

Bioactivity of plant extracts against tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

A. Ndereyimana^{1*}, S. Nyalala¹, P. Murerwa¹ and S. Gaidashova²

¹Department of Crops, Horticulture and Soils, Egerton University. P.O. Box: 536-20115, Egerton, Njoro, Kenya.

²Department of Agriculture Research and Technology Transfer, Rwanda Agriculture and Animal Resources Development Board (RAB), P.O. Box: 5016 Kigali, Rwanda. *E-mail: assinapol@gmail.com

Abstract

Tomato (*Solanum lycopersicum* L.) is economically and nutritionally important in Rwanda, but its production is challenged by the tomato leaf miner (*Tuta absoluta* Meyrick), an invasive pest. Synthetic insecticides which are primarily used for its control, have various drawbacks. Bioactivity of *Tephrosia vogelii*, *Tithonia diversifolia*, *Vernonia amygdalina* and *Phytolacca dodecandra* aqueous extracts was evaluated against *T. absoluta* in laboratory. Leaflets with third instar larvae (3.85 - 5.65 mm) of *T. absoluta* in mines were collected from established tomato field. Aqueous plants extracts were evaluated at a dose of 10 % weight/volume. Sterile tap water and azadirachtin 0.03 % EC were used as negative and positive controls, respectively. Petri-dishes of 9 cm diameter (n=10) were used as bioassay arenas in a completely randomized design with four replications. Data on larval mortality were collected every 24 h for 5 days. Three bioassays were conducted on different dates. Results indicated that tested plant extracts exhibited a capacity to kill *T. absoluta* larvae in tomato leaf galleries with significant difference among them ($P < 0.0001$). The killing capacity increased with exposure time. At 24 h of exposure, *T. absoluta* larvae mortality was in a range of 35.0 - 37.5 % for azadirachtin and 5.0 - 10.0 % for *T. vogelii* while all other aqueous extracts had 0.0 % mortality, except *V. amygdalina* which recorded 2.5 % in bioassay one. In all bioassays, the lowest mortality recorded 5 days after treatments with *T. vogelii*, *T. diversifolia*, *V. amygdalina*, *P. dodecandra* and azadirachtin was 32.2, 2.8, 2.5, 20.5 and 97.5 % while the highest mortality at this time was 35.1, 10.6, 13.3, 24.9 and 100 %, respectively. *Tephrosia vogelii* and *P. dodecandra*, which recorded higher efficacy compared to the other local plants, should be advanced to field evaluation. The observed higher efficacy of azadirachtin to Rwandan population of *T. absoluta* should also be confirmed under field conditions.

Key words: Biopesticides, botanicals, insecticidal plants, *Phytolacca dodecandra*, *Solanum lycopersicum* L., *Tephrosia vogelii*, *Tithonia diversifolia*, *Vernonia amygdalina*

Introduction

Insect pests are one of the important causes of crop production losses all over the world (Silva *et al.*, 2011; Biondi *et al.*, 2018). In particular, tomato leaf miner (*Tuta absoluta*), an invasive pest reported in Rwanda in 2015 (Uzayisenga *et al.*, 2016) causes serious damage to tomato crop resulting in severe yield losses up to 100 % (Desneux *et al.*, 2010). The pest is widespread in all tomato production areas of Rwanda (Uzayisenga *et al.*, 2016). Thus, it is vital to develop effective management strategies against this challenging pest.

Management of insect pests is crucial to ensure good crop productivity. Use of synthetic insecticides, the main method of insect control all over the world (Senthil-Nathan, 2013), often results in pollution of ecosystems, apparition of resistant pest genotypes and new pests and destruction of natural enemies among others (Macharia *et al.*, 2009; Yalçin *et al.*, 2015). Fortunately, there are various plants that possess different chemicals recognised as secondary metabolites which have insecticidal properties and hence have the potential of being used to manage various insect pests (Adeyemi, 2010; Shrivastava and Singh, 2014).

Research on botanical insecticides have been carried out for many years with the main goal to minimize the harmful effects of

synthetic insecticides (Adeyemi, 2010). Azadirachtin is one of the widely known and successful examples of botanical insecticide discovery from plants (Mordue and Alasdair, 2000). It is effective against several pests and comparatively harmless to natural enemies than most of the commonly used synthetic insecticides (Gontijo *et al.*, 2015). El-ghany *et al.* (2016) obtained up to 92 % *T. absoluta* larval mortality caused by azadirachtin. Tomé *et al.* (2013) also observed that azadirachtin is effective against *T. absoluta*. Although high efficacy was obtained with insecticides of plant origin like azadirachtin (Yalçin *et al.*, 2015), they are relatively expensive. Therefore, evaluation and exploitation of extracts of locally available plants against *T. absoluta* is necessary because they are cheap, easy to prepare and contain multiple active components which impede the development of insect resistance (Braham *et al.*, 2012).

