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The Impact of Hexane and Ethanol Extracts of Jatropha Seeds, Arqel Stems and Malathion on Mortality and Fecundity of Tomato Leaf Miner Tuta Absoluta (Lepidoptera: Gelechiidae)

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Abstract The effects of hexane and ethanol organic extracts of jatropha Jatropha curcas seeds, argel (Solenostemma argel (Del.) stem and malathion on mortality and fecundity of the tomato leaf miner Tuta absoluta were evaluated under laboratory conditions at a temperature of 27 ± 2c and a relative humidity of 30 ± 5%. Two-day old adults were tested against four different concentrations of each extract. The mortality results showed that all tested concentrations gave significantly higher mortality percentage than that of the control. The LC50 values of the ethanolic extract of jatropha seeds after 24, 48 and 72 hrs of exposure were 6.406, 2.278 and 2.493 respectively whereas those of the hexane extract were 6.219, 5.629 and 3.139 respectively. The results also showed that the LC50 values obtained by jatropha seeds ethanolic extracts after 48 and 72 hours were lower than their counterparts of the hexane extract which may be suggest that the active ingredient in jatropha seeds is easily extracted in ethanol rather than in hexane. The LC50 values of ethanol extracts of argel stems after 24, 48 and 72 hrs were 17.716, 8.336 and 6.860 respectively whereas those of the hexane extract were 26.191, 26.191 and 13.571 respectively. The mortality results generated by the 15% hexane and ethanol extract of both jatropha seeds were not significantly different from the standard, malathion, after 48 and 72 hrs of exposure which indicates the potency of these extracts. The results also showed that all tested concentrations of hexane and ethanol extracts of both jatropha seeds and argel stems resulted in a significantly lower fecundity of the treated insects than that of the control; however, there was no significant difference in fecundity among insects treated with different concentrations.

Keywords Tomato Leaf Minor, Tuta Sbsoluta, Botanical Extracts

1. Introduction

The tomato leaf miner Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a neotropical oligophagous pest of solanaceous crops (Lietti et. al. 2005). It is considered as one of the most important lepidopterous pests associated with tomato production in South America (Torres et al. 2001; EPPO 2005; Desneux et al. 2010). Recently, it has been detected in various parts of the Sudan where it greatly jeopardized tomato production. In fact, it is now considered as a national pest and a serious threat to tomato production in the Sudan. The seriousness of this pest stems from the nature of its damage where the larvae mine the leaves, flowers, shoots and fruits of tomato in addition to its rapid reproductive rate. Under favorable environmental conditions it can produce about 12 generations in one year and the female can potentially lay up to 300 eggs during its life span (Clarke, 1962). In addition to the direct damage to the tomato fruits, the larvae can penetrate both, leaves and stems forming galleries and mines which can potentially act as a good source for secondary infection by various plant pathogens.

Effective chemical control is difficult because this pest feeds internally; however, Salas (2004) reported that mass trapping as well as lure and kill applications of pheromones have been used successively in combination with bio-rational control tactics for management of Tuta absoluta. Chemical control has been the main measure used against T. absoluta since it was reported in South America, but unfortunately frequent and intense applications of synthetic insecticides lead to its development of insecticide resistance accompanied by environmental pollution. Reduced efficiency and control failure of some insecticides against T. absoluta have been reported in South America whereas resistance to abamectin and deltamethrin has been reported in Argentina (Lietti et al. 2005). Siqueira et al. (2000) has also reported that T. absoluta reduced susceptibility to

abamectin, cartap, methamidophos, and permethrin. Recently, biological control and natural insecticides are investigated in most countries where Tuta absoluta is present.

The objective of this study is to evaluate the effect of hexane and ethanol extracts of jatropha Jatropha curcas seeds, argel (Solenostemma argel (Del.) stem and malathion on mortality and fecundity of the tomato leaf miner Tuta absoluta.

2. Materials and Methods

2.1. Rearing Method

A considerable number of adults of tomato leaf miner were collected from unsprayed tomato fields in Shambat area (Sudan) and brought to the laboratory where they were reared in plastic cages 31×20×19 cm and covered with muslin clothes. Each plastic cage was supplied with tomato plants and a beaker filled with sugar solution and a piece of cotton soaked into it for adults feeding. The insects were left to multiply until a sufficient number was collected for conducting the bioassay.

