

Sudan University of Science and Technology



College of Graduate Studies

Biology, Ecology and Control Trials on the Tomato Leaf

Miner (*Tuta absoluta*)(Meyrick) (*Lepidoptera*:

***Gelechiidae*)**

بيولوجية , وبيئة , وتجارب لمكافحة نفاقة أوراق الطماطم

Tuta absoluta

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Requirements for the M. Sc. Degree in Plant Protection

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الاية

بسم الله الرحمن الرحيم

قِيلَ أَيُّهَا النَّبِيُّ اسْمُ زُورٍ مِّثْلُ مَا اسْمُ عُوَالِهِ إِنَّ الَّذِينَ تَدْعُونَ مِنْ
عِندِ اللَّهِ لَنْ يَخْلُقُوا ذُبَابًا وَلَا يُجِئُهُمْ عُوَالُهُمْ وَلَا يُجِئُهُمْ عُوَالُهُمْ وَلَا يُجِئُهُمْ عُوَالُهُمْ وَلَا يُجِئُهُمْ عُوَالُهُمْ
مِنْهُ ضَعُفَ الطَّالِبُ وَالْمَطْلُوبُ (٧٣)

سورة الحج الاية (73)

Dedication

To My Family...with love

To My Mother & Father,

To My Sister & brothers

To My Friends.

To everyone who Teach me in my life

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ENGLISH ABSTRACT

This study was carried on the infested areas by the tomato leaf miner (*Tuta absoluta*) and its main host plants in Khartoum State during 2012-2013, for estimation of damage percentage, to know the life cycle of the insect and to know the effects of extracts Neem, jatropha on the eggs, of Neem, jatropha and Hargal on the larvae of *T. absoluta*.

The results of the study indicated that there were three areas highly infested, five areas moderately infested and three areas of low infestation in Khartoum State. Also, the results of the study showed that, the total percentages of damage on tomato crop in Four Months in Abu Halima were 40, 50, 70 and 80% damage in fruits only, 52%, 87.5%, on leaves, 72% on unripe fruits and 45% on stem, and there were no preferred parts for the insects, which feed on all plant parts.

The results of the study showed that, the adult of the tomato leaf miner mate after a short period of emergence, that ranges from 4 to 7 days. The number of eggs was 61-64 eggs per female, with a mean of 55.5 eggs. The stages of egg, larva and pupa were completed within 9 ± 1.0 , 31.2 ± 2.6 , and 6.1 ± 1.5 days, respectively, at the temperature range of $(19-21)^\circ\text{C}$. The percentage of insect life span from egg to adult was $64.7 \pm 1.7\%$, and the mean mortality rate in egg was $21.3 \pm 1.6\%$. At the temperature range of $(30-34)^\circ\text{C}$, the stages of egg, larva and pupa were completed within 4 ± 1.0 , 13 ± 2.1 and 6 ± 2 days, respectively.

The results of the study showed that, the percentages of egg mortality by plant seed extracts from Neem and Jatropha, sprayed after 24 hrs. by the concentrations, 62.5, 125, 250, 500, 1000 mg/L, had slight effects on eggs,

with mortality ranging between 22 – 26%. With the larvae, percentages of mortality, at concentrations of 2000 ,4000 ,8000 و 6000 mg/L, of Argel, Neem and jatropa extracts were, 46.5 -23, 46.5-33, 48.5-33, respectively, in the first day, while 100% mortalities were obtained in 4th day.

الملخص

أجريت هذه الدراسة للتعرف على المناطق المصابة بنافقة اوراق الطماطم والعوائل الاساسية لها فى ولاية الخرطوم فى خلال الاعوام 2012 - 2013 , وتقدير نسبة الضرر فى النبات والتعرف على دورة حياة الحشرة ومعرفة تأثير مستخلصات نباتى (النيم والجatroفا) على البيض و (النيم والجatroفا والحرجل) على اليرقات .

أوضحت الدراسة ان هناك ثلاثة مناطق مصابة اصابه شديدة , وخمسة مناطق مصابة اصابة متوسطة وثلاثة مناطق مصابه اصابة خفيفة فى ولاية الخرطوم .

ايضا توصلت الدراسة الى ان نسبة الضرر الكلية فى محصول الطماطم فى اربعة اشهر فى ابو حليلة على التوالى كانت 40,50,70,80 % نسبة الضرر فى الثمار فقط , 52.0 و 87.5% فى الاوراق % , و 72% فى الثمار الخضراء , وفى الساق 45% , و لم تكن هنالك اجزاء تفضلها الحشرة بل تتغذى على جميع اجزاء النبات .

توصلت الدراسة الى ان نافقة اوراق الطماطم تتزاوج بعد فترة قصيرة من خروجها لحشرة كاملة وتتراوح الفترة بين 4-7 ايام . عدد البيض للانثى 61-64 بمتوسط 55.5 بيضة . يكتمل طور البيضة ،اليرقة والعذراء خلال 1.5±6.1, 2.6±31.2, 1.0±9يوميا , على التوالى عند مدى حرارى (19-21) درجة مئوية . نسبة بقاء الحشرة من البيضة حتى الحشرة الكاملة 1.7±64.7% , ومتوسط نسبة الموت فى البيضة 1.6±21.3% اما عند (30-34) درجة مئوية يكتمل طورى اليرقة والعذراء 2±13, 1.0±4 , 2±6 , 1. خلال 6 يوم , على التوالى .

توصلت الدراسة ان نسبة الموت للبيض بواسطة مسخلصات بذور الجatroفا و النيم الذى تم رشه بعد 24 ساعة بالتركيزات الاتية 1000، 500، 250، 125 و 62.5 ملجم لليتر من المستخلص كلاهما لم يؤثر على البيض ، وكانت القيم تختلف بين 22 , 26, وكانت نسبة الفقس عالية وكانت نسبة الموت لليرقات بتركيزات 2000,4000,6000,8000 ملجم لليتر من من مستخلص الحرجل والنيم والجatroفا كانت القيم على التوالى 33-48.5, 33-46.5 و 23-46.5 فى اليوم الاول اما فى اليوم الرابع كانت نسبة الموت 100% .

CHAPTER ONE

INTRODUCTION

Tomatoes (*lycopersicum esculentum mill*) are subject to attack by a large number of insect pests from the time plants first emerge in the seed bed until harvest. Aphids, flea beetles, leaf miners, and spider mites threaten young plant-bed tomatoes. In the field, flea beetles, aphids, leaf miners, stink bugs and fruit worms cause minimal damage to the foliage. However, severe damage may result either from their feeding on fruits or by spreading certain diseases. Whiteflies, leaf miners, and spider mites are more likely to infest greenhouse crops than beetles, grubs, or caterpillars. Occasionally, moths enter through holes in screens or fans and lay eggs in the greenhouse. Even in Greenhouse, tomatoes have many of the same pests as field tomatoes. Tiny pests, such as aphids, screened Armyworms, fruit worms and Hoppers may be brought into the greenhouses on plants.

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) is a moth native from South America, where it's considered important Pest of tomato crops (Filho *et al.*, 2000). In 2006, it was accidentally introduced to Spain from where it spread very quickly in many other countries of the western Pale- artic Region (Desenex, 2010). Tomato leaf miner larvae feed inside the mesophyll tissue hollowing it out. They can also penetrate young stems and fruits. Its damages are heavy both in greenhouse and in field crops. Although tomato (*Lycopersicum esculentum*) is the preferred host plant, *Tuta absoluta* (Meyrick), is an oligophagous insect pest that feeds on solanaceous plants, tomato, potato, eggplant. Tobacco and some weeds (Notz, 1992 ; Anonymous, 2011). The larval stage does not enter diapause as long as nutrition is available, no over wintering was observed In South Europe and North Africa (EPPO, 2005). In favorable weather conditions, 8-10 generations can occur in a single

year. In Sudan, *T. absoluta* is recorded as a serious problem to tomato and potato crops after the official report in 2011, and an outbreak infestation observed with a damage ranging from 5% and up to 80% (Mohamed, and Siam, 2011). Many control trials were made in the countries affected by *T. absoluta*, with chemical control as the first treatment (Siqueira, *et al.*, 2000, Lietti, *et al.*, 2005). In Spain, treatment with recommended insecticides is performed when the number of trapped counts reaches 40 adults per day. However, due to the development of resistance, chemical control has shown only limited efficacy even after varying the types of pesticides and increasing the application frequencies (Lietti *et al.*, 2005; Siqueira *et al.*, 2000). Also, control with pheromone traps and water traps was applied (Al-Zaidi, 2009, Mohamed and Siam, 2011).

Concerning problems and hazards of insecticide application, many studies for the tomato leaf miner control were carried out using plant extracts (e.g., Trindade *et al.*, 2000; Moreno *et al.*, 2011; Nilahyane *et al.*, 2012; Braham and Hajji, 2012, Ghanim and Abdel ghani, 2014). In the same line, the present study was made to evaluate insecticidal effects of two plant seed extracts from Neem (*azadirachta indica*) and Jatropha (*Jatropha curcus*), against eggs and larvae of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) in the laboratory.

The Objectives of this study were:-

- 1- General survey for the distribution, host range and evaluation of the damage of *Tuta absoluta* in selected sites in Khartoum State, by using Geographical Position System (GPS) and Geographical Information System (GIS).
- 2- Studies on the Biology and Ecology of *Tuta absoluta*.
- 3- laboratory trials for the control of *tuta absoluta* using plant extracts.

CHAPTER TWO

2- LITERATURE REVIEW

2.1 The Tomato Leaf miner *Tuta absoluta* (Meyrick)

2.1.1 Classification:

Phylum : Arthropoda

Class : Insecta

Order : Lepidoptera

Superfamily : Gelechioidea

Family : Gelechiidae

Subfamily : Gelechiinae

Genus : *Tuta*

Species : *absoluta*

Full Name : *Tuta absoluta* (Meyrick, 1917)

Preferred Name : Tomato leaf miner

Other Names : Tomato leaf miner moth, Tomato borer, tomato fruit moth (Vergas, 1970).

2.1.2 Common names :

Tomato borer, South American tomato moth, tomato leaf miner, South American tomato pinworm (English); polilla del tomate, polilla perforadora, cogollero del tomate, gusano minador del tomate, minador de hojas y tallos de la papa (Spanish); traça-do-tomateiro (Portuguese).

2.1.3 Note on taxonomy and nomenclature:

Tuta absoluta was originally described as *Phthorimaea absoluta* (Meyrick, 1917). The genus was successively changed to *Gnorimos chema* (Clarke, 1962) and *Scrobipalpula absoluta* (Povolny 1964). The correct name of the species is now *Tuta absoluta* (Povolny, 1994)

2.1.4 History:

The tomato leaf miner, *Tuta absoluta* is a neotropical oligophagous pest of solanaceous crops (Liette *et al.*, 2005). It was reported since the early 1980s from Argentine, Brazil and Bolivia (Estay, 2000). The insect rapidly invaded many European and Mediterranean countries. It was first reported from Eastern Spain in late 2006 (Urbaneja and Molla 2009), from where it spread very quickly in many other countries of the Western pale- arctic regions (Desneux, 2010). Also, it was reported from more than 9 countries in the Middle East (Russell IPM, 2009). Later, its distribution extended to African countries, Morocco, Algeria and Tunisia (EPPO, 2008 & 2009), and to Libya, Egypt and Sudan (Russell IPM, 2009) .

In Sudan, *Tuta absoluta* was realized as a serious problem to tomato and potato crops after the official report in 2011, and the outbreak of infestation observed with a damage range from 5% and up to 80% (Mohamed, and Siam 2011).

2.1.5 World Distribution :

Albania, Algeria, Argentina, Bolivia, Brazil, Bulgaria, Chile, Colombia, Cyprus, Ecuador, Egypt, France, Germany, Greece, Iraq, Israel, Italy, Jordan, Kosovo ,Libya, Malta, Morocco, the Netherlands, Palestinian Authority (West Bank), Panama, Paraguay, Peru, Portugal, Saudi Arabia, Serbia, Spain, Switzerl

and, Tunisia, Turkey, the United Kingdom, Uruguay, and Venezuela (EPPO, 2007; 2009; 2010 ; Korycinska and Moran, 2009).

2.1.6 Pest Description :

2.1.7 Adult Stage :-

The adult moths are small, with a body length of around 7 mm. They are with brown or silver color, with black spots on the narrow wings. The antennae are long, and the legs and palps are ringed with black and brown”, (Korycinska and Moran, 2009).

2.1.8 Egg Stage :

Small (0.36 mm long and 0.22 mm wide), cylindrical, creamy white to yellow. (EPPO, 2005). incubation period of *T.absoluta* egg ranged from 4-6 days(EPPO, 2005).

2.1.9 Larval Stage :

Larva: Creamy in colour with dark head, becoming greenish to light pink in the second to fourth instars. First instars is 0.9 mm long and fourth is 7.5 mm long (EPPO, 2005). The last instars has a black line behind the head (van Deventer, 2009).