Over two thousand plant species are reported to have insecticidal properties (Shivakumar *et al.*, 2013) and studies have shown higher bioactivity of extracts from some plants such as *Acmellaoleracea* (Asteraceae) and *Thymus vulgaris* (Lamiaceae) against *T. absoluta* larvae (Moreno *et al.*, 2012; Nilahyane *et al.*, 2012). Screening different plants to assess their potential against insect pests, including *T. absoluta*, would contribute to sustainable pest management while preserving environment.

Locally available plant materials, such as *Tephrosia vogelii* (Leguminosae), *Tithonia diversifolia* (Asteraceae), *Vernonia amygdalina* (Asteraceae) and *Phytollacca dodecandra* (Phytollaccaceae), are known to exhibit the features required for an ideal botanical insecticide (Adeniyi *et al.*, 2010; Olaitan *et al.*, 2011; Mkenda *et al.*, 2015; Raja *et al.*, 2015) but their potential was not evaluated against *T. absoluta*. Crude extracts of the above-mentioned plants have shown the efficacy against various pests of different crops (Olaitan and Abiodun, 2011; Onunkun, 2012; Mkenda *et al.*, 2015; Raja *et al.*, 2015). Further exploration is needed to broaden their use in IPM of various crops in Rwanda. Furthermore, there is scarce information on their efficacy against *T. absoluta*. Finding the indigenous plant species with insecticidal properties along with a simple preparation technology would benefit more local farmers. The main aim of this study was to determine the bioactivity of four aqueous extracts from locally available plants (*T. vogelii*, *T. diversifolia*, *V. amygdalina* and *P. dodecandra*) against *T. absoluta* in the framework of finding options for IPM of this pest in Rwanda.

Materials and methods

Collection of plant materials and preparation of extracts:

Leaves of *T. diversifolia*, *T. vogelii*, *V. amygdalina* and *P. dodecandra* were collected from various regions in Rwanda where they grow naturally. They were washed to remove sand, dust and chemical contaminants, then dried under shade (to prevent denaturation of active chemicals) for two weeks and subsequently ground, using an electric grinder, into fine powder which was packaged in biodegradable plastic bags. In a litre of boiled water, 100 g of powder for each plant species were added separately. The powder was left in boiled water for 12 hours and then filtered with muslin cloth. The respective extracts were made to a volume of one litre using cold water to give 10 % weight/volume (w/v). This solution was ready for use with no further dilution. Previous work had shown that 10 % w/v of *T. vogelii* water extract was highly effective to certain insects (Adebayo *et al.*, 2007).

Collection of *T. absoluta* larvae: Tomato leaflets containing *T. absoluta* larvae in galleries were collected from a tomato field which was established in an area of high occurrence of *T. absoluta* in Rwanda. This field was located in Rweru Sector of Bugesera District, Eastern Province, on latitude 02°31'974"S, longitude 030°26'853"E and at an altitude of 1342 m above sea level. Cloth bags were used to transport the collected leaflets from the field to the laboratory where they were used in bioassays for a maximum of a half-day (12 h) after their collection.

Laboratory bioassays: Three bioassays were carried out on different dates in the Biological Control Laboratory at Rwanda Agriculture and Animal Resource Development Board (Yan *et al.*, 2010). Each tomato leaflet, with one-third instar larvae (3.85 - 5.65 mm) of *T. absoluta* in galleries, was dipped for 3 seconds (Cherif *et al.*, 2018) in 10 % w/v solution of respective plant extract and then positioned in a Petri-dish lined with three moistened filter paper discs. Each experimental unit was composed of ten Petri-dishes of 9 cm diameter which received the same treatment. Apart from the four plant extracts, azadirachtin 0.03 % EC (5 mL L⁻¹) and water were used as positive and negative controls, respectively. The Petri-dishes were sealed with parafilm and kept at a temperature of 25 ± 2 °C. Each bioassay was conducted in a completely randomised design with four replications.