2.2. Collection of Plant Material

The plant materials used in this study were collected from two different places. *Jatropha Curcas* seeds were obtained from the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) whereas argel (Solenostemma argel) stems were obtained from a retail store in Shambat area, Khartoum North, Sudan.

3. Preparation and Extraction of Test Products

3.1. Jatropha Seeds

Jatropha seeds were individually examined and carefully selected according to their condition. Damaged seeds were discarded whereas those in good condition were cleaned, shelled and subsequently the seed kernels and hulls were manually separated and shade-dried. After complete dryness, the seeds were crushed and ground into a very fine powder using a common domestic grinder.

3.2. Argel Plant (Hargel)

The argel stems were freed from foreign materials such as stones, sand and dust, before being brought to the laboratory for further investigation. The leaves were thoroughly cleaned, left to dry under shade for 5 days and then crushed into very fine powder using a common domestic grinder. The powder obtained was kept carefully and used later for extraction.

3.3 Extraction Method

The extraction processes were conducted at the Department of Pesticides Alternatives at the Environment and Natural Resource Research Institute (ENRRI), National Research Center (NRC). 600 grams of each of the previously prepared powder of jatropha seeds and argel stems were divided into three equal parts each of which consists of 200 grams. Each part was kept separately in a thimble which was placed in an extraction chamber of a soxhlet extractor apparatus, and then extracted with 500 ml of ethanol (99.7%) for each sample. The extraction continued for 6 hours and the ethanol solvent was removed off the crude extract by rotary evaporator as seen in plate (1). The obtained crude extracts of jatropha seeds and argel stems were weighed, carefully stored and eventually used in the preparation of the stock solution and the subsequent desired concentrations. The same procedure was followed for the extraction of jatropha seeds and argel stems using hexane as an organic solvent.

Plate (1) Soxhlet and Rotary Evaporator

3.4. Preparation of the Concentrations

According to volumetric law, 5%, 10%, 15% and 20% concentrations were prepared from each extract by dilution from the crude extract. The recommended dose of malathion 57% was used as standard in this study.

3.5. Bioassay Procedure

Twelve Petri dishes were lined with sterile filter papers. Then 2 mls of each concentration were added to each petri dish and rotated in such a way that an even distribution was achieved. The insects were placed in a refrigerator for 3-5 minutes to reduce their activity so that they can be easily handled and sexed using a magnifying glass. Ten insects (5 males and 5 females) were placed in each of the treated dishes for 4 hrs. Each treatment was replicated three times in a completely randomized block design. The treated insects were then moved into cages containing dampened soil and covered with fresh tomato leaves for eggs oviposition. Each cage was supplied with a small beaker filled with 10% sugar

solution and covered with a piece of dampened cotton for adult feeding. Mortality counts were taken and recorded after 24, 48 and 72 hours of exposure. Egg counts were taken as of the fourth day and the subsequent days.

3.6. Statistical Analysis

The data obtained was statistically analyzed according to analysis of variance (ANOVA) whereas Duncan's multiple range tests was used for means separation. EPA probit analysis program for calculating LC/EC values, version 1.5 software, was adopted to compute LC50 values for each plant extract used in this study.

4. Results and Discussion

4.1. Mortality

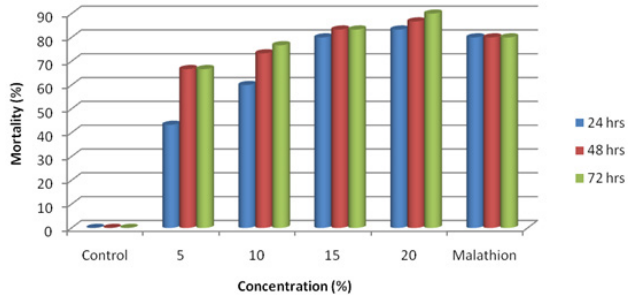

Figure 1. The Effect of Ethanol Extract of Jatropha Seeds on Mortality of Adults of Tomato Leaf Miner T. Absoluta.

