Lilly's scientists identified the organism producing the biologically active substances. They determined that this was a new species of actinomycete products division. (Mertz and yao1990, Thompson1997 and Crouse1998).

2.4.2 Symptomology

Sensitive insects exposed to Spinosad exhibit unique symptomology that is typified by a general paralysis accompanied by loss of body fluid resulting in flaccid paralysis. Under close examination, minute tremor of the mandibles and crochets can be seen. The onset of paralysis is quite rapid for a biological material. The length of time required was 81 minutes before 50 percent of third instar *Heliothis virescens* larvae treated topically with 10mg of technical material failed to respond to a hot needle probe. That can be compared to 25 minutes for cypermethrin treated larvae. However, intoxicated insect remain on the plant for one to two days without feeding; whereas, insects treated by excitatory compounds, such as pyrethroids or organophosphates, tend to fall off the plants more rapidly.

2.4.3 Mode of action

In insect, the mode of action of Spinosad is associated with excitation of the insect nervous system (Salgado 1998). Spinosad uniquely alters the function of nicotinic and GABA-gated ion channels (Salgado 1998, Watson unpublished data), in a manner consistent with the observed neuronal excitation. However, spinosad does not interact with known binding sites for other nicotinic or GABAergic insecticides such as neonicotinoids, fiproles, avermectins and cyclodiens. These data indicated that spinosad acts through a unique insecticidal mechanism (Salgado1998).

2.4.4 Registrations

Spinosad has been approved for use by registration authorities in more than 30 countries and the first registrations were granted during late 1996 and early 1997 for cotton, almonds, vegetables, and turf and ornamentals. Since that time, many additional crop and non-crop uses have been approved worldwide, ranging from Australian cotton to Japanese crucifers to Mexican tomatoes to Chilean stone fruit to Israeli melons. Spinosad products have found utility for pest management for a wide variety of crops, and in the U.S. alone use has been approved on more than 150 crops. In addition to crop uses, Spinosad is also approved for non-crop uses on turfgrass and ornamental plants, for livestock pest control, and fire ant control.

2.4.5 Reduced risk classification

Spinosad has been classified by U.S. Environmental Protection Agency (EPA) as a reduced risk pesticide product. This classification affords preferential registration and expedited label expansions to select products that meet the Agency's stringent criteria and pose less risk to public health and the environment than available alternatives. Spinosad has been classified by EPA as a reduced risk insecticide product because of its:

- Low acute mammalian toxicity
- Low toxicity to fish and wildlife
- Compatibility with integrated pest management and lack of beneficial insect disruption

2.4.6 Ecotoxicology

Spinosad is not acutely toxic to terrestrial birds and wildlife or to fish and most aquatic invertebrates. Laboratory studies indicate that some free-swimming and sediment-dwelling aquatic invertebrates may be sensitive to long-term exposure to spinosad. Under field conditions, this sensitivity may be mitigated by the rapid dissipation of spinosad which occurs from the water column as well as sorption and binding of that small portion of residues organisms.

2.4.7 Environmental fate

2.4.7.1 Fate in soil

Spinosad degrades readily in the soil environment and is non-persistent. Primary mechanisms of degradation are sunlight photolysis and microbial breakdown. Under field conditions, Spinosad dissipates rapidly from soil surfaces with observed half-lives of less than 1 day (Hale,1996).

2.4.7.2 Fate in water

In natural water systems Spinosad rapidly dissipates, with the primary route of degradation involving sunlight photolysis. A water column half-life of less than 1 day has been observed in artificial pond systems under outdoor condition (Saunders, 1997).

2.4.7.3 Fate in plants

Residues of Spinosad present on plant surfaces dissipate at a moderate-to-rapid rate, primarily due to sunlight photolysis. Dissipation half-lives of 2 to 16 days have been observed for residues on leaf and fruit surfaces, the rate dependent on the amount of sunlight received and degree of shading (Saunders, 1997).

CHAPTER THREE

MATERIASLS AND METHODS

3.1 Field survey

The present survey was carried out in greenhouses in three different locations in Khartoum state namely Elselat, AbuHalima and wad Elnaaeem, during the period July-September 2013. Random samples of larval stage of *Tuta absoluta* were collected from different tomato plants at the selected areas. Three greenhouses were selected from each area. Twenty plants were selected from each glasshouse at random. Three leaves were collected randomly from the upper, middle and bottom of the selected plants. A total of 60 plants were collected from each area. The samples from each area were brought to laboratory for calculation.

3.2 Laboratory studies

These experiments were conducted under laboratory conditions at College of Agricultural Studies of Sudan University of Science and Technology at Shambat during 2013.

