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# Effects of Botanical Extracts of Neem(*Azadirachta indica*) and Jatropha (*Jatropha curcus*) on Eggs and Larvae of Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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**Abstract:** Laboratory experiments were conducted to investigate the insecticidal effects ofNeem (*Azadirachta indica*) seeds ethanolic extract and Jatropha (*Jatropha curcus*) seeds petroleum ether extract against eggs and larvae of tomato leaf miner (*Tuta absoluta*).Newly laid eggs of tomato leaf miner were treated topically by five concentrations (1000, 500, 250, 125 and 62.5 mg/L) of each plant seed extract. Meanwhile, the larvae were exposed to four concentrations (8000, 6000, 4000 and 2000 mg/L) of each plant seed extract. The mean percentage mortality of eggs and larvae were recorded daily for 4 days. Results indicated that, each plant seed extract caused significant mortality to eggs and larvae of *T. absoluta* after 4 days in comparison to control. After 4 days, around 25% and 18% of egg mortalities were obtained with the different concentrations of Neem and Jatropha extracts, respectively. On the other hand, larval mortalities ranging between 33- 46.7% and 23.5 - 48.5% were obtained after 24 hours with Neem and Jatropha seed extracts, respectively. Also, higher larval mortalities, up to 100%, were obtained with the two extracts after 4 days of treatments.

**Key Words:** *Azadirachta indica*, *Jatropha curcus*, *Tuta absoluta*, Sudan.

## Introduction

Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae) is an important pest of solanacious crops native to South America (Filho *et al*., 2000). Tomato leaf miner larvae feed inside the mesophyll tissues hollowing it out. Also, they can penetrate young stems and fruits. Many control programs were investigated in many countries affected by *T. absoluta*, with chemical control as the first treatment (Siqueira, *et al.*, 2000; Lietti *et al*., 2005). In 2006, *T. absoluta* was accidentally infested Spain from where it spread very quickly in many other countries of the western Palearctic Region (Desenex, 2010). In Spain, treatment with recommended insecticides was performed when the number of trapped males reached 40 adults per day. However, due to the development of resistance, chemical control has shown limited efficacy even after using different types of pesticides and increasing the application frequencies (Siquira *et al.*, 2000; Lietti *et al*., 2005). Also, control with pheromone traps and water traps was applied and showed high potentials (Al-Zaidi, 2009; Mohamed and Siam, 2011). Many natural enemies were reported from different countries among them the predatory nesibug *Nesicordious tenius* and two parasitic Hymenopteran belonging to Braconidae (Mahmoud, 2012). In Sudan, *T.absoluta* is recorded as a serious problem to tomato and potato crops after the official report in the country in 2011 with infestation levels from 5% and up to 80% (Mohamed *et al*., 2011). Concerning problems and hazards of insecticide applications, many studies to control tomato leaf miner were carried out using plant extracts (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 initiated to evaluate the insecticidal effects of seed extracts of Neem (*Azadirachta indica*) and Jatropha (*Jatropha curcus*), against eggs and larvae of tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) in the laboratory.

## Materials and Methods

**Plant Species used in the study:** Extracts of seeds of Neem (*A. indica*) and Jatropha (*J. curcas*) were chosen to be applied against immature stages of *T. absoluta*.Seeds of both plants were collected from Shambat area and Forestry Research Center, Soba, respectively. Seeds of each plant were cleaned, dried, decorticated and ground to a fine powder by an electric blender (Moulinex). The powder of each plant was kept in tight closed vials for later use.

**Preparation of the plant extracts:** The extraction of Neem and Jatropha seeds was made at the Department of Pesticides Alternatives, at the Environment and Natural Resource Research Institute (ENRRI), National Research Center (NRC). Hundred grams of each plant powder were used for the extraction process. Extraction of Neem was made by ethanol, and that of Jatropha was prepared using petroleum ether.

**Bioassays of** *T. absoluta* **eggs:** In these tests, five concentrations (viz., 1000, 500, 250, 125 and 62.5mg/L)of each of Neem and Jatropha seed powder extracts were prepared from the stock solution. Five groups of 40-50 eggs of *T. absoluta* were prepared in Petri-dishesand were topically treated with the different concentrations of each extract. Also, similar groups of eggs were used as control groups, and were treated with ethanol or petroleum ether according to the type of the extract. The ovicidal effects of the extracts were tested by recording egg mortality at each concentration daily for 4 days. Changes of egg colour from creamy to dark and eventually to black were the main criteria of egg mortality.