Table 1. Effect of Ethanol Extract of Jatropha Seeds on Mortality of Adult of Tomato Leaf Miner Tuta. Absoluta

Conc. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
5	43.3 (6.6)c0)	66.7 (8.2)c	66.7 (8.2)b
10	60 (7.8)b	73.3 (8.6)bc0)0	76.7 (8.8)ab
15	80 (9.0)a	83.3 (9.2)ab	83.3 (9.2)a
20	83.3 (9.2)a	86.7(9.3)a	90.0 (9.5)a
Malathion	80 (9.0)a	80 (9.0)ab	80.0 (9.0)a
Control	0 (0.7)d	0 (0.7) a	0 (0.7)c
SE+	0.7	0.8	0.8
C. V. (%)	5.7	4.6	5.1

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

As seen in Table (1) and Fig. (1), all concentrations of ethanol extracts of jatropha seeds gave significantly higher mortality percentages than that of the control after 24 hrs, 48 and 72 hrs of exposure. Moreover, the two highest concentrations (15% and 20%) gave higher mortality percentages than malathion after 48 and 72hrs of exposure. Generally it has been noticed that as the concentration increases the mortality percentage increases. Even the second highest concentration (15%) gave an equal mortality percentage to that of malathion after 24 hrs of exposure which is an indication of its effectiveness.

As seen in Table (2) and Fig.(2), all concentrations of ethanol extracts of argel stem gave significantly higher mortality percentage than that of the control after 24, 48 and 72hrs of application. The results also show that there was no significant difference in mortality percentages among the different concentrations after 24hrs of application .The malathion 57% gave significantly higher mortality percentage than all tested concentrations after 24, 48 and 72 hrs of exposure.

Table 2. Effect of Ethanol Extract of Argel Stems on Mortality of Adults of Tomato Leaf Miner Tuta Absaluta

Concs. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
5	40(6.4)b	46.7(6.9)c	46.7(6.8)d
10	43.3(6.6)b	50(7.1)c	53.3(7.3)cd
15	46.7(6.9)b	53.3(7.3)bc	60(7.8)bc
20	53.3(7.3)b	60(7.8)b	63.3(8.0)b
Malathion	80(9.0)a	80(9.0)a	80(9.0)a
Control	0(0.7)c	0(0.7)d	0(0.7)e
SE+	0.6	0.7	0.7
C. V. (%)	8.6	5.0	5.4

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

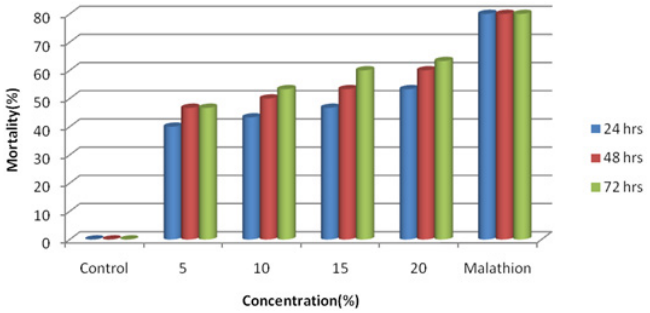

Figure 2. The Effect of Ethanol Extract of Argel Stem on Mortality of Adults of Tomato Leaf Miner T.Absoluta.

The mortality results exhibited in Table (3) and Fig.(3) showed that all tested concentrations of hexane extract of jatropha seeds gave significantly higher mortality percentages than the control after 24, 48 hrs of exposure. The results also show that the 20% concentration gave higher mortality percentage than malathion after 24, 48 and 72 hrs of exposure; moreover there was no significant difference in mortality percentage between the 15% concentration and malathion throughout the experiment which is an indicative of the effectiveness of the 15% concentration.

Table 3. Effect of Hexane Extract of Jatropha Seeds on Mortality of Adults of Tomato Leaf Miner Tuta Absaluta

Conc. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
5	50 (7.1)c	50(7.1)d	63.3(8.0)c
10	53.3 (7.3)c	63.38.0)c	70(8.4)bc
15	66.7(8.2)b	66.7(8.2)bc	76.7(8.8)abc
20	86.7(9.3)a	90(9.5)a	90 (9.5)a
Malathion	80(9.0)ab	80(9.0)ab	80(9.0)ab
Control	0(0.7)d	0 (0.7)e	0(0.7)d
SE+	0.7	0.7	0.7
C. V.(%)	7.0	6.5	5.7

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

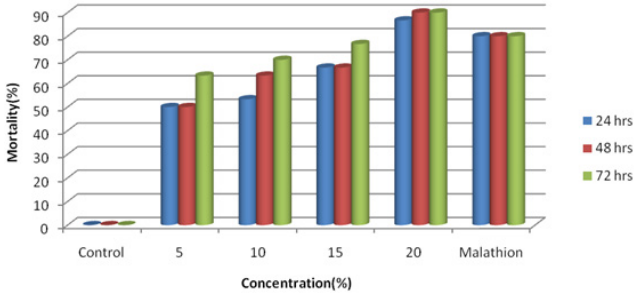

Figure 3. The Effect of Hexane Extract of Jatropha Seeds on Mortality of Adults of Tomato Leaf Miner T. Absoluta.

