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Bio-rationale Management of Tomato Leaf Miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) Using Extracts of Neem (*Azadirachta indica A. Juss*) Jimsonweed (*Datura stramonium* L) and Periwinkle (*Vinca rosea* L.)

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Recently, tomato leaf miner (TLM) Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae), is the most notorious pest of tomato worldwide causing severe reduction of yields in both protected and open fields. In Sudan, the pest was recorded to attack all tomato cultivation areas causing considerable damage affecting income of poor farmers. Various pesticides of different active ingredients are applied excessively and routinely to control T. absoluta in tomato. As known, reliance on insecticide cause adverse impacts in human health, create insect resistance as well as affect environment and natural enemies. This study is initiated to evaluate the efficacy of extracts of 3 botanicals as alternative to synthetic insecticides against T. absoluta. A randomized Complete Block Design field experiment was conducted at Takroof experimental farm of Kassala and Gash Research Station, Sudan during season (2019/2020). The experiment consisted of Neem seed oil, water extracts of Datura, Water extract of Vinca, Dancid®; synthetic insecticide as standard, and non-treated treatment as control. Each treatment was replicated thrice Data included number of infested plants/plot, number of mines/plot, percentage of infested fruits and yield (Ton/ha) were compiled, variance among treatments (ANOVA) was statistically analyzed and means were separated using Duncan Multiple Range Test (DMRT) applying SAS 9 a computer based program. The results of this study, revealed high significant difference between treatments in the number of infested plants/plot, number of active mines/plots for the three assessment times of the post spray counts. Water extract of Datura and Neem seed oil were better than Dancid® and Vinca in reducing the number of infested plants/plots and number of active mines/plots. All test products were significantly same in reduction the percentage of infestation of fruits and they were less than the control. Also high significant difference was observed between treatments on yield. Plots treated with Neem seed oil obtained the highest yield followed by WE of Datura, Dancid®, WE of Vinca with 11.2, 9.9, 9.7, 8.8 (Tons/ha) while the un-treated plots obtained the lowest yield (3.8 Tons/ ha). For their potency, cheapness, easy processing and environmental friendly characters, Neem seed oil, WE of Datura, and WE of Vinca are recommended to be used to control T. absoluta in tomato crop.

Keywords: *Tuta absoluta neem seed oil, Datura, periwinkle, aqueous extracts*

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Introduction

Tomato (*Lycopersicon esculentum*, mill) is one of the most leading vegetable crops worldwide. Tomato ranks second to onion among the most important vegetable grown in Sudan. The main production areas of tomato in Sudan are Gezira Khartoum, Kassala and Northern States (Elassi 2001). Tomato crop has been reported to be attacked by various serious insect pests and disease, that have the potential to drastically reduce its yield such as yellow leaf curl virus disease which is transmitted by whitefly, *Bemisia tabaci.* Leaf miners, *Liriomyza spp*, the African boll worm (ABW) *Helcoverpa armigera* are among the most important insect pests of tomato since long time. Worldwide tomato crop witnessed a highly voracious invasive pest; Tomato fruit borer (*Tuta absoluta),* that can completely rescind the crop. Tomato is the main host plant of *T. absoluta* but the pest also attacks other crop plants belonging to the nightshade family, including potato, eggplant pepper and tobacco. It is known from many solanaceous weeds, including *Datura stramonium Lycium chilense* and *Solanum nigrum (*Biondi et al. 2018, Desneux 2010, Mohammed et al. 2015, Mansour et al. 2018). Tomato fruit borer is originated to South America and due to the importation of tomato to Europe. It invaded Europe, Middle East, North Africa and spread all offer the African content within very short period. In Sudan *T. absoluta* was officially recorded as injurious pest of tomato in 2011 but its impact on tomato is very devastating when compared to other insect pests. The feeding of larvae causes significant damage on leaves, stems, buds and fruits leading to yield losses up to 100% if the pest is not controlled (Desneux et al. 2010). Recently, this pest is one of the main reasons that decline tomato yield in Sudan. Outbreak of Tomato fruit borer was observed in many states Sudan causing a damage up to 80% (Mohammed and Khalid 2011). Management of insect pests is crucial to ensure good crop productivity. Experiments have revealed some promising agents of biological pest control for this moth, including *Nabis pseudoferus*, a species of damsel bug, *Bacillus thuringiensis* and *Beauveria bassiana* (Molla et al. 2011, Guedes et al. 2019). The entomo-pathogenic nematodes *Steinernema feltiae*, *Steinernema carpocapsae*, and *Heterorhabditis bacteriophora* were found very effective for the control of *T. absoluta* (Husin and Port 2021). Buragohain et al. (2021) reported that the use of commercial formulations of *Bacillus thuringiensis* reduced the infestation of leaves and fruits by *T. absoluta*. The sex pheromone of *T. absoluta* has been found to be highly attractive to male moths (Mohammed et al. 2013, Mahmoud et al. 2020). Application of insecticides is the main method used to control *T. absoluta* in Asia and sub-Saharan Africa, including Sudan, due to absence of other management techniques. As reported by (Lietti et al. 2005) resistance of *T. absoluta* to insecticides continued to build further year after year. In Turkey, the population of *T. absoluta* was found to be highly resistant to organophosphate, pyrethroid, indoxacarb, spinosad, chlorantranilprole and metaflumizone (Yalçin et al. 2015).