2.1.10 Pupal Stage:

Pupae are brown, duration: 9–11 days Estaly ,2000,and EPPO,2005, in the labrotary (at aconsant temperature f 25°c and 75% percent R.H.)to studies life cycle

2.1.11 Biology and Ecology:

T. absoluta has a high reproductive potential with a single female laying approximately 260 eggs in its life time (EPPO, 2005). Females mate once a day for several days (Uchoa-Fernandes *et al.*, 1995) . Females Their lay eggs individually falling on the night on the upper third of the plant (Coleacp PIP 2013). using pheromones to attract males. A calling behavior sequence has been observed by virgin females under laboratory generations per year can range from several up to 10-12 (EPPO, 2005). *T.absoluta* has been observed to have five generations per year in Argentina (Korycinska and Moran, 2009). Larvae prefer leaves, stems, buds, or the calyx over tomato fruit (FERA, 2009). Diapause in larvae does not occur unless food is unavailable (EPPO, 2005). Development stops between 6 and 9°C [42.8 and 48.2°F] (, Barrientos , Apablaza, Norero, and Estay. 1998) Betancourt.,1996), depending on the life stage (Potting *et al.*, 2009).Eggs are laid on the aerial parts of host plants (EPPO, 2005). *T. absoluta* goes through four larval instars (Vargas, 1970). Pupation places are dependent on environmental conditions and can occur in the soil, on leaf surfaces, or in mines (EPPO, 2005). Larvae may be covered by a cocoon when pupating on or in host plants (van Deventer, 2009). Depending on environmental conditions, the lifecycle may take 29-38 days (EPPO, 2005). In laboratory experiments, the development of *T. absoluta* averaged 76.3 days at 14°C, 39.8 days at 19.7°C and 23.8 days at 27.1°C (Barrientos , Apablaza, Norero, and Estay. 1998). In a greenhouse with a year-round tomato crop, *T. absoluta* could have approximately 9 generations (Potting *et al.*, 2009). Adults are nocturnal and may be found hiding between leaves during the day (EPPO, 2005; FERA, 2009). The moth species may survive colder climates in greenhouses, possibly spreading to field crops during warmer months (Potting *et al.*, 2009).

2.1.12 Diapause:

The larval stage does not enter diapause as long a nutrition is available, never wintering was observed in South Europe and North Africa(EPPO., 2005). a pupate in the soil ,although pupation may also occur on the leaves.

2.1.13 Major hosts:

Tomato leaf miner feeds mainly on solanaceous hosts; however, other hosts may be attacked occasionally. All of its known hosts are reported here *Solanum lycopersicum*(tomato),*Solanu tuberosm* (potato), *Solanum melongena* (eggplant), *Capsicum annuum* (pepper), *Nicotiana tabacum* (tobacco),*Solanum nigrum*, *Datura stramonium*, *Solanum eleagnifolium*, *Physalis peruviana*. *Solanum bonariease*, *Solanum sisymbriifolium*. *Datura ferox*, *Lycium sp.*and *Malva sp* (Vargas, 1970; NAPPO-PAS, 2008; Korycinska and Moran, 2009, Potting *et al.*, 2009).

2.1.14 Pathogens vectored :

This pest is not currently known to vector any pathogens or other associated organisms. However, damage by *T. absoluta* may lead to invasion by secondary pests.

2.1.15 Pest importance:

Tuta absoluta is considered a key pest in many areas where it is present, including Latin America (EPPO, 2005; Anonymous, 2010). A key pest is one that occurs regularly and will cause economic losses if left uncontrolled. In Latin America, *T. absoluta* can lead to reduction in yield and fruit quality through direct feeding and introduction of secondary pathogens. Some consider *T. absoluta* to be the major limiting factor in tomato production in South America

(Frrarea *et al.*, 2001). It is known as the most devastating tomato pest in Brazil, at times causing 100% loss of production (Filho *et al.*, 2000). Tomatoes may lose their commercial value when severely attacked. Losses of 50-100% have been reported for tomato, mainly during low rainfall. *T. absoluta* can potentially become a pest of tomatoes in both field and greenhouses (EPPO, 2005). In introduced areas like Spain, high crop losses have occurred (EPPO, 2008).

Larvae are difficult to control because they develop within the plant, making this species hard to control with pesticides. Intensive use of pesticides to control *T.absoluta* has led to pesticide resistance in this species (Van Deventer, 2009) .

2.1.16 Biological control :

The following bio-agents could be used to control *Tuta absoluta*

Genes	Family	Order	Reference
<i>Trichogramma pertiosum</i>	Trichogrammatidae	Hymenoptera	Cabello <i>et al.</i> , 2009a
<i>Trichogramma achaeae</i>	Trichogrammatidae	Hymenoptera	Cabello <i>et al.</i> , 2009a
<i>Macrolophus pygmaeus</i>	Miridae	Heteroptera	Cabello <i>et al.</i> , 2009a
<i>Nesidiocoris tenuis</i>	Miridae	Hemiptera	Cabello <i>et al.</i> , 2009a

The (Cabello *et al.*, 2009a). egg parasitoid *Trichogramma achaeae* has been identified as a candidate for biological control of the South American Tomato Pinworm, *Tuta absoluta*. On greenhouse a high efficacy, 91.74 % of damage reduction was obtained when releasing 30 adults/ plant (= 75 adults/ m²) every 3-4 days on August and September of 2008 in the south east of Spain (Cabello *et al.*, 2009). The use of biological pest control, the damsel bug *Nabis pseudoferus*, is being studied to be applied in Spanish greenhouses. Two semi field bioassays on tomato plants, under controlled conditions, have shown an important reduction in the number of eggs of *Tuta absoluta*, between 92 and 96 %, when releasing 8 or 12 first stage nymphs of *Nabis pseudoferus* per plant (Cabello *et al.*, 2009).

2.1.17 Microbial control :

Bacillus thuringiensis var. *kurstaki* have exhibited satisfactory efficacy against *Tuta absoluta* larval infestations in Spanish outbreaks. Delayed application of *Bacillus thuringiensis* may cause higher insect mortality if the insects become more susceptible to the pathogen after a longer period of feeding on the resistant crop. It is reported that in a combine application of mass release of *Trichogramma pertiosum* and *Bacillus thuringiensis* resulted fruit damage only 2 % in South America. Entomopathogenic fungus *Metarhizium anisopliae* could be caused female's mortality up to 37.14%. Laboratory studies indicated *Beauveria bassiana* could cause 68% larval mortality.

2.1.18 Botanical insecticides

Neem seed extract, Azadiractin acts as contact and systemic insecticide against *Tuta absoluta*. soil application larval mortality was recorded 48.9-100%. Application of Neem oil in ad axial surface of the foliage causes 57-100% larval mortality. However, it was reported that application directly on larvae cause

52.4-95% mortality (Goncalves-Gervasio and Vendramin, 2008). Effect of extracts the mortality of egg There was no effect of the toxic extract ethanolic neem seed, Eggs tomato leaf mine The incubation of eggs for all treatments, averaged 4.32 ± 0.02 days, not statistically significant difference between them, the eggs showed light yellow initially, and brown or reddish near hatching in all treatments, similar to Haji *et al.* 1988) when studying the biology of the tomato pinworm in laboratory. Neem cause high mortality in caterpillars of tomato. pinwourm. Schmutterer(1990).

2.1.19 Chemical control :

Chemical control has been the main control measure used against *Tuta absoluta* since it was reported in South America. In Brazil tomato growers carried out up to 36 insecticide applications to control *Tuta absoluta* within one cropping season. Frequent intense application of insecticides lead to developmental insecticide resistance. Reduce efficiency and control failure of the insecticides used against *Tuta absoluta* have been reported in South America. Resistance to Pyrethroid and Methamidophos and, Cartap has been reported in Brazil, Chile and Argentina. However, there are active ingredients have been found to be effective against *Tuta absoluta* larval infestations in Spanish outbreaks; Imidacloprid, Indoxacarb and Spinosad. It is also reported that Deltamethrin has provided effective 'knock-down' of adult in Spain.

2.1.20 Good Agricultural practices :

Good Agricultural Practices includes cultural practice rotation with non-solanaceous crops, plugging, adequate fertilization, irrigation, destruction of infested plants and post hare vest plant debris. There are a number of cultural control measures that aid the eradication of this pest. Crop rotation, crop removal and the selective removal and destruction of infested plant material are important

cultural control practices that would help eradication of this pest in green houses. The wild host plants should also be d to prevent the further build up of a potential population. (EPPO2005).

2.1.21 Integrated pest management :

IPM strategies are being developed in South America to control *Tuta absoluta*. Various active substances can be applied n combination with bio-rational control tactics. The integrated control method recommended employs, in order, (1) massive trapping before planting, (2) clearing the soil of crop residues, (3) the application of imidacloprid in the irrigation water 8-10 days after planting, (4) the application of either spinosad or Indoxacarb if occasional individuals of *Tuta absoluta* are observed, and (5) elimination of the remnants of the crop immediately after the last fruits have been harvested (Robredo Junco, f., and Herrero 2008). In case of pheromone trap catches less than ten moths per trap per week control treatment treatments are recommended to be carried out mainly with bio-rational products, such as *Bacillus thuringiensis* and Azadirachtin. In case of pheromone trap catches more than ten moths per trap per week control treatment treatments are recommended to be carried out by combining bio-rational insecticides with synthetic chemical insecticides. In low population densities mass trapping of the pest with pheromone baited water traps has also proved to be an effective control measure in Spanish outbreaks.

An Average of 30-40 pheromone baited water traps should be placed per hectare. Mass trapping provide an environmentally friendly control measure .

2.1.22 Pheromone Based control strategy :

2.1.23 Monitoring :

Pheromone trap based on Qlure-TUA gives early warning of infestation and also exhibits the density of the insect accurately in low population to medium level infestation. In heavy infestation, Qlure -TUA tends to give high level of capture which makes data collection difficult. For this application Qlure-TUALD is specifically designed to give lower capture rate to make data collection in heavily infested fields manageable.

2.1.24 Mass Trapping

Qlure -TUA with its high capture rate is ideal for mass trapping of *Tuta absoluta* particularly in for protected tomato cultivation. It helps to reduce population in greenhouses particularly if insect exclusion nets and tight doors were used. 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.

2.1.25 Lure and Kill :

Lure and Kill is a very promising approach to control the male adults of *Tuta absoluta* with minimum amount of insecticide application. This will reduce the mating incidence and therefore reducing the number of viable eggs. Based on sustained release matrix, Lure and kill product can release the pheromone over a long period normally over 6-8 weeks while sustaining the activity of the contact

insecticide throughout the same period. Lure and Kill technique normally is a single application provides safe yet constant control over a long period of time. Pheromones of other pests many be incorporated to reduce the need to other insecticide applications . It's targeted application in specific locations leaves over 99% of the plant source insecticide free providing a safe environment for beneficial insect to develop and to participate in the overall control of Strategy. Several trials are taking place to evaluate this technology and its viability to different Tomato production methods. Lure and kill should always be considered as a part of an Integrated Pest Management program.

2.2 *Jatropha curcas* L:

Jatropha is a genus of approximately 175 succulent plants .The name derived from Greek words, iatros= physician and trophe =nutrition, and hence the common name physic nut. *Jatropha* is native to Mexico and Central America (Heller, 1996, Morton, 1997, and Little, *et. al.*, 1974).

2.2.1 Classification :

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Subfamily: Crotonoideae

Genus: *Jatropha*

Species: *curcas*

2.2.2 Description:

Jatropha or physic nut can grow to a height of about 3 to 5 meters. If the conditions are favorable they can grow to height of about 8 to 10 meters, with spreading branches and stubby twigs with smooth grey bark Normally five roots are formed from seeds, one tap root and 4 lateral roots. Leaves are deciduous broad and usually simple alternate but apically crowded, ovate, acute to acuminate, basally cord ate, deeply palmate 3 to 5 lobed, green or pale green in color. Flower consist of several to many in greenish cymes ,which are yellowish, bell shaped are formed terminally on branches. Fruits are small capsule-like round fruit that about 2.5-4cm in diameter. They are green and fleshy when immature.As seed there are: 2 to 3 black seeds and each one is about 2cm long (Morton, 1997, and little, *et. al.*, 1974)).

2.2.3 Varieties :

2..2.4 Cape verde variety:

These are small seeds and the weight of,1000 grains is about 682 g. Length of seed is about 16.8 mm. This variety is found almost in all countries of the world, except Central America (Becker and Makkar, 1998).

2.2.5 Nicaragua variety:

This variety is different from the Cape Verde variety by having larger leaves, which have a more rounded form and by having larger seeds. The weight of 1.1000 grains is about 878 g and the length of the seed is about 20.3 mm. The yield of the trees seems to be the same because there are less fruits on a tree than with the Cape Verde variety (Becker and Makkar, 1998).