**Bioassays of** *T. absoluta* **Larvae:** Groups of 15-20 recently hatched larvae of *T. absoluta* were placed in Petri-dishes, each petri-dish contained a piece of fresh tomato leaf, previously immersed for 5 seconds in each of the different concentrations of the

extracts. Similar groups of larvae, were used as control with each plant extract, and placed in petri-dishes, each contained fresh tomato leaf, treated with ethanol or petroleum ether according to the type of the extract. In each concentration, larval mortality was observed and recorded daily for 4 days. Criteria of larval mortality: the larval colour changes to dark, then larva becomes sluggish, and finally died.

**Experimental design and data analysis:** The experiments were conducted in the laboratory, at a temperature of 25 – 27 $C^{\circ}$ and a 50 ±10% RH. Also, the experiments were arranged in complete randomized design, replicated three times for each concentration. Obtained data were transformed using ($\sqrt{\times}$ + 0.5). Analysis of variance (One Way ANOVA) was computed using SPSS program (version 20) and means were separated using Tukey test.

## Results

**Effect of botanical extracts of Neem and Jatrophaon eggs of** *T. absoluta***:** After 4 days of application, about 24 – 26 % egg mortality was observed due to application of different concentrations of Neem seed extracts when compared to control (5.1%). No significant differences were found between the effects of different concentrations of Neem extract on eggs of *T. absoluta* (Table 1). Similarly, the different concentrations of Jatropha seed extracts showed (18 – 25%) mortality on eggs of *T. absoluta* when compared to control (3.8%). Also, no significant differences were observed between different concentrations of Jatropha (table 2).

**Effect of botanical extracts of Neem and Jatrophaon larvae of** *T. absoluta***:** Effects of the two plant extracts on the larvae of *T. absoluta* are shown in Tables 3 and 4.After 24 hours, larval mortality ranged between 33- 46.6% was obtained with the 4 concentrations of the Neem seed extract, and by the $4^{th}$ day, higher larval mortality up to100% was obtained. In Table 4, the results also showed that, Jatropha seed extract induced larval mortality between 23% - 48.5% for all concentration after 24 hours. By the $4^{th}$ day, high percentage larval mortality, between 85% - 100% was obtained with different concentrations.

## Discussion

The conventional use of synthetic pesticides during the past decades and their efficacy against different pests has led to their wide acceptance along the world. However, their extensive uses have resulted in certain drawbacks and hazards, including, persistence, toxicity to non-target organisms, pest resistance and environmental pollution (Siquira *et al.*, 2000; Lietti *et al.*, 2005). 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 and 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 Solanacious plants since its introduction to the country in 2010(Mohamed *et al*., 2011). Accordingly, many trials were made for its control and suppression of damage through application of chemicals, botanicals and pheromones (Mohamed and Siam, 2011). In the present study, two plant seed extracts from Neem and Jatropha were applied for egg and larval control of *T. absoluta*. No effect was noticed on the eggs after 24 hrs. of topical application of the two extracts. However, within 4 days, the different concentrations of the two plant extracts caused an egg mortality ranging between 20 – 26% with no significant differences between the concentrations. On the other hand, most of the remaining eggs hatched normally after 4 – 6 days of treatment, indicating no effect on the egg viability by the plant

extracts. 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. The results of the present experiment are quite similar to that of (Trindade *et al*., 2000), who applied Neem seed extract against eggs and larvae of *T. absoluta*. Their results showed that, although some egg mortality occurred, the extract did not affect the viability of the eggs. 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 (Arthur, 2002) and application of biological control trials with egg parasitoids (Faria *et al*., 2008). In addition, there are new records of bio control agents of *T. absoluta* (Mahmoud, 2012). The results of the larval control trials, in comparison, showed high efficacy of the plant extracts against *T. absoluta* larvae, with higher larval mortalities, up to 100%, obtained 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 84-100% 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 *Acomellaoleracea* 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 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 a cheap, 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 botanical pesticides.