Studies on botanical pesticides have been conducted for many years and proofed high performance on controlling insect pests as well as reducing the negative impacts of synthetic insecticides (Adeyemi 2010). Fortuitously, various plants contain compounds of insecticidal properties avail their use to manage various insect pests (Adeyemi 2010, Shrivastava and Singh 2014).

The use of botanicals; especially Neem *Azadirachta indica* which consists of Azadirachtin, a complex of tetranortri-terpenoid, indicated their efficacy as anti-feedant, toxic and repellent to *T. absoluta* without any incidence of resistance (Kona et al. 2014, Yalçin et al. 2015). In Sudan, many insecticides and three types of pheromones were recommended by the National Committee of Pests and Diseases to be used to control *T. absoluta* (Mohammed et al. 2013, Mahmoud et al. 2020). Intercropping of tomato plant with coriander was reported to reduce infestation of tomato by *T. absoluta* (Mahmoud et al. 2020). Laboratory experiments proofed that, the use of Neem seeds ethanoic extract and Jatropha (*Jatropha curcus*) seeds petroleum ether extract caused significant mortality to eggs and larvae of *T. absoluta* after 4 days (Kona et al. 2014). The insecticidal activities of aqueous extracts of five local plants (Neem *Azadirachta indica,* Garlic *Allium sativum*, Argel *Solenostemma sp.*, Coriander *Coriandrum sativum* and Khella *Ammi visnaga* (L.) plants reduced significantly the number of infested leaflets, the number of mines and the number of fruits infested by *T. absoluta* under greenhouse condition (Mahmoud et al. 2020). Due to development of pests' resistance, hazards to human health, adverse effects on environment and natural enemies, looking for safer alternative to insecticides is highly required. Sudan is a large country with diverse ecosystem contained big numbers of wild plants of medicinal values. Neem trees are widely grown for shade and as building materials which avail utilization of its products in medicine, cosmetics as well as in agriculture with free or low cost to poor communities. This study is a part of a program commenced to integrated management program to determine the impact of Neem seed oil, water extract of Datura and water extract of Vinca on *T. absoluta* as well as the effect of their use on yield of tomato compared to synthetic insecticide and untreated treatments.

Materials and Methods

The experiment was conducted at the experimental farm of Kassala and Gash Research Station, (Takroof area), Kassala state, Sudan in the winter season of (2019/ 2020). The tomato accession HSD 10655 provided by Plant Genetic Resource Center of the Agricultural Research Corporation was sown in the nursery of the station in October prior to transplanted to the field in November. The land was ploughed, harrowed, leveled and allocated to plots measured ($3x4m^2$) with two beds (Mastaba, $3x1.4m^2$). Plants were sown on both sides of the bed an intra row spacing was 0.6m and inter row spacing was 0.3m between plant holes. All cultural practices were followed as ARC standards.

Collection of Plant Materials and Preparation of Extracts

Seeds of Neem *Azadirchta indica A. Juss* and leaves of periwinkle, *Vinca rosea L.*, and Datura, *Datura stramoniumn, L* were collected from Kassala Research Farm. Leaves of Vinca and Datura were dried under shade to prevent denaturation of active chemical compounds. Ten grams of powder leaves were

soaked in 1 liters of water and then boiled for 20 minutes and left for 24 hours, then filtered with muslin cloth and kept as a stock solution for later use.

For extraction of Neem seed oil, Neem seed were soaked in water for 12 hours and were manually decorticated then left for 24 hours to dry under shaded area. Decorticated Neem seeds were pressed in local-made expeller to produce oil.