2.2.6 Nontoxic Mexican variety:

The weight of 1000 grains is between 524 g and 901 g. This Non –toxic variety of jatropha could be a potential source of oil for human consumption, and the seed cake can be a good protein for human as well as for live stocks (Becker and Makkar, 1998).

2.2.7 Distribution :

Though native to America the species is almost tropical now and it is widely planted as a medicinal plant. It is listed as a weed in Brazil, Fiji, Honduras, India, Jamaica, Panama, PuertoRico, and Salvador. The plant was spread as a valuable hedge plant to Africa and Asia by Portuguese traders. (Holm, *et al.*, 1979).

In Sudan, *Jatropha* is found in many areas such as Khartoum state, in Central Sudan, Kassala State in the East and Kordofan State in the West. It is also dominant in the Southern States especially in Bahr Eljebel and Bahr Elgazal State where the farmers use it as hedges to protect houses and gardens. *Jatropha* project was in Kutum, North Darfur, with participation of the German Development service (List and Horhammer, 1969-1979, Henning, 2001).

2.2.8 Ecological requirements:

Jatropha will have its best result when it is planted in the rainy season. So it would be easy to prepare the seedlings during the dry season and make them ready before the rainy season. *Jatropha* plant grows on a wide range of climates and soils and can be established even in marginal poor soil. In fact, *Jatropha curcus* grows almost anywhere, even on gravelly, sandy and saline soils. It can also thrive on the poorest stony soil and grow in the crevices of rocks (James, 1984).

Jatropha are found in tropical and subtropical zones and also in regions that have lower temperature and it has the capacity to withstand mild frost. The jatropha trees can live with the minimum water content and can live for several months without water by shedding its leaves to reduce the transpiration losses. (Duke and Wain, 1981).

2.2.8 Cultivation:

Growth can occur readily from cuttings or seeds. Cuttings roots is so easy and the plant can be used as energy-producing material as for living fences post. (Morton, 1977 and Little, *et al.*, 1974).

2.2.8 Yield and Economics :

According to Gaydou, *et al.*, 1982) seed yields approach 6-7 MT/ha with 37% oil. They calculated that such yields could produce the equivalent of 2,100-2,800 liters fuel oil /ha in Madagascar where, they have 10,000 ha of purging nut and each producing 2,400 oil/ha for a potential production of 24,000,000 liters. The plants yield more than four times as much fuel per hectare as soybean as and more than ten times that of maize (Fitzgerald, 2006) .

2.2.9 Chemistry:

Per 100g, the seed is reported to contain 6.6g water, 18,9g protein 38,0g fat, 33.5g total carbohydrate, 15,5g fiber and 4.5g ash (Duke and Atchley, 1984). Leaves, which show anti-leukemic activity, contain a-amyrin, b-sitosterol, stigmasterol , and comp sterol, 7-Keto-6 sitosterol, stigmast-5-ene-3-6, 7-a-diol (Morton, 1981).Leaves contain isovitexin and vitexin, Saccharose, raffinose, stachyose, glucose, fructose, galactose, protein and oil. Oleic and linoleic-acid (List & Horhammer, 1969-1979), Curcasin, arachidic, linoleic-, myristic-, oleic-, palmitic-, and steric-acids are also reported (Perry, 1980).

2.2.10 Seeds and its toxicity:

The seeds of physic nut are a good source of oil, which can be used as a diesel substitute. However, the seed of *J. curcas* are in general toxic to humans and animal. Curcin, a toxic protein isolated from the seeds, was found to inhibit protein synthesis invitro studies. The high concentration of phorbol esters present in *Jatropha* seed has been identified as the main toxic agent responsible for *Jatropha* toxicity. (Adolf, et al., 1984, Makkar, *et al.*, 1997). Several cases of *J. curcas* nut poisoning in humans after accidental consumption of seeds have been reported with symptoms of giddiness, vomiting and diarrhea and condition even death have been recorded (Becker and Makkar, 1998).

Ionizing radiation treatment could serve as a possible additional processing method for inactivation or removal of certain anti nutritional factors such as phorbol esters, phytates, saponins and lectins (Siddhuraju, *et al.*, 2002). It is not possible to destroy phorbol ester by heat treatment because they are heat stable and can withstand roasting temperature as high as 160c for 30 minutes. However, it is possible to reduce its concentration in the meal by chemical treatments. This treatment is promising, but in economic terms it is expensive to produce *Jatropha*

2.2.11 Uses of *Jatropha* :

(Areqheore, *et al.*, 2003). Martinez- Herrera, *et al.*, (2006) studied the nutritional quality and the effect of various treatments to inactivate the anti-nutritional factors in defatted *Jatropha* kernel meal of both toxic and nontoxic varieties from different regions of Mexico. Complete removal of the toxins is therefore necessary before *Jatropha* oil can be used in industrial application or in human medicine. The oil must be completely innocuous before it is used commercially.

2.2.12 As hedge:

Jatropha is an excellent hedging plant for protection of agricultural fields against damage by livestock as it is unpalatable to cattle and goats. (Sherchan , *et al.*, 1989).

2.2.13 As Green manure and Fertilizer :

The seed cake is an excellent source of plant nutrients. In a green manure trial with rice in Nepal, the application of 10 tons of fresh physic nut biomass resulted in increased yield of many crops. Another use of Jatropha seed cake is as a fertilizer and its properties are more comparable with those of other organic fertilizer. (Sherchan , *et al.*, 1989).

2.2.14 As Food :

The physic nut seed is eaten in certain regions of Mexico once it has been boiled and roasted (Delgado and parado, 1989).

2.2.16 Soap :

The glycerin that is a by-product of biodiesel can be used to make soap .Soap can also be produced from *Jatropha* oil itself .In either case the process produces a soft,durable soap which is well adopted in household or small-scale industrial activity(Watt and Breyer-Brandwijk, 1962).

2.2.17 Pesticide :

The oil and aqueous extracts from oil have potential use as insecticides, such extract have been used in the control of pests of pulses, potato & corn (Kaushik and Kumar, 2004).Methanol extracts of Jatropha seeds which

contain biodegradable toxins are being tested in Germany for control of bilharzia-carrying water snails.

2.2.18 As an energy source :

The oil from *Jatropha* is regarded as a potential fuel substitute. Air New Zealand and Houston based continental Airlines have run tests in January 2009 for demonstrating the viability of *Jatropha* oil as jet fuel. Japan Airlines also conducted test flights in Jan. 2009 as well. Researchers at Daimler Chrysler Research explored the use of *Jatropha* oil for automotive use, concluding that although *Jatropha* oil as fuel has not yet reached optimal quality, it already fulfills the European norm for biodiesel quality".(Daimler Chrysler, 2004).

2.2.19 Medicinal uses :

All parts of *Jatropha* (seeds ,leaves & bark) have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1995; Duk,1988).The oil has a strong purgative action and it is also widely used for skin diseases and to soothe pain such as that caused by rheumatism. The oil is used as cathartic purgative and for the treatment of skin ailments (Duke, 1988).The leaves & latex are used in healing of wounds, refractory ulcers , septic gums and as a styptic in cuts.Roots are used in decoction as a mouth wash for bleeding gums and toothache.It is also used for eczema ,ring worm and,scabies (Perry ,1980; Duke and Atchley, 1984). Latex are used to dress sores and to treat ulcer and inflamed tongues and it is also effective against Plasmodium falciparum, P.vivax ,P.ovale, and P.malariae. (Perry, 1980). The seeds are used to treat arthritis, gout and jaundice. Leaves are regarded as anti-parasitic and are applied to scabies and as arubefacient for paralysis, rheumatism. It is also applied to hard tumors (Hart well, 1967-1971).

2.2.20 Biotic factors :

Agricultural Hand book lists the following agents as affecting *Jatropha curcas*:

- *Clitocybe tabescens* (root rot).
- *Colletotrichum gloeosporioides* (leaf spot).
- *Phakospora jatrophiicola* (rust).

Spodoptera litura (fab) Boursin (Lepidoptera: Noctuidae) is reported as a pest of *Jatropha curcas* L. in India.

2.2.21 As Botanical insecticides :

Higher plants are extremely rich in biologically active secondary metabolites over 80% of all known alkaloids, phenols another secondary metabolite were produced by higher plants (Elsiddig , 2007).

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 and be can readily accepted by the majority of farmers, governmental organization 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 have been used for a considerable time in small scale subsistence and also in commercial agriculture.

2.3 Argel (Solenostemma argel) :

Kingdom: Plantae

Class: Magnolopsida

Order: Gentianales

Famaily:Asclepiadaceae

Genus : *Solenostemma*

Species : *argel*

Scientific Name: *Solenostemma argel* (Del) Hayne

English Name:Hargal .

2.3.1 Description :

It is an erect perennial shrub that reaches up to 1.5-2 feet in height with numerous branches carrying opposite decussate leaves; the leaves lanceolate to oblong –ovate, with acute or sub-acute apex and cuneate base, the leaf petiole is thick .

Fruit are solitary follicles, thick , ovoid, lanceolate, acuminate at the apex and they are very hard with dark purple colour .Seeds are reticulate ,ovoid and they are channel down at one face, they are minutely tuberculate bearing and apical tuft hair (Elkamali , 1991) .

2.3.2 Distribution:

Solenostemma argel is a desert plant ,which is of wide spread in central and north ,s parts of the Sudan ,Egypt ,Libya, Chad, Algeria, Saudi Arabia and

Palestine. However, Sudan is regarded as the richest source of this plant (Orange, 1982).

2.3.4 Locality:

Solenostemma argel grows wild or cultivated in north Sudan, in the area extending from Dongle to Barber tribe, whose Capital town is Ubo Hamad, is famous for Argel production and wild collection (Elkamali, 1991).

2.3.5 Chemical constituents of Argel

Elkamali (1991) conducted a photochemical screening of Also Argel constituents of the leaves, stem and roots at the pre-flowering and flowering stages. Results of photochemical screening showed the presence of a number of chemical groups (Flavonoides, tannins, sterols *triterpens*, *saponins*), the major constituents were saponins.

2.3.6 Insecticidal activity of *solenostemma argel* :

Hag-Eltayeb (2005) reported that, argel aqueous extract was effective in control of the larvae of mosquitoes *Culex spp* and *Anopheles spp*. under laboratory conditions. Also Argel water extract when tested under laboratory conditions against faba bean beetle, *Buruchidius incarenatus* at 2.5%, 5%, and 10 %, gave 60.1%, 66.7% and 75.8% mortality of the adult insects, respectively (Mohamed, 2004).

2.4 Neem (*Azadirachta indica*) :

Kingdom: Plantae

Division: Magnoliophyta

Order: Sapindales

Family: Meliaceae

Genus: Azadirachta

Species: indica

Scientific Name : *Azadirachta indica*

Neem has Homeopathic medicine (Kausik and Bandyey., 2002 and Girish, and Shankara, 2008). Neem is a member of the Mahogany Family (Rembold, 1996). Neem and its leaves used for the treatment of various diseases including eczema, ringworm, acne, anti-inflammatory activities, anti-hyperglycemic and also treat chronic wounds, diabetic foot and gangrene. It also removes toxins from the body; neutralize the free radicals present in body and used as blood purifier. Recently it is reported as anticancer and used for hepatorenal protective activity and hypolipidemic effects (Kumar & Gupta, 2002). Neem is distributed widespread in the world. The Chemical constituents of Neem contain many bioactive compounds including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is a mixture of seven isomeric compounds (Verkerk & Wright, 1993). To clean wounds, soothes, swellings and erases skin problems, boiled Neem is used. Neem leaves have been demonstrated to have vast properties like as immunomodulatory, anti-inflammatory, anti hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, anti mutagenic and anticarcinogenic (Hoque *et al.*, 2007). El-Mahmood *et al.*, (2010) observed the anti bacterial effects of crude extract of Neem seed against pathogens involved in eyes and ear infections.

CAPTER THREE

3- MATERIALS AND METHODS

3.1 Main materials and Equipments used in the study :

Materials:

The main materials used in this study were:

Muslin cloths, Argel leaves, Filter papers, Cotton ,Jatropha seeds (Plate. 2), Neem seeds, Petroleum ether, methanol 99.7%, Distilled water, Sand, Soap, Pheromones (Russell IPM : *T.absoluta*-100, PH-937-100H) (Plate. 6).

Equipment :

Also, the equipment used in the study included Plastic cages, Glass cages, Hand Sprayer, Petri dishes, Brushes, Gloves, , Hand Lenses, Glass Pipette, Camera,(plate 1.) Timer (Plate 3),Light traps (Plate. 4), and Pheromones traps (plate.5)



Plate No. 1. Equipment Used in The Study



Plate No.2. . *Jatropha curcas* Seeds



Plate No.3. Timer

3.2 General survey of the leaf miner, *Tuta absoluta* in Khartoum State :

This pest was newly introduced into the country with imported tomatoes, and was not known previously. As high damage was reported by this pest, especially in Khartoum State, a general survey was carried out to determine the incidence and percentage of damage by the pest in different areas in the State.