3.3 Insect culture

Random samples of infested tomatoes plants at the selected areas were collected and brought to laboratory for insect rearing. Each of the collected leaves were kept in plastic cages (30x20x20 cm).The cages were covered with muslin cloth plate (4), and the bottom of these cages were filled with sand and moistened with water as pupation media. After emergence the adults were transferred to other rearing cages. The adults were fed on sugar solution (10%).

3.4 Preparation of neem ethanolic extract

Ripened mature fruits of the neem tree *Azadirachta indica* A. Juss which had dropped to the ground under trees, were collected from Shambat area. Fruits were washed with tap water and the seeds were left to dry under shade for 5 days. Obtained seeds, were crushed, in an electronic blender to a fine seed powder for extraction.

3.5 Extraction method

Extraction process was conducted at department of Pesticides Alternative of the Environment and Natural Research Institute (ENRRI), National Research Center (NRC). Ten grams of each of the previously prepared powder of neem seed kernel was wrapped separately with filter paper and placed in an extraction chamber of a soxhelt extractor and extracted with 250 ml ethanol solvent for each sample. The extraction continued until the extracts were completely colourless. Then the solvents were removed off the crude extracts by rotary evaporator (plate3). The materials obtained were carefully stored in a refrigerator at 5^{o} C until used for experiment.

3.6 Bioassay procedure

These experiments were conducted under laboratory conditions at College of Agricultural Studies of Sudan University of Science and Technology at Shamba. Ten larvae were used for each treatment of the seeds ethanolic extracts of neem, and each treatment was replicated three times. Thirty larvae (three replicates) were treated with *Azadirachta indica* A. Juss for five concentrations (15%, 7.5%,3.75%,1.8% and 0.9%). Thirty larvae (three replicates) were treated with the recommended dose of Spinosad (1 ml Spinosad /liter water) as standard (Tracer (Spinosad) 240 SC at 27.36 g a.i./fed. (0.114 litre/Fed.). Tracer is manufactured and introduced in the Sudan by Chemimport Company ltd). Also, thirty larvae (three replicates) were used as a control which was treated with distilled water only. All treated larvae were kept in Petri-dishes (9 cm) in diameter at temperature range between 27-32 °c. During treatment period the larvae were fed on fresh tomato leaves. The mortality counts were recorded after 24, 48 and 72 hours after application, (plate 5).

3.7 Statistical Analysis

Treatments were arranged in a Complete Randomized Design (CRD). Transformations of data were done using square root. Data were subjected to Analysis of Variance. Duncan's Multiple Range Test was used for means separation. Also, the probit analysis was applied (Finney, 1971) to calculate the LC_{50} for the different concentrations of the neem seed oil.

Plate 2: Soxhlet Extractor Apparatus

Plate 3: Soxhlet and Rotary Evaporator

Plate 4 : Rearing cages.

Plate 5: Experiment Design

CHAPTER FOUR

4. RESULTS

4.1 Field survey

Table1 shows that there was significant difference between mean population of *Tuta absoluta* from the three study areas during July, August and September (2013). The population in July and August was significantly higher than September population in Elselat and Abo Halima was significantly higher than Wad Elnaaeem. The same trend was obcarved in August and September population in Wad Elnaaeem was significantly less than Abo Halima and Elselate.

4.2 laboratory study

4.2.1 Effect of neem extracts on mortality of third larval instars of *T. absoluta*.

Results exhibited in Table (2) and fig. (1) showed that all concentrations tested of the seed ethanolic extract of neem except 0.9 and 1.8% concentrations gave significantly higher mortality percentage than the control after 24hrs of exposure. The mortality caused by the highest concentration used in this study (15%) was comparable and not significantly lower than the mortality caused by the recommended dose of Spinosad even after 48 and 72 hrs of exposure.

After 72 hrs of application, all concentrations of the seeds ethanolic extract of neem generated higher mortality percentages than control. However, the highest concentration (15%) of neem extract was not significantly different from that obtained by standard Spinosad.

4.2.2 Propit analysis

Results obtained in Table5 showed that the Lethal Concentration (LC_{50}) values for seeds ethanolic extracts of neem were 16.3%, 9.7% and 3.2% after 24, 48 and 72 hrs of exposure respectively.

Table (1): Occurrence of *Tuta absoluta* in different areas during different months

	July	August	September
Wad Elnaaeem	++	+	Non
Abo Halima	+++	+++	++
Elselate	+++	+++	++

+++: high infestation.

++: moderate infestation.

+: low infestation.

non: No infestation.