Data collection and analysis: Mortality of *T. absoluta* larvae was recorded at 24, 48, 72, 96 and 120 h after treatment application. Dead larvae outside the gallery were recognised by their inability to move back to ventral position after being positioned on their dorsum. Larvae still inside the leaf galleries were recorded as dead when unable to respond to microscopic light or gentle touch by fine camel's hairbrush on posterior body segment. The number of dead larvae per treatment was represented as percentage mortality by considering the total number of larvae per treatment. Mortality observed in water treatment (negative control) was used to correct mortality in plant extracts and positive control (azadirachtin) treatments using Schneider-Orelli's formula (Püntener, 1981).

Collected data were checked for normality before analysis, using proc-univariate procedures, and were found to be not normally distributed. Arcsine square root transformation was used for data transformation before analysis. Analysis of variance was carried out through PROC GLM procedure. Means of the significantly different treatments ($P < 0.05$) were separated using Tukey's honestly significant difference (HSD) test. The level of significance was fixed at $\alpha = 0.05$. All these procedures were carried out using Statistical Analysis System package (SAS) software version 9.2 (SAS Institute, 2010).

Results

Results on mortality (%) of *T. absoluta* larvae inflicted by 10 % (w/v) aqueous plant extracts and azadirachtin 0.03 % EC (positive control) are presented in Table 1. The tested plant extracts exhibited a capacity to kill *T. absoluta* larvae inside the leaf galleries. In all bioassays, statistical analysis revealed strong evidence ($P < 0.0001$) of significant difference in the bioactivity of evaluated plant extracts against *T. absoluta* larvae. Effect of the studied plant extracts on *T. absoluta* larvae increased progressively with the duration of exposure from 24 to 120 h after treatment application.

Apart from *T. vogelii* which recorded the mortality range of 5.0-10.0 % at 24 h after treatment application; all other aqueous extracts recorded 0.0 % mortality at this time, except *V. amygdalina* which had 2.5 % in bioassay one. At this time, mortality due to azadirachtin ranged between 35.0 and 37.5 % in all bioassays. The lowest mortality recorded at 120 h of exposure to treatments in all bioassays were 32.2, 2.8, 2.5, 20.5 and 94.5 % while the highest mortality at this time were 35.1, 10.6, 13.3, 24.9 and 100 % for *T. vogelii*, *T. diversifolia*, *V. amygdalina*, *P. dodecandra* and azadirachtin, respectively (Table 1).

Azadirachtin, which served as positive control, resulted in higher larval mortality ($P < 0.0001$) compared to all tested botanicals in the three bioassays from 24 to 120 h after treatment application. The efficacy of azadirachtin was followed by that of *T. vogelii*, which was not different from *P. dodecandra* during the period from 72 to 120 h following application of treatments. *Tithonia diversifolia* and *V. amygdalina* were not significantly different in effect on *T. absoluta* larvae except during 120 h post-treatment in bioassay one (Table 1).

Discussion

The bioactivity of evaluated botanicals against larvae of *T. absoluta* is explained by the secondary metabolites produced by

Table 1. Mortality of *T. absoluta* larvae (mean \pm SD) in tomato leaf galleries treated with aqueous plant extracts at 10 % (w/v) and azadirachtin 0.03 % EC (5 mL L⁻¹)