The survey was made during -January, 2012 and September 2013 . A GPS and a GIS were used to determine the study sites in the main localities in Khartoum State : Khartoum, Khartoum North and Omdurman. To determine the presence and damage of the pest in each study Site, a light trap composed of : a plastic container, containing some water with few drops of soap and a torch (Plate. 4), was fixed at a height of 0.5 m in each Green House or open field. The numbers of males and female caught by each trap were recorded daily for one week and were transported to the Plant Pathology Laboratory of the General Plant Protection Directorate, Khartoum North for further study. Also, pheromones traps(plate..5.),were used in some sites .



Plate No. 4. Light Trap used in the Survey



Plate.5. pheromones traps

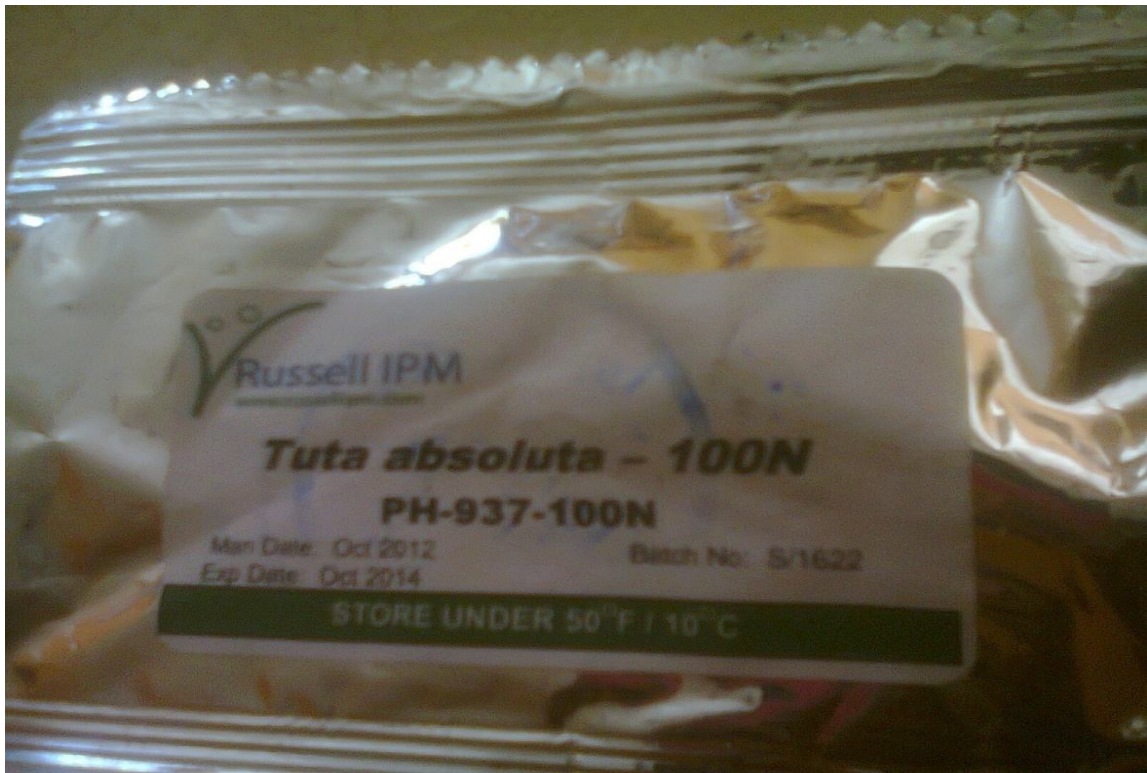


Plate.6. pheromones

3.3 Studies on The biology of The Tomato Leaf Miner: *Tuta absoluta*

3.3.1 Rearing of the insects :

Tomato fruits infested with *T. absoluta* were collected and obtained from different locations including : Khartoum, Wad Medani, Dongola and from Kassala State. Specimens collected were brought to the Plant Pathology Laboratory, and were kept in plastic cages (29× 28×22 cm & 29×22× 18 cm).

The temperature in the laboratory was maintained between 30-34°C in summer, and temperature in winter was (19-21)°c. A light sand layer was placed at the bottom of each cage to facilitate pupation.

All cages were examined daily for emergence of the adults of *T.absoluta*. After adult emergence, the mating was observed.

3.3.2 Studies on the egg stage :

After adult emergence, 10 groups, each of one pair of an adult male and a female, were collected and transferred into separate Petri-dishes, and the mating and egg laying behavior was observed daily. To facilitate the egg laying, each Petri-dish was provided with fresh leaves of tomato plant. The tomato leaves in each petri-dish were changed every day, and were examined daily under a Bionocular Microscope to determine :

a- the egg incubation period,

b- the number of eggs laid by each female and hatchability,

3.3.3 Studies on the larval stage:

After hatching, the first instars larvae were kept, each, in a separate Petri-dish, and were provided with fresh tomato leaves, and were examined daily to determine:

a- number of the larval instars,

b- duration of the first and of the other larval instars,

3.3.4 Studies on the pupa and adult stages :

After pupation, the duration of the pupal stage was followed until the emergence of the adult stage of *T.absoluta*. The sex ratio, and the pre-adult mortality were also determined.

3.4 Studies on the nature and percentage of damage of the tomato leaf miner, *Tuta absoluta* :

The tomato seedlings of Hagen variety were transplanted on June 9/2012 in two Green houses in Abu Halima Site, each measuring 9m X 360m. In these green houses, an application of one dose 0.175L./fed. of each of Two insecticides Actara and Romin was applied for the control *T. absoluta* at the beginning of the planting season .Two samples, of 10 plants each, were taken randomly from each Green house during Four months (i.e., August, September, October and November) to know the percentage of infested tomato plant (ripe fruit, unripe fruit, leaves and stems) .

The percentage of damage in each Green house was determined by the following formula :

Percentage of infestation = $\frac{\text{No. of infested plants}}{\text{Total number of plants}} \times 100$

Total number of plants

To determine the nature of damage, each infested plant was examined to record infestation and damage in different plant parts [ripe fruits, leaves (Plate .5), unripe fruits and stems]. This test was performed during the growing period of tomato crop, from August and up to November, 2012.



Plate No. 7 Example of Tomato Leaf Damage at Abu Halima Study Site

3.5 Laboratory trials for the control of the tomato leaf miner,

***Tuta absoluta* using plant extracts :**

3.5.1 Plant Species used in the study :

Extracts of three plants, Neem (*Azadirachta indica*), Argel (*Solenostemma argel*) and Jatropha (*Jatropha curcas*) were chosen for application against immature stages of *T. absoluta*. Neem seeds were collected from Shambat area and were dried, decorticated and ground to a fine powder by an electric blender, (Moulinex). The powder was kept in tight closed vials for later use. Fresh Argel leaves were obtained from the local market, and were washed and dried under laboratory condition for 48hrs. Then, they were ground to a fine powder by the electric blender (Moulinex), and the powder was kept in tight containers, to be used later. Jatropha seeds used in this study were collected from Forestry Research Center, Soba. They were cleaned, dried and were then also ground to fine powder by the electric blender, (Moulinex) .The powders were kept in tight container for later use.

3.5.2 Preparation of the plant extracts :

3.5.2.1 Jatropha seed powder extract (Petroleum ether extraction)

As mentioned, *Jatropha* seeds used were collected from Soba Forestry Research Center. The extraction was conducted at the Department of Pesticides Alternatives at the Environment and Natural Resources Research Institute (ENRRI), National research center (NRC).

Extraction of *Jatropha* seed powder was prepared using petroleum ether, and the extraction process and preparation of the stock solution were made as follows:

100 grams of *J. curcas* seeds were successively extracted with petroleum ether using Soxhlet extractor apparatus (Plate No. 6) . Extraction carried out for about six hours for petroleum ether. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally, extracts allowed to air in Petri dishes till complete dryness and the yield percentages were calculated as follows:

$$\frac{\text{Weight of extract obtained}}{\text{weight of plants simples}} \times 100$$

Yield percent of extracts :

Weight of sample	Petroleum ether	
	Weight of extract	Yield%
100g	50.097g	50.097%

3.5.2.2 Neem seed powder extract :

A ready made Neem seed powder extract was kindly provided by the staff of the ENRRI (NCR) in Khartoum. A stock solution was prepared from the extract concentrate.



PlateNo. 8 Soxhlet and Rotary Evaporator System

3.5.2.3 Argel powder leaves water extract :

The extract was prepared by mixing 100gm of the leaves powder in 100 liters of water, and the mixture was left for 24 Hrs. Then, it was strained through a cotton cloth and kept as a stock solution for later use.

3.5.3 Laboratory Bioassays on the eggs and larvae of *T. absoluta* :

3.5.3.1.1 Laboratory Bioassays on the eggs:

3.5.3.1.2 Bioassay with Jatropha seeds Petroleum ether extract :

In this test, Five concentrations were prepared from the stock solution (1000, 500, 250, 125 and 62. Mg/L). Five groups, of 40-50 eggs, of *T. absoluta* were prepared and topically sprayed with the different concentrations, and the control group was sprayed with Petroleum ether only. Three replicates were prepared with each concentration. The experiment was arranged in CRBD. Treatment containers were kept in the laboratory under a temperature of 25⁰c and 50±10% R.H. The ovicidal effects of the extract were tested by recording egg mortality daily for 4 days. Criteria of egg mortality : the egg colour changes gradually from creamy to dark colour , and finally became black.

3.5.3.1.3 Bioassay with Neem seed Ethanol extract :

In this test, the same procedure of the previous test was followed. Five concentrations were prepared from the stock solution ((1000, 500, 250, 125 and 62. Mg/L). Five groups, of similar number, of *T. absoluta* eggs were also prepared and topically sprayed with the different concentrations, and the control group was sprayed with methanol only. Three replicates were prepared with each concentration, and the experiment was arranged in a CRBD. Treatment containers were kept in the laboratory, under a temperature of 25⁰c and 50±10%

R.H. The ovicidal effect of the extract and the egg mortality were made as in the previous test. To determine egg mortality : the egg colour changes gradually from creamy to dark colour , and finally became black.

3.5.4 Laboratory bioassays on the Larvae of *T. absoluta*:

Based on a preliminary test, a series of 4 concentrations (2000, 4000, 6000 and 8000 Mg/L) were prepared from each of the following extracts :

- 1 .Jatropha seeds Petroleum ether extract
2. Neem seeds Methanol extract,
3. Argel powder leaves water extract.

Groups of 20 recently hatched larvae of *T. absoluta* were placed in Petri-dishes, each contained a piece of fresh tomato leaf, previously immersed for 5 seconds in each of the different concentrations of the extracts. Another group, of 20 larvae, was used as control with each of the different concentrations, and placed in Petri- dishes, each contained fresh tomato leaf, treated with methanol, petroleum ether or water , according to the type of the extract. Two replicates were made with each concentration. The experiment was arranged in a CRBD. The test containers were kept in the laboratory, at a temperature of 25 – 27 °c and a 50 ±10%. R.H. Larval mortality was observed and recorded daily for 6 days. Criteria of larval mortality: the larval colour changes to dark, then larva becomes sluggish, and finally died.

3.5.5 Experiment design

The experiment was conducted using randomized complete Block Design (RCBD).

3. 5.6 Statistical analysis

The data obtained were transformed using $(\sqrt{x+0.5})$. Analysis of variance (One Way NOVA) was computed using SPSS Program (version 20) and means were separated using Tukey test.

CHAPTER FOUR

4- RESULTS

4.1 General survey of the leaf miner, *Tuta absoluta* in Khartoum State

The Survey was made during season Jan., 2012 September 2013 and included 11 sites in Khartoum State : 7 sites in Khartoum North, 1 sites in Khartoum and 3 sites in Omdurman. The positions of the survey were determined by using a GPS and a GIS systems. Within the selected sites, a total of 163 Green Houses were visited and inspected to determine the level of infestation with the leaf miner *T. absoluta* in each site. Most of the Green Houses were planted with tomatoes and other vegetables (e.g., potatoes, beans, cucurbits and egg plant).

The survey sites inspected and their geographical positions are shown in Figure 1. Also, the final results of the survey, and levels of infestation determined after inspection of all sites are shown in Table 1.

These results showed that, the infestation by *T. absoluta* has a wide distribution almost in all areas of Khartoum State. The main host plant noticed was tomatoes (*Lycopersicon esculentum*) as the main host, in addition to potatoes (*Solanum tuberosum*), beans and Datura (*Datura stramonium*).