As seen in Table (4) and Fig. (4), all concentrations of the hexane extract of argel stem gave significantly higher mortality percentages than that of the control after 24, 48 and72hrs of exposure. It is interesting to note that even the highest concentration 20% generated less than 50% mortality after 24, 48 and 72hrs of exposure which is an indication of its poor performance as a chemical control agent.

Table 4. Effect of Hexane Extract of Argel Stems on Mortality of Adults of Tomato Leaf Miner Tuta Absaluta

Conc. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
5	30(5.5)c	30(5.5)c	33.3(5.8)d
10	36.7(6.1)bc	36.7(6.1)bc	36.7(6.1)d
15	43.3(6.6)b	43.3b(6.6)b	50(7.1)c
20	46.7(6.9)b	46.7(6.9)b	63.3(8.0)b
Malathion	80(9.0) a	80(9.0) a	80(9.0)a
Control	0(0.7)d	0(0.7) d	0(0.7)e
SE+	0.6	0.6	0.7
C. V. (%)	8.9	8.9	7.9

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

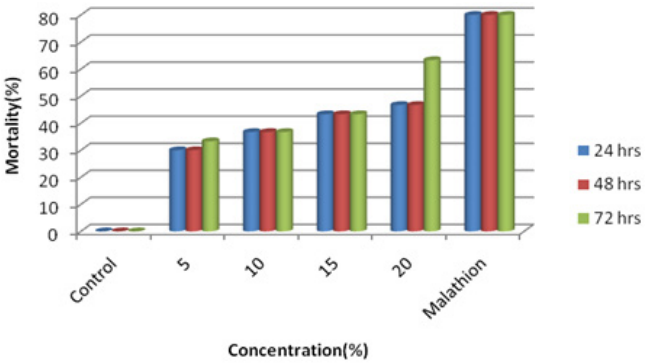

Figure 4. The Effect of Hexane Extract of Argel Stem on Mortality of Adults of Tomato Leaf Miner T. Absoluta

Table 5. LC50 Values of Tested Botanical Extracts Against Tomato Leaf Miner Adults T. Absoluta After 24 Hours Of Exposure

Botanical extract	Slope	Intercept	Chi-square	LC50(%)
Ethanolic extract 2.448 14.880 5.815				
Jatropha seeds	1.976	3.405	0.532	6.406
Argel stems	0.515	4.356	0.148	17.716
Hexane extract				
Jatropha seeds	1.976	3.405	0.532	6.406
Argel stems	0.515	4.356	0.148	17.716

Table 6. LC50 Values of Tested Botanical Extracts Against Tomato Leaf Miner Adults T. Absoluta After 48 Hours Of Exposure

Botanical extract	Slope	Intercept	Chi-squa re	LC50 (%)
Ethanolic extract 2.448 14.880 5.815				
Jatropha leaves	1.136	4.593	0.263	2.278
Neem seeds	0.512	4.528	0.155	8.336
Hexane extract				
Jatropha leaves	1.648	3.762	2.969	5.629
Neem seeds	0.746	3.941	0.021	26.191

Table 7. LC50 Values of Tested Botanical Extracts Against Tomato Leaf Miner Adults T. Absoluta After 72 Hours Of Exposure

Botanical extract	Slope	Intercept	Chi-square	LC50 (%)
Ethanolic extract 2.448 14.880 5.815				
Jatropha leaves	1.309	4.480	0.216	2.493
Neem seeds	0.714	4.402	0.029	6.860
Hexane extract				
Jatropha leaves	1.284	4.361	1.326	3.139
Neem seeds	1.235	3.600	1.144	13.571

4.2. Fecundity

As seen in table (8) insects treated with the different concentrations of hexane extracts of jatropha seeds gave significantly lower fecundity than that of the control on the 4th, 5th, 6th and 7th day indicating the great impact of the tested extracts on fecundity.