Treatments	<i>T. absoluta</i> larvae mortality (%)				
	24h	48h	72h	96h	120h
Bioassay One					
<i>T. vogelii</i>	7.5 \pm 5.0b*	15.0 \pm 5.8b	18.3 \pm 4.9b	28.5 \pm 5.7b	35.1 \pm 8.1b
<i>T. diversifolia</i>	0.0 \pm 0.0c	0.0 \pm 0.0c	2.5 \pm 5.0b	2.8 \pm 5.6c	2.8 \pm 5.6d
<i>V. amygdalina</i>	2.5 \pm 5.0bc	5.0 \pm 5.8bc	5.3 \pm 6.1b	5.6 \pm 6.4c	11.8 \pm 0.8c
<i>P. dodecandra</i>	0.0 \pm 0.0c	10.0 \pm 0.0c	10.6 \pm 0.6b	11.5 \pm 0.7bc	20.5 \pm 5.5bc
Azadirachtin	35.0 \pm 5.8a	77.5 \pm 12.6a	81.4 \pm 19.1a	94.5 \pm 6.4a	100.0 \pm 0.0a
<i>P</i> ($\alpha = 0.05$)	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
Bioassay Two					
<i>T. vogelii</i>	5.0 \pm 5.8b	18.1 \pm 5.5b	25.6 \pm 5.2b	26.4 \pm 6.3b	32.2 \pm 7.4b
<i>T. diversifolia</i>	0.0 \pm 0.0b	2.5 \pm 5.0c	5.0 \pm 5.8c	5.0 \pm 5.8c	10.6 \pm 8.2c
<i>V. amygdalina</i>	0.0 \pm 0.0b	2.5 \pm 5.0c	7.5 \pm 5.0bc	7.8 \pm 5.2bc	13.3 \pm 4.4bc
<i>P. dodecandra</i>	0.0 \pm 0.0b	2.5 \pm 5.0c	12.5 \pm 12.6bc	21.1 \pm 1.3b	21.7 \pm 9.1bc
Azadirachtin	37.5 \pm 9.6.0a	64.2 \pm 5.0a	82.2 \pm 9.3a	92.2 \pm 5.2a	94.5 \pm 6.4a
<i>P</i> ($\alpha = 0.05$)	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
Bioassay Three					
<i>T. vogelii</i>	10.0 \pm 0.0b	17.5 \pm 5.0b	20.3 \pm 7.7b	31.4 \pm 7.4b	33.3 \pm 7.9b
<i>T. diversifolia</i>	0.0 \pm 0.0c	0.0 \pm 0.0d	2.5 \pm 5.0c	5.0 \pm 5.8cd	5.3 \pm 6.1c
<i>V. amygdalina</i>	0.0 \pm 0.0c	0.0 \pm 0.0d	2.5 \pm 5.0c	2.5 \pm 5.0d	2.5 \pm 5.0c
<i>P. dodecandra</i>	0.0 \pm 0.0c	7.5 \pm 5.0c	18.1 \pm 5.5b	18.3 \pm 4.9bc	24.9 \pm 3.7b
Azadirachtin	37.5 \pm 9.6a	75.0 \pm 5.8a	87.2 \pm 4.8a	94.7 \pm 6.1a	97.5 \pm 5.0a
<i>P</i> ($\alpha = 0.05$)	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001

*Mean values followed by different letters in same column are significantly different according to Tukey's test ($P < 0.05$)

these plants, which have various modes of action (Gurjar *et al.*, 2012). *Tephrosia vogelii* contains rotenoid compounds which are mitochondrial poisons that block the electron transport chain and prevent energy production. Rotenone is also known to be stomach and contact poison (Stoll, 2000). The bioactivity of *P. dodecandra* could be associated with the presence of phytochemicals such as saponins, alkaloids, sterols, triterpenoids, phenols, flavonoids and glycosides (Qwarse *et al.*, 2016). Insecticidal activity of *T. diversifolia* is due to some of the sesquiterpenes, diterpenes, monoterpenes and alicyclic compounds in its leaves (Obafemi *et al.*, 2006). Insecticidal properties of *Vernonia amygdalina* are due to its alkaloids, flavonoids, saponins, tannins, phlobatannins, terpenoids and cardiac glycosides (Adeniyi *et al.*, 2010).

As compared to the findings of the present study, other authors reported higher efficacy of the evaluated botanicals against various insect pests. For instance, *T. vogelii*, used at the rate of 10 % w/v, was observed to be as effective as a chemical insecticide, Decis® EC25, in controlling *Maruca testularis*, *Ootheca mutabilis* and *Zonocerus variegatus* on cowpea (Adebayo *et al.*, 2007). Aqueous extracts of *T. vogelii* reduced significantly the pest population for *Maruca vitrata*, *Megalurothrips sjostedti* and *Ripotortus dentipes* in cowpea field (Olaitan and Abiodun, 2011). Cold and hot water extracts of *P. dodecandra* at the rate of 10 g/100 mL caused 100 % mortality of cabbage flea beetle *Phyllotreta cruciferae* in 24 h (Raja *et al.*, 2015). Extracts of *P. dodecandra* were also effective against onion thrips under field conditions (Shiberu *et al.*, 2012). Crude water extracts of *P. dodecandra* leaves resulted in mosquito (*Anopheles gambiae*) egg mortality of more than 80 % (Yugi and Kiplimo, 2017) with higher efficacy than neem and deltamethrin.