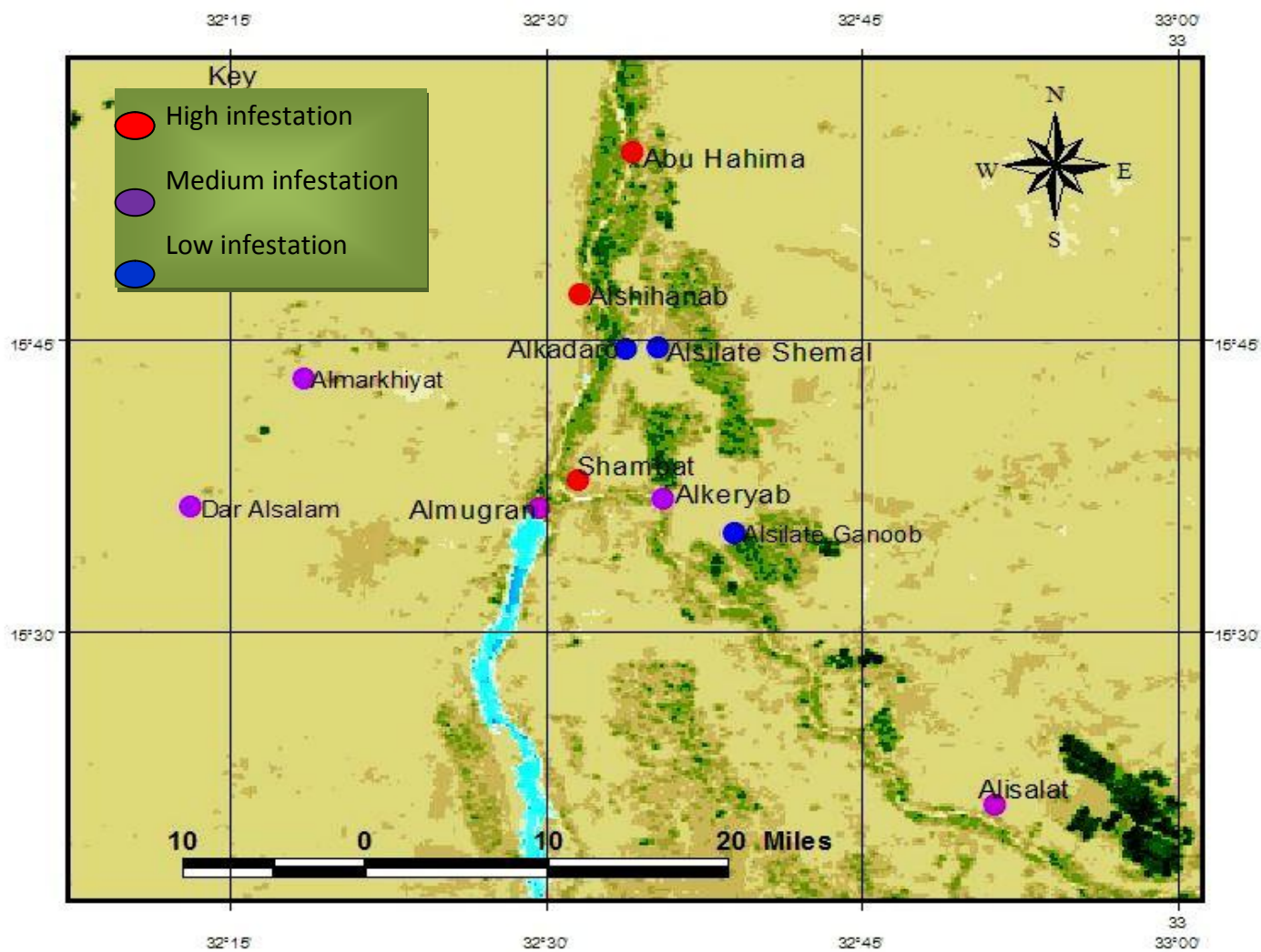


Figure No.1 The main Survey Sites of the tomato leaf miner *Tuta absoluta* in Khartoum State.

Table No.1 General Survey of the leaf miner, *Tuta absoluta* in Khartoum State

Locality	Site	GPS coordinate	Area inspected and No. captured	Level of Infestation
Khartoum	Department Horticulture	"38.80'38°15N/"0.58'31°32E	Green house (5)	Medium
Khartoum north	(Abu halima	"54.41'54°15N/"7.06'34°32E	Greenhouse (50)	<u>High</u>
	Shambat	"38.80'38°15N/"0.58'31°32E	Green house (11)	<u>High</u>
	Elkadro	"25.39'44°15N/"19.36'33°32E	Open field (3)	Low
	Elesilat North	"54.41'54°15N/"59.53'35°32E	Green house (2)	Low
area Eastern				

Nile				
	Elkriab	"18.91'37°15N/"33.64'35°32E	Greenhouse (8)	Medium
	Elesilat South	"17.70 '35°15N/"56.32'39°32E	Greenhouse (4)	Low
Omdrman				
	Al Shihnab	"26.61'47°15N"1.74'32°32E	Open field (250)	High
	Dar Assalam	"9.70'37°15N/59.3'13°32E	Open field (6)	Medium
	El Markhiat	"12.26'42°15N/6.3'22°32E	Greenhouse (6)	Medium

4.2 Studies on the biology and ecology of the tomato leaf miner **,*Tuta absoluta***

4.2.1 Mating and oviposition

Adults of *Tuta absoluta* were found to mate few hours after their emergence from the pupal stage. The biological cycle of this moth depends on temperature. In the present study, the total life cycle obtained was 33 – 43 days at (30 -34)°c, and 56 – 66 days at (19-21) °c, Figure N.O.2) *Tuta absoluta*. Larvae mine in the mesophyll of the leaf, Four larval instars, Pupates in the soil and sometimes in the leaves, Prefers tomato but can complete in other solanaceous plants

At an average temperature of (19-21)° c, the number of eggs laid by the female was (61-64) eggs, reaching a total of about (260) eggs over its life, with a mean of 55.5 eggs per female (Table No. 2).

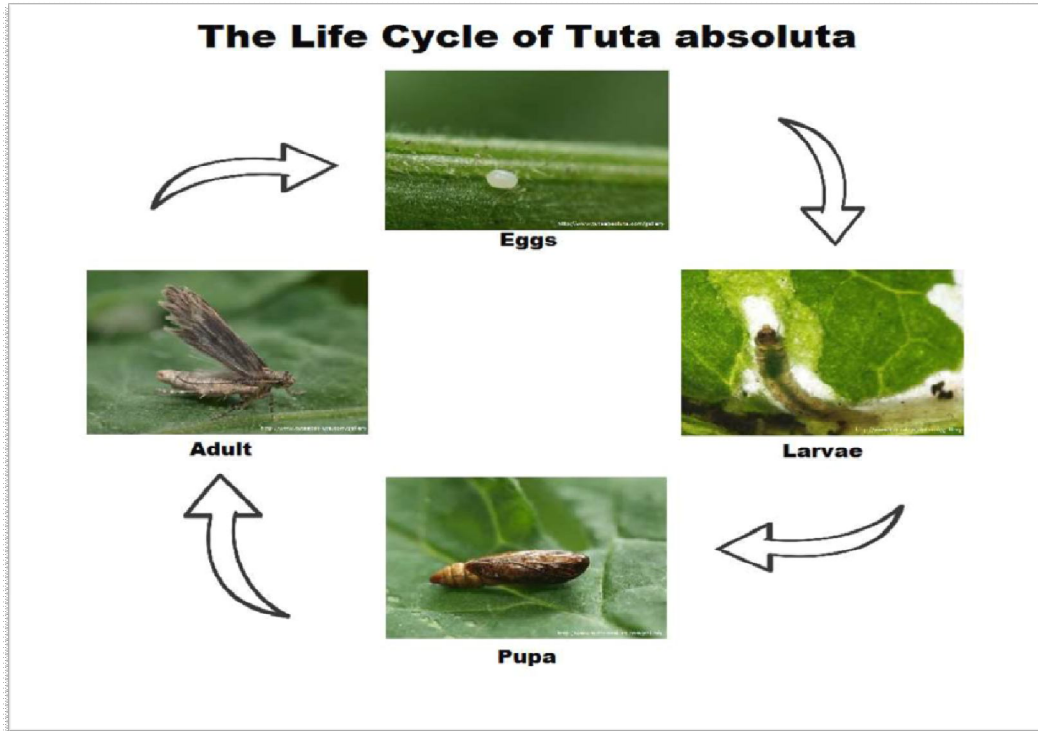


Figure N.O.2 The life cycle of leaf miner *Tuta absoluta*

4.2.2 Studies on the egg stage:

The egg of *T. absoluta* is small, cylindrical. Newly laid eggs are creamy white and turn yellow and then yellow orange(FigureNo.2).During development, mature eggs turn dark and the outline of the larva head capsule can be seen . Incubation period of the eggs ranged between 5.5- 6.5days, with a mean of 6.0 ± 1.5 days at 19-21°C. Egg hatchability ranged from 71-85% with a mean of $78.7 \pm 1.6\%$ (table No. 2).



FigureN.o.3. Eggs of Leaf Miner ,*Tuta absolut*

Table No. 2 Number of eggs / female and hatchability of *T. absoluta* eggs

No. Of Females	No. Of egg laid	No. Of egg hatched	No.Unhatched	Hatchability %	Unhatchability%
10	555	423	132	78.7	20.7
Mean	55.5	42.3	13.2	78.7	20.7
S.E		1.3	0.07	1.6	0.9

4.2.3 Studies on the larval stage

Larvae of *T. absoluta* emerged from the spin side of the egg, and begin to feed shortly after emergence. There are four larval instars, which are well defined and are of different size and color. Their color changes from creamy white in the 1st instars (length about 0.5mm), to deep green in the 2nd and 3rd instars, then to light pink colour in 4th instars (7-9mm in length), with dark head(.fig.N.3) The larval duration differs according to temperature, from 13±2.0 days at (30-34 °c), and 31.2±2.6 days at (19-21)°c, representing the longest among the developmental stages in the life cycle of *T.absoluta*. (Table No. 3).

The larva feed inside the plant leaf, causing minute tunnels, and do not enter diapause as long as food is available.

Table No. 3 Mean duration (in days) of different stages of *T.absoluta*

Stages	Mean(at 34-30 °C)	Mean(at 19-21°C)
Egg	4 ±1.0	6.0± 1.5
Larva	13±2.1	31.2±2.6
Pupa	6±1.0	9±1.0



Figuer.No.4. larvae instars of *T.absoluta*

4.2.4 Studies on the pupa and adult stages :

Newly formed pupae are greenish and turn dark brown as they mature, male pupae are lighter and smaller. Adults are nocturnal and may be found hiding between leaves during the day. They are brown or silvery in color; the wing apex is fringed with specked brown scales .FigureN.o.3. Morphological characteristics of males and females of *T. absoluta* (TableN.o.5).The duration of life cycle of *T. absoluta* from egg to adult was found to be $46 \pm 1.6\%$ at $(19-21)^{\circ}\text{c}$, and it was found that the emerging females outnumbered males by three times (a sex ratio of 3:1) (Table No. 4).

Table No. 4 Total number of eggs laid / female , resulted adults and the durationl percentage of *T. absoluta* (at $19-21^{\circ}\text{c}$.)

No of Females	No. of eggs laid	No. of Emerging adults	% of adult Survival
10	555	371	64.7
Mean	55.5	37.1	64.7
S.E		0.3	1.7



Figuer.N.O.5. Adult of *T.absoluta*

Table No. 5 Morphological characteristics of males and females of *T. absoluta*

Characters	Male	Female
Abdomen	Wider and bulkier	Narrower and pointed posteriorly
Abdominal scales	Grey	Creamy
Life span range	6-7 days	10-15 days

4.3 Studies on the nature and percentage of damage of the tomato leaf miner, *Tuta absoluta* :

Observation of *T. absoluta* damage at Abu Halima Site during 4 months:

According to the daily observation on the tomato leaf miner, eggs are laid on any part of the plant. After hatching, young larvae penetrate into tomato fruits, leaves or stems on which they feed and develop, thus creating conspicuous mines and galleries. Fruits can be attacked as soon as they are formed, and the galleries bored inside them can be invaded by secondary pathogens leading to fruit rot (Figure N.o.4). On leaves, larvae feed only on mesophyll tissues, leaving the epidermis intact. Leaf mines are irregular and may later become necrotic. Galleries in stems alter the general development of the plants. Tomato plants can be attacked at any developmental stage, from seedlings to mature plants. The

pest is generally easily found because it prefers apical buds, flowers or new fruits, on which the black frass is visible.

Pupal position :

After the larva reaches full development, it enters pupation, which was often observed to take place through the same bars that were made by the larva.

total plant infestation: After two months, the percentage of infested plant leaves reached 80.0%, and in stem only 22.0 %. At the end of the four months, the percentage of damage in leaves reached 87.5%, in green fruits 72.5% , in stem 45.0% and in ripe fruits 52.% (Figure No. 2).