Table 2 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 24hrs)

Concentration.(%)	Larval mortality (%)			
	R1	R2	R3	Mean
15	40(6.4)	50(7.1)	50(7.1)	46.7(6.9)a
7.5	20(4.5)	20(4.5)	10(3.2)	16.7(4.0)b
3.75	0(0.7)	10(3.2)	10(3.2)	6.7(2.3)c
1.87	0(0.7)	0(0.7)	0(0.7)	0(0.7)d
0.937	0(0.7)	0(0.7)	0(0.7)	0(0.7)d
SP	50(7.1)	60(7.8)	30(5.5)	46.7(6.8)a
Control	0(0.7)	0(0.7)	0(0.7)	0(0.7)d
C.V. (%)				24.4
SE				0.6

Means followed by the same letter (s) are not significantly different at ($P< 0.05$).
Means between brackets are transformed according to $\sqrt{X + 0.5}$

Table 3 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 48hrs)

Cons (%)	Mortality			
	R1	R2	R3	Mean
15	70(8.4)	60(7.8)	80(9.0)	70(8.4)a
7.5	40(6.4)	30(5.5)	30(5.5)	33.3(5.8)b
3.75	20(4.5)	20(4.5)	30(5.5)	23.3(4.8)bc
1.87	10(3.2)	20(4.5)	20(4.5)	16.7(4.0)cd
0.937	0(0.7)	10(3.2)	20(4.5)	10(2.8)d
SP	80(9.0)	70(8.4)	70(8.4)	73.3(8.6)a
CO	0(0.7)	0(0.7)	0(0.7)	0(0.7)e
C.V.(%)				17.4
SE				0.6

Means followed by the same letter are not significantly different at ($P < 0.05$).
Means between brackets are transformed according to $\sqrt{X + 0.5}$

Table 4 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of the *Tuta absoluta* (Mortality% 72hrs)

Concs.(%)	Mortality (%)			
	R1	R2	R3	Mean
15	100(10)	80(9.0)	100(10.0)	93.3(9.7)a
7.5	70(8.4)	60(7.8)	60(7.8)	63.3(8.0)b
3.75	50(7.1)	40(6.4)	50(7.1)	46.7(6.9)c
1.87	30(5.5)	40(6.4)	30(5.5)	33.3(5.8)d
0.937	30(5.5)	20(4.2)	30(5.5)	26.7(5.0)d
SP	100(10)	90(9.5)	90(9.5)	93.3(9.7)a
CO	0(0.7)	0(0.7)	0(0.7)	0(0.7)e
C.V. (%)				7.1
SE				0.6

Means followed by the same letter are not significantly different at ($P< 0.05$).

Means between brackets are transformed according to $\sqrt{X + 0.5}$

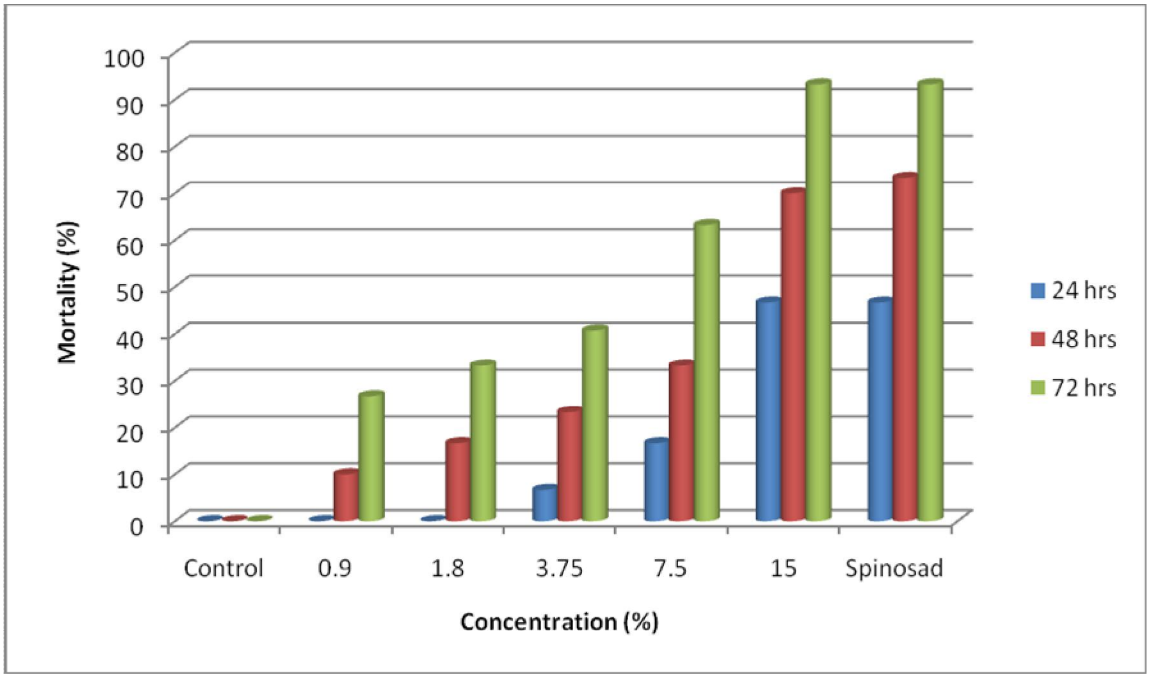

Fig. 1 : Effect of seeds ethanolic extract of neem on the mortality of third larval instars of *Tuta absoluta*.