Experimental Design and Field Observations on Infestation Levels, Active Mines on Leaves and Percentage of Damaged Fruits by T. absoluta

A randomized complete block design (RCBD) was used to lay out the experiment which consisted of five treatments. Treatments were included Neem seed oil (0.72 L/ha), water extract of Vinca (0.96 L/ha) water extract of Datura (0.96 L/ha), Dancid15% EC (0.48 L/ha); standard chemical insecticide, and untreated as control. Each treatment was replicated thrice. All test materials were applied before sunset 3 times during the cropping season using pneumatic knapsack sprayer. Pre and post spray counts were conducted and data representing infestation parameters caused by *T. absoluta* were compiled during vegetative and harvesting periods include:

Number of Tomato Infested Plants/Plot

From each plot 20 plants were randomly selected and inspected for the presence of symptoms of *T. absoluta.*

Number of Active Mines of T. absoluta on Leaves/Plant/Plot

Number of active mines that contain live larvae of *T. absoluta* were determined by inspection of 5 leaves from 5 plants randomly selected from each plot.

Percentage (%) of Fruits Damaged by T. absoluta

On harvesting time, fruits were harvested and separated to damaged and healthy, the percentage of damaged fruits was calculated according to the following formula:

$$\%\ \text{of infested fruits} = \frac{\text{No of infested fruis}}{\text{Total number of fruits}}\ x\ 100\%$$

The three above mentioned parameters were assessed pre spray of test materials and also were assessed after 48h, 72h and week post spray.

Yield Assessment

Yield of tomato of different treatment (Ton/Ha) was calculated.

Statistical Analysis

Compiled data were analyzed using (SAS) statistical computer based package software version 9 and means were separated using Duncan's Multiple Range Test (DMRT).

Results

Effect of Test Products on the Number of Infested Plants/Plot

1st Spray

Number of tomato plants infested by *T. absoluta* was significantly different between treatments after 48 hours, all test products were better than the control. Same results of reduction on number of infested plants was recorded for Neem seed oil, Water extract of Datura as well as Dancid® the chemical insecticides followed by water extract of Vinca. High significant difference was observed after 72 hours post spray in spite of increase of number of infested plants but the result was same for the four treatments including water extract of Vinca with average of 34.5 infested plants while the control reached 57.5 infested plants. After 7 days' number of infested plants was increased significantly compared to 48h and 72h. Untreated plots obtained highest number of infested plants (67.2plants/plot) while Plots treated with water extract of Datura generally were less infested followed by same effects on plots treated with Neem seed oil and Dancid® (the insecticide).

2nd Spray

As demonstrated in Table 1. High significant difference was observed between the number of infested plants/plot after 48h and 72h of the second spray. All test products reduced the number of infested plants/plot less than the control plots. Neem seed oil and WE of Datura were better fluctuated between 18.4 and 22.5 infested plants/plot in reducing the number of infested plants/plot than WE of Vinca and Dancid® (the insecticide). As in the first spray the number of infested plants was slightly increased in treated plots after 7 days (26-33plants/plot) when compared to very high number (75.4) of infested plants/plot recorded for the control. High significant difference was observed between the treatments. Effect of WE of Datura in infested plants/plot was comparable to that of Danicd followed by Neem seed oil and WE of Vinca.

3rd Spray

All test products reduced the number of infested plants/plot with significant differences between treatments for 48h, 72h and 7days post spray count. Water extract of Datura was the best product that reduced the number of infested plants/ plot followed by Neem seed oil and then Dancid® and Vinca. After 7 days of the third spray, all test products were same in their effects and significantly reduced the number of infested plants/plot to (18 to 22) which is better than the control (82.1) infested plants/plot.

Effect of Different Test Products on Active Mine of T. absoluta

1st Spray

In the first count significant difference was observed between treatments for pre spray count as well as for 48h, 72h and 7 days post spray. After 48 h of spray, WE of Datura gave better reduction in active mines (16.7) caused by larvae of *T. absoluta* followed by Dancid®, Neem seed oil and WE of Vinca with 19,21, 24.7 mines while the control recorded (28.3) mines (Table 2). After 72 h Neem seed oil, Dancid® and WE of Datura showed similar effect on the active mines which were less than the control. The number of mines 7 days' post spray was significantly same for all test products which were less than the control.

2nd Spray

In the second spray it was noticed that the number of mines decreased than its situation after 7 days in the first count. Significant differences were observed between treatments on the number of mines for 48h, 72h and 7days post spray. In all post spray counts, Plots treated with WE extract of Datura gave the lowest number of mines (mean=12) followed by Dancid® (mean=12), Neem seed oil (mean=15) and Vinca (mean=17) while the control recorded (mean=36) mines.