FigureN.o.6 *Tuta absoluta* – Fruit Damage

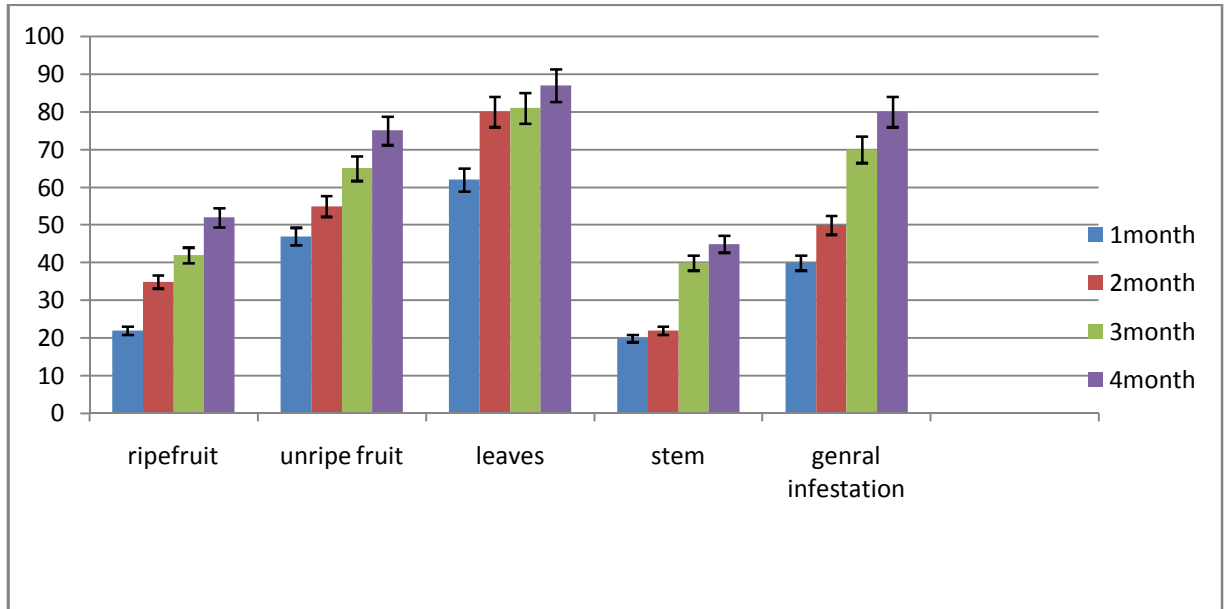


Figure No.7. Percentage of infested tomato plant in four months

4.4 Mortality of eggs of *Tuta absoluta* after Four days of topical application of Neem seeds methanolic extract (at: 25 c°& 50 ±10% RH)

The results of the different concentrations are shown in Table (6), Figure (8) and in Appendix (3). Although the mortality values varied between 20-26% all applications did not affect the egg viability, as a quite number of eggs hatched, with a mean egg incubation period of 4.31 ± 0.02 days., eggs showed light yellow initially, and brown hatching occurred, and no significant differences were noticed between effects of all concentrations.

Table NO.6 Mean percentage mortality of egg of *Tuta absoluta* after four days of topical application of methanolic of Neem seed sextract

Mortality of eggs					
Concentration	1 st day	2 nd day	3 rd day	4 th day	Total%
1000	0 (0.71) b	3 (1.71) ab	7 (2.71) a	12 (3.49) a	24.5
500	2 (1.56) a	6 (2.52) ab	10 (3.23) a	13 (3.64) a	26
250	4 (1.84) a	7 (2.72) a	9 (3.08) a	12 (3.49) a	24.5
125	3 (2.11) a	6 (2.55) ab	9 (3.08) a	10 (3.48) a	25
62.5	4 (2.06) a	6 (2.53) ab	10 (3.24) a	12 (3.49) a	24
Control	2 (1.47) ab	2 (1.47) b	2 (1.47) b	2 (1.47) b	5.1
S.E. ±	0.1	0.1	0.1	0.2	

Means followed the same letter(4)are not significantly different at $p \leq 0.05$ means between brackets are transformed by $(\sqrt{x+0.5})$.

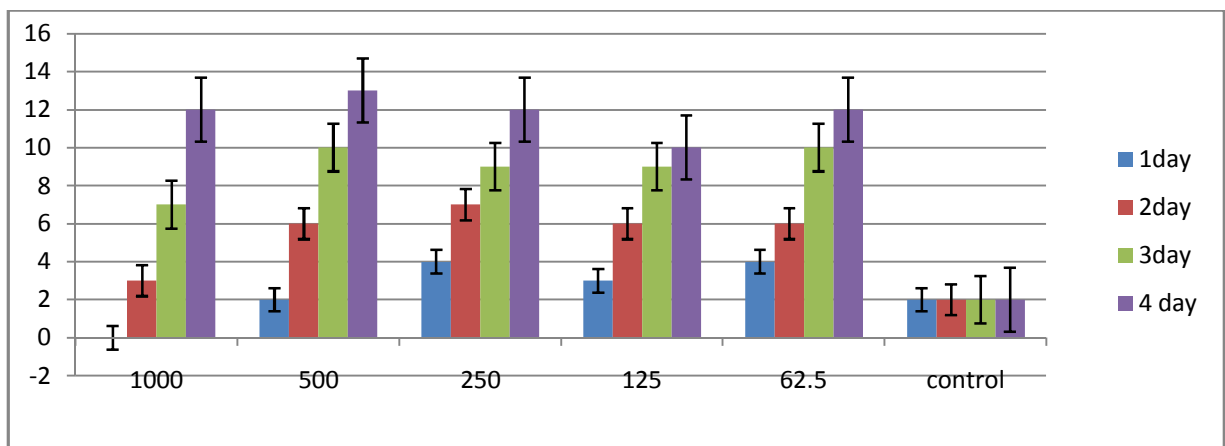


Figure NO.8 Mean percentage mortality of egg of *tuta absoluta* after Four days of topical application of methanolic of Neem seeds extract.

4.5 Mean percentage mortality of eggs of *T. absoluta* after Four days of Topical application of Jatropha seeds extract (at: 25 c° & 50 ±10% RH)

The results of the different concentrations are shown in table (7), fig. (9) and appendix(4). Although the mortality values varied between 18-20%, all applications did not affect the egg viability, as a quite number of eggs hatched , eggs showed light yellow initially, and brown hatching occurred, and no significant differences were noticed between effects of all concentrations.

Table NO.7 Mean percentage mortality of egg of *Tuta absoluta* after Four days of topical application of Jatropha seeds extract

Mortality of eggs					
Concentration	1 st day	2 nd day	3 rd day	4 th day	Total%
1000	2 (1.56)ab	6 (2.52) a	9 (3.08) a	10 (3.2)a	20.4
500	3 (1.86)ab	5 (2.32)ab	7.3 (2.73) a	9 (3.07)a	18
250	3 (1.82)ab	6 (2.50) a	8 (2.90) a	9 (3.07)a	18.4
125	1 (1.17) b	5 (2.29)ab	7 (2.73) a	10 (3.24)a	25
62.5	5 (2.34) a	7 (2.73) a	9 (3.08) a	11 (3.024)a	22
Control	1.7 (1.39) b	1.7 (1.39) b	1.7 (1.39) b	1.7 (1.39) b	3.8
S.E. ±	0.1	0.2	0.2	0.2	

Means followed the same letter(4)are not significantly different at $p \leq 0.05$ means between brackets are transformed by $(\sqrt{x+0.5})$.

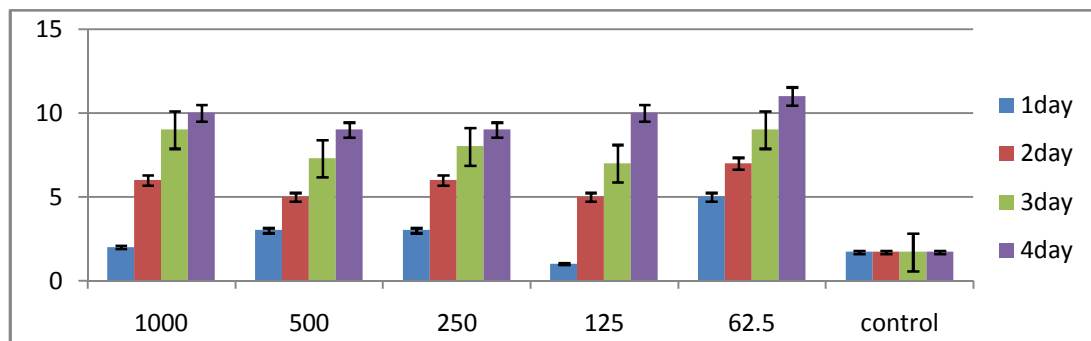


Figure NO.9. Mean percentage mortality of egg of *Tuta absoluta* after Four days of topical application of Jatropha seeds extract.

4.6 Mean percentage mortality of larva of *T. absoluta* after Four days of Topical application of Neem seeds ethanolic extract (at 25°C & 50±10% RH)

The results of the different concentrations in Table (8), Figure (10) and appendix (5) showed that, after 24 hrs. of treatment a larval mortality ranging between 33- 46.6% was obtained with the 4 concentrations of the Neem seeds extract, and by the 4th day, higher larval mortality, between 50% - 100% were obtained at the different concentrations.

Table NO.8 Mean percentage mortality of larva of *tuta absoluta* after Four days of topical application of methanolic extract of Neem seeds

Mortality of larvae					
Concentration	1 st day	2 nd day	3 rd day	4 th day	Total%
8000	7 (2.72) a	7.3 (2.76) a	13.6 (3.76) a	15 (3.94) a	100
6000	7 (2.74) a	7 (2.73) a	9.3 (3.13) ab	13.5 (3.76) a	86.7
4000	5.6 (2.48) a	6.3 (2.60) a	7 (2.84) b	9.3 (3.13)bc	62
2000	5.3 (2.41) a	6 (2.52) a	6 (2.78) b	7.5 (2.52) c	50
Control	1 (1.1) b	1 (1.1) b	1 (1.1) c	1 (1.1) d	6.7
S.E. ±	0.1	0.1	0.2	0.2	

Means followed the same letter(4)are not significantly different at $p \leq 0.05$ means between brackets are transformed by $(\sqrt{x+0.5})$.

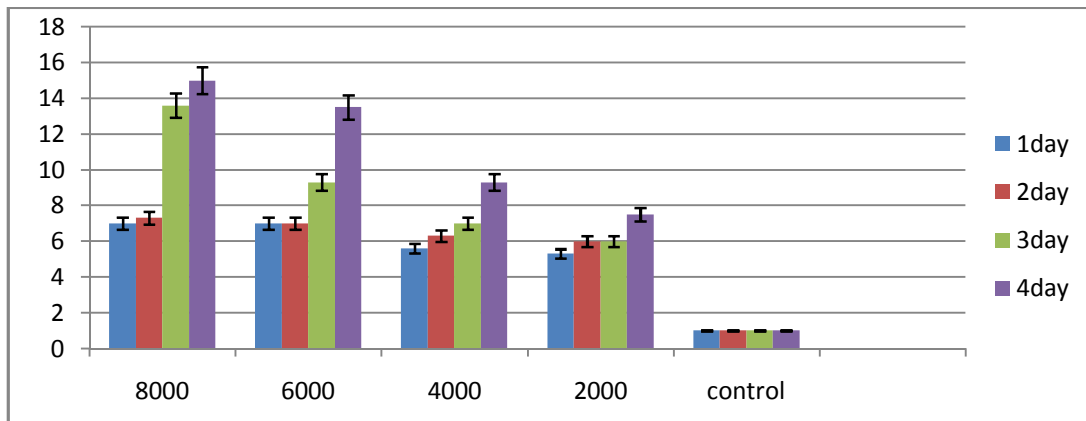


Figure NO. 10. Mean percentage mortality of larva of *T. absoluta* after Four days of topical application of methanolic extract of Neem seeds.

4.7 Mean percentage mortality of larva of *Tuta absoluta* after four days of topical application of petroleum ether of *jatropha* seeds at: 25 c°& 50 ±10% RH)

The results of the different concentrations are shown in table (11), figure. (10) and appendix (6). Results showed that, the treatment with *Jatropha* seed extract induced larval mortality between 23% - 48.5% at all concentration after 24 hrs. of application. By the 4th day, high percentage larval mortality, between 85% - 100% was obtained at the different concentrations.

Table NO. 9 Mean percentage mortality of larva of *Tuta absoluta* after Four days topical application of petroleum ether of *Jatropha* seeds extract

Mortality of larvae					
Concentration	1 st day	2 nd day	3 rd day	4 th day	Total%
8000	9.7 (2.27)b	12 (2.37) b	18 (3.83) b	20 (4.22) a	100
6000	9 (2.67)ab	10 (2.84)ab	12.3 (3.83) b	18 (4.01) a	90
4000	6.7 (3.06) a	7.7 (3.23)ab	11.3(3.58)ab	17 (4.29) a	85
2000	4.7 (3.18) a	7 (3.53) a	11.3 (4.29) a	17.4 (4.53) a	87
Control	1.3 (1.27) c	1.3 (1.27) c	1.3 (1.27) c	1.3 (1.27) a	6.5
S.E. ±	0.2	0.2	0.2	0.2	

Means followed the same letter(4)are not significantly differentatp≤0.05means between brackets are transformed by($\sqrt{x+0.5}$).