Table 5 : LC_{50} values of seeds ethanolic extracts of neem against third larval instars of the tomato leaf miner.

Exposure time	Slope	Intercept	Chi-square	LC_{50} (%)
24 hrs	2.6	1.8	0.6	16.3
48 hrs	1.4	3.6	3.1	9.7
72 hrs	1.5	4.2	4.1	3.2

CHAPTER FIVE

DISCUSSION

In the last four decades many botanical formulations have proven to be potent and effective as many as conventional synthetic pesticides even at low concentrations. In fact, botanical insecticides have drawn great attention as major control agents in organic farming. Higher plants are extremely abundant with biologically active secondary metabolites .Over 80% of all known Alkaloids, Terpenoids, Phenols and other secondary metabolite were produced by higher plants (Siddig, 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Kelany, 2001). Stoll (2000) demonstrated that the use of plant extracts to control destructive insects is not new. Rotenone, Nicotine and Pyrethrin has been used for a considerable time in small scale subsistence and also commercial agriculture.

The results obtained revealed that there is significant difference between mean population of *Tuta absoluta* from the three areas during July, August and September. The population in July and August was significantly higher than September. The population in Elselat and Abu Halima was significantly higher than Wad Elnaaeem. This may be attributed to variation in tomato varieties, time of sowing and cultural practices in different locations. The present results are in line with Ali (2014) (personal communication).

The present study is the lethal effect of seed ethanolic extract of neem *Azadiracta indica* against the tomato leaf miner *Tuta absoluta*, was evaluated the highest concentration of seed ethanolic extracts of *Azadiracta indica* (15%) induced a high mortality percentage of 93.3%, and was not significantly different from Spinosad after 72 hours of exposure. Similar results were obtained by Dawood (2001) reported that neem water extracs at 1Kg/liter water reduced the number of onion thrips by 63.5% under the field condition. Also, Mohammed (2002) reported that neem seeds showed a good performance against *Aphis gossypii, Bemisia tabaci*, and *Empoasca lybica* on Okra.

Goncalves-Gervasio and Vendramin, (2008) in South America found that neem seed extract, Azadiractin acts as contact and systemic insecticide against *Tuta absoluta*. In a soil application 48.9-100% larval mortality was recorded. Application of neem oil in adaxial surface of the foliage causes 57-100% larval mortality. However, it is reported that application directly on larvae caused 52.4-95% mortality by the same authors. Similar results were found by Braham and Hajii (2012) who obtained 87% mortality of *Tuta absoluta* larvae after 12 day where neem seed extract were used as 100 cc/hl. Abrahim (2013) found that neem seeds contain active compounds which can be used as alternative insecticides for controlling fruit flies.

The results also demonstrate the efficacy of Spinosad (Tracer) against tomato leaf miner larvae. In fact, it scored a high mortality percentage (100 %) among tested third larval instars of *Tuta absoluta*. Spinosad, is a mixture of spinosyns A and B, and is derived from the naturally occurring actionomycete *Saccharopolyspora spinosa* (Sparks *et al.,* 1998). Because of its unique mode of action, involving the postsynaptic nicotinic acetylcholine and Gamma-aminobutyric (GABA) receptors, spinosad has strong insecticidal activity against many insects pests particularly Lepidoptera (Salgado, 1998). The present results are in agreement with Braham and Hajii (2012) who found that Spinosad gave 93.75% mortality against *Tuta absoluta* larvae after 12 days.

CONCLUSION AND RECOMMENDATIONS

The population of tomato leaf miner reaches the peak during, July and August and declined in September in the three areas. Tomato plant was the most preferred host. This study clearly demonstrates that the neem seeds ethanolic extracts have a lethal effect on the larvae of the tomato leaf miner.

Based on the above mentioned results, neem seeds ethanolic extracts can be recommended to be used as a control agent for *Tuta absoluta*. However, further experiments should be conducted to evaluate the effects of neem seeds with other organic solvents and also in other insect pests. Finally, a comprehensive study should be made in order to determine the active ingredients.

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