Furthermore, *T. diversifolia* extracts have been observed to be effective in field conditions against aphids and flower beetles of common bean (Mkenda *et al.*, 2015). Aqueous leaf extract

of *T. diversifolia* caused 100 % mortality of acrobat ant (*Crematogaster lineolata*), an insect pest of honeybees (Olufemi *et al.*, 2015). The efficacy of 1.0 and 1.5 % *T. diversifolia* hot water leaf extract was the same as a chemical insecticide, Permethrin, against oviposition of *Sitophilus zeamais* (Onekutu *et al.*, 2015). *V. amygdalina* was observed to be toxic to common bean aphids (Kawuki *et al.*, 2005) and bean weevil, *Acanthoscelides obtectus* (Adeniyi *et al.*, 2010). Water extracts of *V. amygdalina* leaves caused reduction in the population of two flea beetles (*Podagrica unifirma* and *P. sjostedti*) at 55 %, in okra (Onunkun, 2012).

Lower efficacy of plant extracts obtained in this study, as compared to the previous findings on other insects, could be due to the water extraction method used. This method was selected because the study was aiming to find indigenous plant species with insecticidal properties along with a simple preparation technology to benefit the local farmers. Furthermore, it is advised that initial screening of plants for possible bioactivity should begin by water (universal solvent) extracts; then extraction using different organic solvents may follow (Gurjar *et al.*, 2012). Other methods of extractions would likely give better results. For example, Fan *et al.* (2011) demonstrated the relationship between efficacy of plant extracts and type of solvents used in extraction. The same authors reported that toxicity of *Piper nigrum* fruit extracts against second instar larvae of tobacco army worm (*Spodoptera litura*) varied with the solvents used and decreased in the order of hexane (LD₅₀: 1.8 mg g⁻¹) > acetone (LD₅₀: 18.8 mg g⁻¹) > chloroform (LD₅₀: NA, the toxicity was very low). Similarly, Arora *et al.* (2017) obtained higher antifeeding activity of *Paederia foetida* L. (Rubiaceae) against *S. litura* larvae with methanol extracts compared to water extracts. Furthermore, Olufemi *et al.* (2015) reported that efficacy of N-hexane and methanol extracts from *T. diversifolia*, *Azadirachta indica*, *Ageratum conyzoides* and *Carica papaya* against *Aethina tumida*,

Galleria mellonella and *Achroia grisella* was significantly higher than that of water extracts. Higher efficacy with organic solvents as compared to water could be due to their difference in polarity. Organic solvents, such as N-hexane and methanol, are less polar than water, and this facilitates some organic compounds to be easily dissolved in them (Widyawati *et al.*, 2014). Thus, further studies should be carried out using various solvents other than water to evaluate the potential of these indigenous plants against *T. absoluta*.

Lower efficacy obtained with evaluated aqueous extracts could also be attributed to the concentration used (10 % w/v). According to Olaitan *et al.* (2011), levels of plant extract concentrations determine their efficacy against a given pest. In our research, the fixed concentration utilized was for screening purpose; concentrations higher than 10 % w/v would have resulted in higher mortality levels. Thus, further research should be continued with *T. vogelii* and *P. dodecandra* which were superior to others in efficacy.

Higher efficacy recorded by azadirachtin in the present study corroborates the earlier findings by different researchers who obtained high mortality of *T. absoluta* larvae treated with this botanical insecticide. For instance, El-ghany *et al.* (2016) obtained up to 92 % *T. absoluta* larval mortality using azadirachtin. Similar results were obtained by Tomé *et al.* (2013). Azadirachtin was proved to be effective against several other pests, such as *Brevicoryne brassicae*, *Sitophylus oryzae*, *Tribolium confusum*, *Epilachna paenulata*, and comparatively harmless to natural enemies than most of the commonly used synthetic insecticides (Gontijo *et al.*, 2015). The recorded azadirachtin efficacy may be attributed to its various mode of action such as enzyme inhibition, growth inhibition, feeding-deterrence and insecticidal activity among others (Senthil-Nathan, 2013). To the best of our knowledge, this is the first report of azadirachtin efficacy to Rwandan population of *T. absoluta*; this efficacy should be confirmed under field conditions of Rwanda.

Conclusively, the evaluated aqueous plant extracts displayed potential insecticidal properties and differed significantly against third instar larvae of *T. absoluta*. Further study should be carried out using solvents other than water to evaluate the potential of these indigenous plant against *T. absoluta* in laboratory conditions. *Tephrosia vogelii* and *P. dodecandra* which differed in efficacy from other studied aqueous plant extracts should be further evaluated under field conditions. Finally, the observed higher efficacy of azadirachtin to Rwandan population of *T. absoluta* should be confirmed under field conditions of Rwanda.

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