Table1. Effect of ethanolic extract of Neem seed on mortality of eggs of *T. absoluta*

| Concentration | No of eggs | Mortality | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | Total % |
| 1000 | 49 | $0^{b}$ (0.7) | $3^{bc}$ (1.7) | $7^{a}$ (2.7) | $12^{a}$ (3.5) | 24.5 |
| 500 | 50 | $2^{ab}$ (1.6) | $6^{ab}$ (2.5) | $10^{a}$ (3.2) | $13^{a}$ (3.7) | 26 |
| 250 | 49 | $4^{a}$(2.1) | $7^{a}$ (2.7) | $9^{a}$ (3.1) | $12^{a}$ (3.5) | 24.5 |
| 125 | 40 | $3^{a}$(1.7) | $6^{ab}$ (2.5) | $9^{a}$ (3.1) | $12^{a}$ (3.5) | 25 |
| 62.5 | 50 | $4^{a}$ (2.1) | $6^{ab}$ (2.5) | $10^{a}$ (3.2) | $12^{a}$ (3.5) | 24 |
| Control | 39 | $2^{ab}$ (1.5) | $2^{c}$ (1.5) | $2^{b}$ (1.5) | $2^{b}$ (1.5) | 5.1 |
| S.E. ± | | 0.2 | 0.2 | 0.2 | 0.2 | |

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

Table 2. Effect of Jatropha seed petroleum ether extract on the mortality of *T. absoluta* eggs

| Concentration | No of eggs | Mortality | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | Total % |
| 1000 | 49 | 2 [b] (1.6) | 6 [a] (2.5) | 9 [a] (3.1) | 10[a] (3.2) | 20.4 |
| 500 | 50 | 3 [ab] (1.7) | 5 [a] (2.3) | 7 [a] (2.7) | 9 [a] (3.1) | 18 |
| 250 | 49 | 3 [ab] (1.7) | 6 [a] (2.5) | 8 [a] (2.8) | 9 [a] (3.1) | 18.4 |
| 125 | 40 | 1 [b] (1.2) | 5 [ab] (2.3) | 7 [a] (2.7) | 10 [a] (3.2) | 25 |
| 62.5 | 50 | 5 [a] (2.3) | 7 [a] (2.7) | 9 [a] (3.1) | 11 [a] (3.4) | 22 |
| Control | 45 | 1.7 [b] (1.4) | 1.7[b](1.4) | 1.7 [b] (1.4) | 1.7 [b] (1.4) | 3.8 |
| S.E. ± | | 0.1 | 0.2 | 0.2 | 0.2 | |

Means followed by the same letter(s) are not significantly different at P≤0.05
Means between brackets are transformed by (√ × + 0.5).

Table 3. Effect of Neem seed ethanolic extract on larvae of *T. absoluta*

| Concentration | No of larvae | Mortality | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | Total % |
| 8000 | 15 | 7[a] (2.7) | 7[a] (2.7) | 13 [a] (3.8) | 15[a] (3.9) | 100 |
| 6000 | 15 | 7[a] (2.7) | 7 [a] (2.7) | 9 [a] (3.1) | 13 [ab] (3.8) | 86.7 |
| 4000 | 15 | 5 [a] (2.3) | 6[a] (2.5) | 7[ab] (2.7) | 9 [bc] (3.1) | 62 |
| 2000 | 15 | 5 [a] (2.3) | 6 [a] (2.5) | 7 [b] (2.7) | 7[c] (2.7) | 50 |
| Control | 15 | 1[b] (1.1) | 1[b] (1.1) | 1[c] (1.1) | 1[d] (1.1) | 6.7 |
| S.E. ± | | 0.2 | 0.2 | 0.2 | 0.3 | |

Means followed by the same letter(s) are not significantly different at P≤0.05
Means between brackets are transformed by (√ × + 0.5).

Table 4. Effect of ether seed extract of Jatrophaon larvae of *T. absoluta*

| Concentration | No of larvae | Mortality | | | | |
|---|---|---|---|---|---|---|
| | | 1st day | 2nd day | 3rd day | 4th day | Total % |
| 8000 | 20 | 9 [a] (3.1) | 12[a] (3.5) | 18 [a] (4.3) | 20 [a] (4.5) | 100 |
| 6000 | 20 | 9[a] (3.1) | 10 [ab] (3.2) | 12 [ab](3.6) | 18[a] (4.3) | 90 |
| 4000 | 20 | 7 [ab] (2.7) | 8 [b] (2.8) | 11 [b] (3.4) | 17[a](4.0) | 85 |
| 2000 | 20 | 5 [b] (2.3) | 7 [b] (2.7) | 11 [b] (3.4) | 17 [a] (4.0) | 87 |
| Control | 20 | 1.3[c] (1.3) | 1.3[c] (1.3) | 1.3[c] (1.3) | 1.3 [b] (1.3) | 6.5 |
| S.E. ± | | 0.2 | 0.2 | 0.3 | 0.3 | |

Means followed by the same letter(s) are not significantly different at P≤0.05
Means between brackets are transformed by (√ × + 0.5).

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