Table 8. Effect of Ethanol Extract of Jatropha Seeds on Fecundity of Adults of Tomato Leaf Miner Tuta Absaluta

Conc. (%)	Fecundity (No. of eggs) Exposure time (days.)			
	4th	5th	6th	7th
5	1.7b	0.7b	0.3b	0.0b
10	0.7b	0.3b	0.0b	0.0b
15	0.3b	0.3b	0.0b	0.0b
20	0.0b	0.0b	0.0b	0.0b
Malathion	0.0b	0.0b	0.0b	0.0b
Control	25.0a	22.0a	22.0a	21.7a
ES±	0.6	0.4	0.4	0.3
C. V. (%)	23.4	20.0	20.0	17.3

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

As seen in table (9) Insects treated with the different concentrations of hexane extracts of argel stem gave significantly lower fecundity than that of the control on the 5th, 6th and 7th day which also show a good performance of the tested extracts on fecundity.

Table 9. Effect of Ethanol Extract of Argel Stems on Fecundity of Adults of Tomato Leaf Miner Tuta Absaluta

Conc. (%)	Fecundity (No. of eggs) Exposure time (days.)				
	4th	5th	6th	7th	8th
5	2.0b	1.0b	0.7b	0.7b	0.0b
10	1.3bc	1.0b	0.7b	0.3b	0.0b
15	1.0bc	0.7b	0.3b	0.3b	0.0b
20	1.0bc	0.3b	0.3b	0.3b	0.0b
Malathion	0.0c	0.0b	0.0b	0.0b	0.0b
Control	25.0a	22.0a	22.0a	21.7a	20.0a
ES±	0.6	0.6	0.5	0.5	0.2
C. V. (%)	20.3	23.3	21.3	20.0	12.3

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

As seen in table (10) insects treated with the different concentrations of hexane extracts of jatropha seeds gave significantly lower fecundity than that of the control on the 4th, 5th, 6th, 7th and 8th day.

As seen in table (11) insects treated with the different concentrations of hexane extracts of argel stem gave significantly lower fecundity than that of the control on the 4th, 5th, 6th, 7th and 8th day

Table 10. Effect of Hexane Extract of Jatropha Seeds on Fecundity of Adults of Tomato Leaf Mainer Tuta Absaluta

Conc. (%)	Fecundity (No. of eggs) Exposure time (days.)				
	4th	5th	6th	7th	8th
5	1.3b	0.7b	0.7b	0.3b	0.0b
10	0.3b	0.7b	0.7b	0.3b	0.0b
15	0.7b	0.3b	0.0b	0.0b	0.0b
20	0.3b	0.0b	0.0b	0.0b	0.0b
Malathion	0.0b	0.0b	0.0b	0.0	0.0b
Control	25.0a	22.0a	22.0a	21.7a	20.0a
ES±	0.6	0.6	0.6	0.4	0.2
C. V.(%)	21.5	25.4	25.0	19.0	12.3

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

Table 11. Effect of Hexane Extract of Argel Stems on Fecundity of Adults of Tomato Leaf Miner Tuta Absaluta

Conc. (%)	Fecundity (No. of eggs) Exposure time (days.)				
	4th	5th	6th	7th	8th
5	1.7b	1.7b	1.0b	0.7b	0.0b
10	1.3bc	1.3bc	0.7b	0.7b	0.0b
15	1.0bc	0.7bc	0.7b	0.3b	0.0b
20	0.7bc	0.3bc	0.3b	0.0b	0.0b
Malathion	0.0c	0.0c	0.0b	0.0b	0.0b
Control	25.0a	22.0a	22.0a	21.7a	20.0a
ES±	0.5	0.5	0.5	0.4	0.2
C. V. (%)	18.5	19.6	22.0	19.2	12.3

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

The mortality generated by the two highest concentrations of jatropha seeds ethanolic used in this study (15% and 20%) induced high mortality percentages of 83.3.% and 90.0% respectively, with no significant differences compared to malathion after 72 hours of exposure. Working with the fruit fly Bactrocera invades, Ahmed (2012) reported that the hexane extracts of Jatropha seeds gave significantly higher mortality percentage of than the ethanol extracts. The highest concentrations of argel stem ethanolic and hexane extracts used in this study (20%) induced the same mortality percentage (63.3%) after 72 hours of exposure. Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos et al., 1980; Hamed, 2001). Sulieman et al. (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties.

All concentrations of jatropha Jatropha curcas seeds, and argel Solenostemma argel Del. stems ethanolic and hexane

extracts induced significantly lower fecundity than the control after 24, 48, and 72 hours of exposure.

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