3rd Spray

High significant difference was observed between treatments on the number of mines for 48h, 72h and 7days of the 3rd post spray counts (Pr ≥F 0.0001). The number of mines decreased gradually and successively according to time elapse for all test products while vice versa was reported for the control. Table 2 demonstrated same reduction effect of number of mines due to use of WE of Datura, Dancid® and Neem seed oil followed by WE of Vinca for the three post spray counts. Effect of test products on the percentage of tomato fruits infested by *T. absoluta.* Results displayed in Table 3 showed that there was significant difference between treatments on the percentage of fruit damage. Statistically equal reduction to the percentage of damaged fruits was recorded for WE of Vinca, Neem seed oil, Dancid®, WE of Datura ranged between 26.8 and 31.0% while the control recoded the highest fruit damage percentage (49.4%) according to the infestation by *T. absoluta.*

Table 1. *Effect of Water Extracts of Wenca, Water Extract Datura, Neem Seed Oil and Dancid® on Mean Number of Plants Infested by T. absoluta/ Plot on Tomato Plants*

Treatment	Pre-spray	1st Post-spray			2nd Post-spray			3rd Post-spray		
		48hrs	72hrs	7days	48hrs	72hrs	7days	48hrs	72hrs	7days
Dancid®	40.9	32.7b	36.4b	38.2cb	29.0b	30.0b	26.0c	22.1cb	20.6b	18.4b
WE of Vinca	44.3	40.2 ba	37.1b	43.9b	31.9b	26.5cb	33.4b	25.8b	20.5b	22.0b
WE of Datura	40.5	29.5b	31.9b	36.03c	18.4c	20.8c	26.4c	14.4d	14.4c	19.2b
Neem seed oil	41.7	32.9b	33.5b	41.3cb	21.8c	22.5c	28.8cb	17.1cb	17.3cb	19.5b
Control	44.0	49.2a	57.5a	67.2a	53.7a	67.9a	75.4a	59.0a	71.0a	82.1a
C.V	11.0	16.2	7.6	7.0	11.8	10.6	7.3	14.0	10.5	9.0
SE±	1.12	2.23	2.66	3.1	3.4	4.7	5.1	4.4	5.8	6.7
Pr ≥F	0.6603	0.0517	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. *Effect of Water Extracts of Wenca, Water Extract Datura, Neem Seed Oil and Dancid® on Mean Number of Active Mines of T. absoluta/ Plot on Tomato Plants*

Treatment	Pre-spray	1st Post-spray			2nd Post-spray			3rd Post-spray		
		48hrs	72hrs	7days	48hrs	72hrs	7days	48hrs	72hrs	7days
Dancid®	24.0b	19dc	17.7c	19.0b	13.7dc	12.7c	12.7c	8.7c	8.0c	7.7c
WE of Vinca	28.8a	24.7b	25.3b	22.3b	21.3b	19.7b	17.7b	16.3b	13.7b	11.7b
WE of Datura	23.7b	16.7d	18.7c	20.3b	11.7d	12.7c	13.3cb	8.0c	8.0c	6.7c
Neem seed oil	28.2a	21.0c	20.3c	21.3b	16.0c	15.3cb	16.0cb	10.3c	9.7c	7.3c
Control	27.1a	28.3a	32.3a	36.2a	31.3a	36.3a	39.3a	34.3a	38.7a	45.3a
C.V	5.4	8.3	7.3	9.1	10.3	13.3	11.6	7.8	13.5	11.1
SE±	0.6	1.2	1.5	1.8	1.9	2.4	2.7	2.6	3.2	4.0
Pr ≥F	0.0123	0.0010	<0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Note: WE= Water extract. Data with same letter are significantly equal.

Table 3. *Effect of Water Extract of Vinca, Water Extract of Datura, Neem Seed Oil and Dancid® on (%) of Fruits Damaged by T. absoluta*

Treatment	Fruits Damage (%)
Dancid®	28.03b
WE Vinca	26.8b
WE Datura	31.0b
Neem seed oil	27.1b
Control	49.4a
C.V	27.2
SE±	3.0
Pr≥F	0.0595

Note: WE= Water extract. Data with same letter are significantly equal.

Effect of Different Test Products Used to Control T. absoluta on Yield of Tomato

The obtained results of yield of tomato (Tons/hectare) due to the use of some products to control *T. absoluta* revealed high significant difference (Pr≥F 0<0.0001) between all test products and control. All test product improved yield 2 to 3 times better than the control. Neem seed oil obtained the highest yield (11.2 Ton/ha) among all test products followed by WE of Datura (9.9), Danicd® (9.7), WE of Vinca (8.8 Tons/ha) while the least yield (3.8 Tons/ha) was recorded to the control (Table 4).