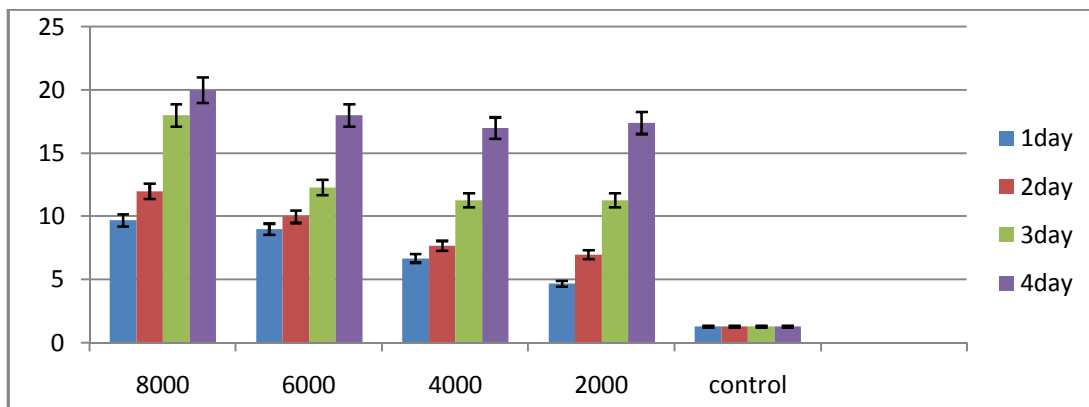


Figure NO.10 Mean percentage mortality larva of *Tuta absoluta* after Four days of topical application of petroleum ether of *Jatropha* seeds extract

4.8 Mean percentage mortality of larva of *T. absoluta* after Four days of topical application of Argel leaves extract (At: 25°c & 50± 10% RH)

The results of different concentrations are shown in table (11),figure (7) and appendix (8). Results showed that, the treatment with Argel seed extract induced larval mortality between 53- 50% at all concentration after 24 hrs. of application. By the 4th day, high percentage larval mortality, between 50% - 100% was obtained at the different concentration

Table NO. 10 Mean percentage mortality of larva of *Tuta absoluta* after Four days of topical application of *Argel* leaves extract

Mortality of larvae					
Concentration	1 st day	2 nd day	3 rd day	4 th day	Total%
8000	9.7 (3.18) a	9 (3.08) b	18.3 (4.34) a	19 (4.41) a	95
6000	9 (3.06) a	10 (3.23) b	12 (3.51) b	18 (4.29) a	90
4000	10 (3.24) a	14 (3.79) a	14.3 (3.85) b	20 (4.53) a	100
2000	7 (2.72) a	7.4 (2.80) b	8.8 (3.05) c	10 (3.23) b	50
Control	0 (0.71) b	0 (0.71) c	0 (0.71) d	0 (0.71) c	0
S.E. ±	0.3	0.3	0.3	0.4	

Means followed the same letter(4)are not significantly differentat $p \leq 0.05$ means between brackets are transformed by $(\sqrt{x+0.5})$.

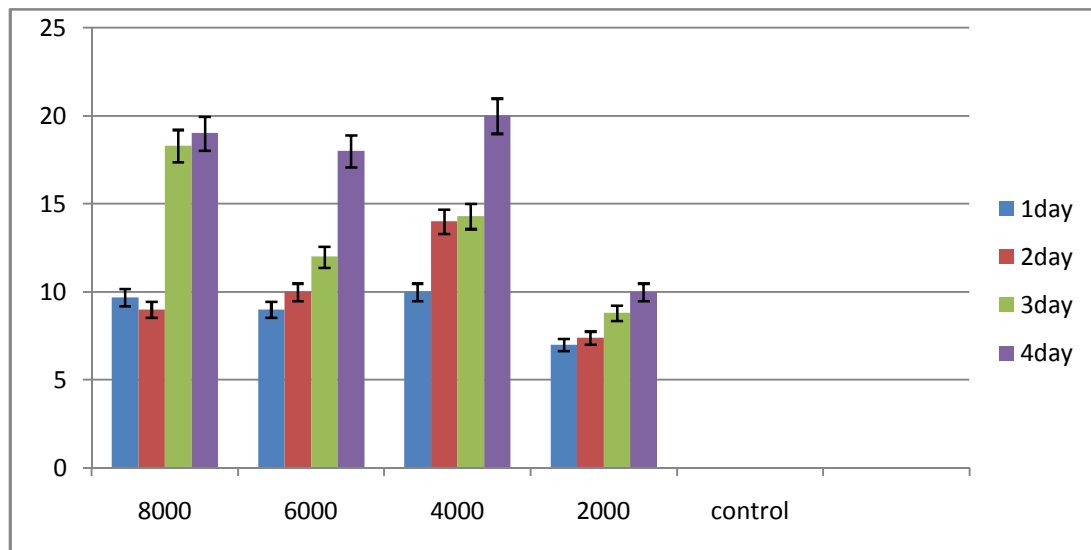


Figure NO.11. Mean percentage mortality of larva of *Tuta absoluta* after Four days of topical application of *Argel* leaves extract

CHAPTER FIVE

DISCUSSION

General Survey of the leaf miner, *Tuta absoluta* in Khartoum State :

The survey carried out in Khartoum State covered 11 sites: 1 sites in Khartoum Locality, 7 in Khartoum North Locality and 3 in Omdurman Locality. Within these sites, a total of 163 Green Houses and open farms, planted with tomatoes and other vegetables, were investigated.

The results of the survey showed that, all sites were infested with *T. absoluta*, with varying degrees of infestation (high infestation was found in 3sites, moderate infestation in 5 sites, and low infestation in 3 sites (as shown in Table No. 1). In the survey, the main host plants found were tomatoes, in addition to potatoes (*Solanum tuberosum*), egg plant (*Solanum melongena*) , beans (*Phaseolus vulgaris*), water melon (*Citrulus vulgaris*) and the weed Datura . These results are in agreement with those of Fernandez and Montagne (1990) who conducted host preference studies in a laboratory in Venezuela. They found that, the tomato cultivar “Rome Gigante” was the preferred oviposition host and the best host for larval development when compared to egg plant, tobacco and potato. Among the alternate hosts were Datura (*D. stramonium* and Tobacco (*Nicotiana glauca*).

The survey also indicated that, adults of *T. absoluta* are nocturnal in habits, and sometimes are found hiding between leaves during the day. These findings are similar to that of Fernandez and Montagne (1990) who stated that, adults of *T. absoluta* usually rest among leaves during the day. Also in this survey, it is noticed that, *T. absoluta* adults have a low flight range. This results is similar to that of Al Zaidi (2009) who reported that, *T. absoluta* adults do not fly very high, and placed the traps at a height of 0.3 – 0.6 m above host plants

Biology of the tomato leaf miner *T. absoluta* :

In the present study, mating was observed to occur shortly after adult emergence, mostly on the same day of emergence, at a temperature of 19 –21°C . A somewhat similar results were reported by Fernandez and Montagne (1990) who mentioned that, mating usually occur the day after adult emergence. The range of eggs laid by a female in the present study was 50 – 64, with a mean of 55.5 eggs per female , reaching a total of 260 eggs over its life. It is reported that, the female of *T. absoluta* can lay up to 260 eggs in a life time (CABI, 2011). In the present study, the total life cycle obtained was 33 – 43 days at 30 -34 °c, and 56 – 66 days at 19-21°C. Vargas (1970) mentioned that, at a constant temperature of 25 °c, *T. absoluta* completed a generation in 28.7 days, while Barrientos Apablaza, Norero, and Estay (1998) reported that, the development of *T. absoluta* from egg to adult requires 39.8 days at 19.7 °c and 23.8 days at 27.1 °c.

The description and colour changes of *T. absoluta* eggs from time of being laid till hatching reported in this study were similar to those mentioned by Estay (2000). Also, the range of the egg incubation period obtained in this study was 4 ±1.0 - 6.0 ±1.5 days, which are in agreement with Fernandez and Montagne (1990), who found that the egg incubation period of *T. absoluta* eggs ranged between 4.4 – 5.8 days.

Four larval instars were observed in the present study, which are similar to the finding of Estay (2000), who reported that *T. absoluta* complete 4 well defined larval instars of different size and colour. The larval stage which is the damaging stage, was found to be the longest compared to other stages. At 19-21°C, the larval stage took 31.2± 2.6 days, and at 30-34 °c took 13± 2.0 days. In comparison,(Barrientos , Apablaza, Norero, and Estay (1998) who recorded that, at 27.1°C, *T.absoluta* larvae completed their development in 11 to 13 days.

In the present study, the pupal stage took 6 ± 2.0 days at an average temperature of 30-34 °c. which is similar to the results of , Barrientos , Apablaza, Norero, and Estay. (1998), as they mentioned that, at 27.1 °c the pupae emerged as adults in 5 to 8 days.,

Damage of *T. absoluta* in tomatoes :

Vargas (1970) mentioned that, Larvae of *T. absoluta* are capable of penetrating and mining tomato tender shoots. Mohamed and Siam (2011) mentioned that, *T. absoluta* is recorded in Sudan as a serious problem to tomatoes and potatoes , and infestation outbreaks were observed with a damage ranging from 5% up to 80%. At Abu Halima site, observations made in this study on the damage by *T. absoluta* during tomato growing season showed that, all plant parts were infested with varying degrees, from 45% damage in stems, up to 75% and 87% in fruits and leaves, respectively. These results are in agreement with Pastrana (2004) who stated that, the larvae of *T. absoluta* mine leaves, shoots, flowers and fruits of tomato plants.

Laboratory trials for the control of the tomato leaf miner, *Tuta absoluta* , using plant extracts :

The conventional use of synthetic pesticides during the past decades and their efficacy against different pests have led to their wide acceptance along the world. However, their extensive use have resulted in certain drawbacks and hazards including, persistence, toxicity to non-target organisms, pest resistance and environmental pollution. Consequently, researchers have directed attention towards application of more safe and environmentally friendly compounds. They focused on the use of botanical extracts, oils and plant powders, which are cheap, of short persistence and of low mammalian toxicity. A number of references (Stoll, 2000 & Hiiesaar *et al.*, 2001) indicated that, many of these plant

materials show a broad spectrum of activity against insect pests, such as lethal, antifeedant, repellent and growth regulatory effects.

The tomato leaf miner, *Tuta absoluta*, a new pest in Sudan, has caused extensive damage to tomato and other Solanaceous plants since its introduction to the country in 2010 (Mohamed and Abdelgamel (2011)). Accordingly, many trials were made for its control and suppression of damage through application of chemicals, botanicals and pheromones

In the present study, two plant seeds extracts from Neen and Jatropha were applied for egg control, three extracts, Neen, Jatropha and Argel were applied for larval control of *T. absoluta*.

Effect of plant extracts on *T. absoluta* eggs :

The results of the egg control trials showed that, none of the plant extracts has caused any clear effects on the eggs. No effect was noticed on the eggs after 24 hrs. of topical application of the two extracts. Also, no significant difference was detected between the slight egg mortality (20-26%) occurred at the different concentrations after 4 days of treatment. In addition, no effect was noticed on the egg viability, as many eggs hatched normally after an incubation period of 4-6 days. The results of the present experiment are quite similar to that of Trindade *et al.*, (2000), who applied Neem seed extract against egg and larvae of *T. absoluta*. Their results showed that, although some egg mortality occurred, the extract did not affect the viability of the eggs. It can be supposed that, the hard shell of the eggs may be the main factor for their protection against effects of the plant extracts. There are no previous records on the effects of insecticides or plant extracts on *T. absoluta* eggs. The only methods of control practiced were through application of irradiation (Arther, 2002) and application of biological control trials with egg parasitoids (Faria *et al.*, 2008).

Effect of plant extracts on *T. absoluta* larvae :

The results of the larval control trials, in comparison, showed high efficacy of the plant extracts against *T. absoluta* larvae. After 24 hrs., larval mortalities ranging between 35-48.5%, 33-46%, and 23-48.5% were obtained with Argel leaves extract, Neem seed extract and Jatropha seed extract, respectively. Also, higher larval mortalities, up to 100%, were obtained with the three extracts after 4 days of treatments.

Many previous studies reported effective larval control of *T. absoluta* with botanical materials. Trindade *et al.*, (2000), reported that application of 4 concentrations of Neem seed extract against young larvae of *T. absoluta* resulted in 50-100% mortality after 4 days. 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 *Acomela oleracea* was the most active against *T. absoluta* larvae. Also, 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 ghani (2014), used 5 plant extracts against 2nd instar larvae of *T. absoluta*. Their results showed that, Chinaberry showed the highest effects on *T. absoluta* larvae, followed by Geranium, Onion and Garlic. It is clear from the present study, and previous studies mentioned, that the different plant extracts tested proved high insecticidal activity against *T. absoluta* larvae. Considering the high risks and hazards and toxicity of chemical insecticides, these natural plant extracts constitute valuable, safe and environmentally friendly alternative compounds in the field of insect pest

control. Surely, more investigations, with new plant materials in the future, will constitute an asset in the field of plant pesticides.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

The tomato leaf miner, *Tuta absoluta* is considered an important Pest of tomato crops. In recent years, it spread very quickly in many countries around the world. In Sudan, *T.absoluta* was recorded as a serious problem of tomato in different localities in Khartoum State, and also in many parts in the country.

Concerning the biology and ecology, the results of the study showed that, The leaf miner *T. absoluta* has high reproductive efficiency. It has a relatively longer larval stage duration (31.2 ± 2.6 days) compared to other developmental stages. Also, The larvae do not enter a diapause as long as there is an availability of food. The larval feeding behavior usually takes place inside the tunnels. The results also indicated that, the leaf miner *T.absoluta* have a wide range of host plants among a diversity of families of field crops, vegetables and grasses.

The extracts of Neem seeds and petroleum ether of jatropha seeds did not affect *T. absoluta* egg viability. Trials with the larval stage showed that, Ethanolic extracts of Neem seeds, petroleum ether of Jatropha seeds extract and Argel leaves extract caused high mortality among larvae of the tomato leaf miner.

RECOMMENDATIONS

1-The tested extracts of the three plants (Neem and Jatropha seeds and Argel leaves) are recommended for the control of the larval stage of *Tuta absoluta*, as they showed no effect against the egg stage. These natural plant extracts constitute valuable, safe and environmentally friendly alternative compounds in the field of insect pest control .

2- It is recommended to carry out good agricultural operations and precautions to prevent injury of the tomato leaf miner from the beginning of agriculture up to harvesting time, and application of Pheromone traps and Sticky traps to predict and control the appearance of the leaf lesions.

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APPENDICES

Appendix 1. The full number of eggs\female and egg hatchability of *T. absoluta*

Female NO.	NO. of egg laid	Egg Hatch	Unhatch	Hatchability%	unHatchability%
1	58	43	15	74	25.8
2	52	40	12	76	23.7
3	60	35	25	85	41.6
4	52	44	8	84	15.3
5	55	42	13	76	23.6
6	60	51	9	85	15
7	61	48	13	78	21.3
8	56	40	16	71	28.5
9	52	39	13	75	25
10	49	41	8	83	16.3
Mean	55.5	42.3	13.2	78.7	20.7
S.E		1.3	0.07	1.6	0.9

Appendix 2. The total number of eggs laid per female, resulted adults and the survival percentage of *T.absoluta*

Female NO.	Resulted adults	NO .of egg laid	Survival%
1	37	58	74
2	36	52	76
3	44	60	85
4	40	52	84
5	34	55	76
6	43	60	85
7	40	61	78
8	32	56	71
9	32	52	75
10	33	49	83
Mean	37.1	55.5	78.7
S.E	3.0	1.3	1.6

Appendix 3. Mean percentage mortality of egg of *tuta absoluta* after forth days of topical application of methanolic Neem seeds extract Table (6)

Title: topical

Function: ANOVA-1

ANALYSIS OF VARIANCE TABLE

first ,second ,third and forth day by treatment /statistics descriptive

/missing analysis /post hoc=Duncan alpha 0.05).

First day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	4.024	.0805	3.920	0.024
Within	12	2.464	0.205		
Total	17	6.488			

1	Number	SD	SE
1	3.00	0.0000	0.0000
2	3.00	.18800	0.32565
3	3.00	013867	024002
4	3.00	0.25667	044456
5	3.00	0.34854	0.60368
6	3.00	0.41.73	0.71141
Total	18.00	0.14561	0.61776

Table (1)2nd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	4.090	.818	2.55	0.085
Within	12	3.845	0320		
Total	17	7.935			

Descriptives

1	Number	SD	SE
1	3.00	0.93038	0.5371
2	3.00	.50123	0.28939
3	3.00	036529	0.21090
4	3.00	0.19502	0.11260
5	3.00	0.35799	0.20667
6	3.00	0.71141	0.41073
Total	18.00	0.68322	0.16104

Table (1)3rd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	6.939	1.388	9.347	.001

Within	12	1.782	0.148
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Total	17	8.720	
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Descriptives

1	Number	SD	SE
1	3.00	0.51433	0.29695
2	3.00	.26558	0.15333
3	3.00	0.0000	0.00000
4	3.00	0.16000	0.09238
5	3.00	0.15503	0.08950
6	3.00	0.71141	0.41073

Total	18.00	0.71621	016881
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Table (1)4th day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	10.526	.2.105	6.520	0.004
Within	12	23.875	0.323		

Total	17	14.40-1			
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Descriptives

1	Number	SD	SE
1	3.00	0.70437	0.40607
2	3.00	.55048	0.31782
3	3.00	.70437	0.40607
4	3.00	0.29547	0.17059
5	3.00	0.22053	0.41073
6	3.00	0.71141	0.21694
Total	18.00	0.92039	

Appendix .6 Mean percentage mortality of egg of *tuta absoluta* after four days of topical application of petroleum ether of jatropha seeds extract Table (7)

Title: topical

Function: ANOVA-1

ANALYSIS OF VARIANCE TABLE

first ,second ,third and fourth day by treatment /statistics descriptive

/missing analysis /post hoc=Duncan alpha 0.05).

Table (7)first day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	2.536	0.507	2.893	0.061
Within	12	2.104	0.175		

Descriptives

1	Number	SD	SE
1	3.00	0.32362	0.18684
2	3.00	.27033	0.15607
3	3.00	.51764	0.29886
4	3.00	0.4394	0.25374
5	3.00	0.21417	0.12365
6	3.00	0.6058	0.34977
Total	18.00	0.52239	0.12313

Table (2)2nd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	3.309	0.662	2.427	0.097
Within	12	3.272	0.273		
Total	17	6.580			

Descriptives

1	Number	SD	SE
1	3.00	0.50276	0.29027
2	3.00	0.39184	0.22623
3	3.00	0.60715	0.35054

4	3.00	0.62092	0.35849
5	3.00	0.32924	0.19009
6	3.00	0.60582	0.34977

Total 18.00 0.62216 0.14664

Table(2) 3rd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	8.411	1.682	13.233	0.000
Within	12	1.526	0.127		
Total	17	9.937			

Descriptives

1	Number	SD	SE
1	3.00	0.50276	0.29027
2	3.00	0.39184	0.22623
3	3.00	0.60715	0.35054
4	3.00	0.62092	0.35054
5	3.00	0.32924	0.35849
6	3.00	0.60582	0.34977
Total	18.00	0.62216	0.14664

Table(2) 4th day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	6.181	1.236	12.069	0.000
Within	12	1.229	0.102		
Total	17	7.411			

Descriptives

1	Number	SD	SE
1	3.00	0.2746	0.1585
2	3.00	0.2746	0.1585
3	3.00	0.2746	0.1585
4	3.00	0.15449	0.8920
5	3.00	0.38160	0.22032
6	3.00	0.60582	0.334977
Total	18.00	0.76454	0.18020

Appendix .6 Mean percentage mortality larva of *tuta absoluta* after forth days of topical application o fNeem methanlic extract

Title: topical

Function: ANOVA-1

ANALYSIS OF VARIANCE TABLE

first ,second ,third and forth day by treatment /statistics descriptive

/missing analysis /post hoc=Duncan alpha 0.05)

Table(3)first day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	5.603	1.401	11.384	0.001
Within	10	1.230	.123		
Total	14	6.834			

Descriptives

1	Number	SD	SE
1	3.00	0.11795	0.06810
2	3.00	0.11795	0.06810
3	3.00	0.0000	0.00000
4	3.00	0.36878	0.21292
5	3.00	0.67188	0.83791
Total	18.00	.69865	0.18039

Table (3)2nd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	5.952	1.488	6.591	0.007

Within	10	2.258	.226
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Total	14	8.210	
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Descriptives

1	Number	SD	SE
1	3.00	0.50276	0.29027
2	3.00	0.28893	0.16681
3	3.00	0.18302	0.10567
4	3.00	0.55477	0.32030
5	3.00	0.67188	0.38791

Total	15.00	.76579	0.19773
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Table(4) 3rd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	11.691	2.923	15.556	0.000
Within	10	21.879	.226	.188	
Total	14	13.570			

Descriptives

1	Number	SD	SE
1	3.00	0.38647	0.22313
2	3.00	0.42551	0.24567
3	3.00	0.24136	0.13935
4	3.00	0.3154	0.18195
5	3.00	0.67188	0.38791
Total	15.00	.98452	0.25420

Table(3)4th day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	15.817	3.954	.578	0.000
Within	10	1.609	.161		
Total	14	17.426			

Descriptives

1	Number	SD	SE
1	3.00	0.24136	0.13935
2	3.00	0.20495	0.11833
3	3.00	0.0000	0.00000

4	3.00	0.07188	0.38791
5	3.00	0.11567	0.28801

Total 15.00 1.11567 .28807

Appendix 7 Mean percentage mortality of larva of *tuta absoluta* after .forth day topical application of petroleum ether of jatroph seeds extract

Title: topical

Function: ANOVA-1

ANALYSIS OF VARIANCE TABLE

first ,second ,third and forth day by treatment /statistics descriptive

/missing analysis /post hoc=Duncan alpha 0.05).

Table(4)first day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	7.105	.4776	11.877	0.001
Within	10	1.496	0150		
Total	17	8.601			

Descriptives

1	Number	SD	SE
1	3.00	0.12926	0.07463
2	3.00	0.211129	0..2199
3	3.00	0.49354	0.28495

4	3.00	0.32088	0.18526
5	3.00	0.58304	0.33662

Total	15.00	0.78382	0.2023

Table(4)2nd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	9.144	2.286	15.768	0.000
Within	10	1.450	0.145		

Total	17	10.549			

Descriptives

1	Number	SD	SE
1	3.00	0.32924	0.19009
2	3.00	0.42551	0.24567
3	3.00	0.27464	0.15556
4	3.00	0.14154	0.08172
5	3.00	0.58304	0.33662

Total	15.00	0.8698	0.2246

Table(4)3rd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	15.400	3.850	24.858	0.000
Within	10	1.548	0.155		
Total	17	16.949			

Descriptives

1	Number	SD	SE
1	3.00	0.3816	0.22032
2	3.00	0.3816 0	0.22032
3	3.00	0.2116	0.112217
4	3.00	0.31304	0.18116
5	3.00	0.58304	0.33662
Total	15.00	1.10029	28409

Table(4) 4th day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	21.897	5.474	30.978	0.000

Within	10	1.767	0.177
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Total	17	23.664	
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Descriptives

1	Number	SD	SE
1	3.00	0.44831	0.25883
2	3.00	0.41225	0.23801
3	3.00	0.41558	0.23994
4	3.00	0.0000	0.00000
5	3.00	0.58304	0.33662

Total	15.00	1.30011	0.3396
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Appendix 8. Mean percentage mortality of larva of *tuta absoluta* after fourth days of topical application of Argel leaves extract

Title: topical

Function: ANOVA-1

ANALYSIS OF VARIANCE TABLE

first ,second ,third and fourth day by treatment /statistics descriptive

/missing analysis /post hoc=Duncan alpha 0.05).

Table (5)first day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	13.633	3.408	33.651	0.000

Within	10	1.013	0.101
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Total	17	14.646	
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Descriptives

1	Number	SD	SE
1	3.00	036878	21292
2	3.00	01544	0.8920
3	3.00	0.4935	0.28495
4	3.00	0.32088	0.18526
5	3.00	0.0000	0.00000

Total	15.00	1.02281	0.26409
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Table(5)2nd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	16.777	4.1948	48.561	0.000
Within	10	.864	0.086		

Total	17	17.641	
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Descriptives

1	Number	SD	SE
1	3.00	0.36269	0.20940

2	3.00	0.47438	0.27388
3	3.00	0.27464	0.15856
4	3.00	0.0000	0.00000
5	3.00	0.0000	0.00000

Total 15.00 1.12252 0.28983

Table (5)3rd day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	23.937	5.984	141.215	0.000
Within	10	.424	0.042		

Total 17 24.361

Descriptives

1	Number	SD	SE
1	3.00	0.11921	0.6882
2	3.00	0.19699	0.11373
3	3.00	0.31588	0.18237
4	3.00	0.24310	0.14035
5	3.00	0.0000	0.00000

Total 15.00 1.31912 0.34060

Table (5) 4th day

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	31.109	7.777	182.215	0.000
Within	10	.427	0.043		
Total	17	31.845			

Descriptives

1	Number	SD	SE
1	3.00	0.27464	0.15856
2	3.00	0.0000	0.00000
3	3.00	0.3178	0.18116
4	3.00	0.19884	0.11480
5	3.00	0.0000	0.00000
Total	15.00	1.50085	0.38752