Table 4. *Effect of Application of Water Extracts of Vinca, Water Extract of Datura, Neem Seed Oil and Dancid® to Control T. absoluta on Tomato Yield (Tons/ha)*

Treatment	Yield (Tons/hectare)
Dancid®	9.7b
WE Vinca	8.8c
WE Datura	9.9b
Neem seed oil	11.2a
Control	3.8d
C.V	4.2
SE±	0.6
Pr≥F	0<0.0001

Note: WE= Water extract. Data with same letter are significantly equal.

Discussion

In the last seven decades many botanical formulations have proven to be potent and effective as many as conventional synthetic pesticides even at low concentrations. In fact, botanical insecticides have drawn great attention as major control agents in organic farming. However, their extensive uses have resulted in certain drawbacks and hazards, including, persistence, toxicity to non- target organism, pest resistance and environmental pollution (Siqueira et al. 2000, Lietti et al. 2005). Many studies have been focused on the use of botanical extracts includes oil, powder, ethanoic or aqueous extract for their effectiveness, cheapness, short persistence and low mammalian toxicity. Stoll (2000) and Hiiesaar et al. (2001) reported that many of plant materials show abroad spectrum of activities

against insect pests include lethal, anti-feedant, repellent and growth regulatory effects and Azadirachtin is well known effective botanical insecticide extracted from Neem plant (Mordue and Alasdair 2000). The tomato leaf miner, *T. absoluta*, anew pest in Sudan, has caused excessive damage to tomato in all parts of country since 2010 (Mahmoud et al. 2020).

In this study, the aqueous extracts of Datura and Vinca and Neem seed oil were applied to tomato plots infested by *T. absoluta* to determine their effect on number of infested plants/plot, active mines/plot and fruit damage/plot as well as their effect on yield of the crop. The results revealed significant difference among the test plant extracts compared to standard and control for three consecutive sprays for the above mentioned parameters. The results of using neem oil are in agree with findings of Coelho and Deschamps (2014) who stated the insecticidal and anti-feedant effect of neem on caterpillars of *T. absoluta*. Also the results of using neem oil is in accordance with findings of Illakwahhi and Srivastava (2019) who reported the potency of use of neem oil as insecticide and as synergist to increase activity of abamectin to control *T. absoluta* and reduce its resistance to abamectin. Study conducted by Tindade et al. (2000), reported that 84-100% control was achieved using different concentrations of Neem seed extract against young larvae of *T. absoluta*. On other hand, results of this study proved that plots treated with neem seed oil gained the highest yield, the same results of increasing yield due to use of neem seed oil was mentioned by Abbasi et al. (2003). Plots that's were treated by WE of Datura obtained similar results to Dancid® (the insecticide) in reducing the infestation of *T. absoluta* which is in accordance with findings of Buragohain et al. (2021) who stated similar reduction of the infestation of *T. absoluta* between neem extract and the standard insecticides without affecting the yield. In the current study, the effect of WE of Datura on reducing the number of infested plant/plot, number of active mines and percentage of infested fruits as well as increase yield was recorded which give the product the opportunity to be used as alternative to pesticides to control *T. absoluta* and increase yield of tomato crop (Habib et al. 2011). Abbasipour et al. (2011), reported that the seed and leaves extracts of *D. stramonium* are effective to control *Tribolium castaneum* while (Moreira et al. 2004) reported that, hexane and alcoholic extracts of *D. stramonium* had no insecticidal activity against larvae of *Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae).

In spite of its effectiveness in controlling *T. absoluta*, Datura contains atropine, hyoscyamine, and scopolamine, which can produce poisoning with a severe anticholinergic syndrome which cause hallucinogenic and euphoric effects to human (Trancă et al. 2017). For the mentioned reasons further studies on residual effects of Datura is highly required. WE of Vinca is comparable to the insecticide in many post spray counts are comparable to other test products and much better than the control in the number of infested plant/plots and number of active mines and same as other test products on percentage of infested fruits. This results of Vinca is in agree with results of mortality and repellency caused by its powder and aqueous extracts against Faba bean beetle, *Bruchidius* incarnates (Boh.) under laboratory condition (Mohammed 2004).

Conclusions and Recommendations

Use of Neem seed oil, WE of Datura and WE of Vinca as alternative to insecticides is effective to control *T. absoluta*. The extract of the three products reduced the number of infested plants/plot, number of active mines/plot as well as reduced the percentage of infested fruits and increased tomato yield.

Based on the above mentioned results, authors would like to recommend to the farmers use of Neem seed oil at (0.72 L/ha and use of WE of Datura and WE of Vinca at (0.96 L/ha) and for management of *T. absoluta*. Further studies for residual effect of WE of Datura is necessary and also preparation of formulations of the botanical extracts is required to encourage their use by farmers

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