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Field efficacy of entomopathogens and plant extracts on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting tomato in Rwanda

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ABSTRACT

Following its outbreak in 2015, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) immediately became one of the major threats to the food chain in Rwanda and, therefore, sustainable management options are needed to address the situation. Two field trials were established on 3rd April and 28th June 2019 to study the efficacy of entomopathogens and plant extracts on *T. absoluta* infestation in Rwanda. Similar procedures were followed and nine treatments were evaluated, including: entomopathogenic nematodes (EPNs) (*Steinernema* sp. RW14-M-C2a-3 and *Steinernema* sp. RW14-M-C2a-3), commercial formulations of entomopathogenic fungi (EPFs) [Metatech®WP: *Metarhizium anisopliae* (Metsch.) Sorok, Strain FCM Ar 23B3), Beauvitech® WP: *Beauveria bassiana* (Bals.) Vuill., Strain J25], plant extracts of *Tephrosia vogelii* and *Phytolacca dodecandra*, azadirachtin 0.03% EC, imidacloprid as positive control and water as negative control. The entomopathogens and azadirachtin significantly reduced leaf and leaflet damages compared to the plant extracts and the controls. However, leaf damage increased with time and reached the maximum level (100%) in 9–10 weeks after transplanting in all the treatments. In both trials, the maximum leaflet damage observed with entomopathogens and azadirachtin in 10 weeks after transplanting varied between 59.7% and 74.7% with the marketable fruit yield of 12.4–16.2 t ha^{-1}; while leaflet damage in positive control ranged 80.0%–92.1% with marketable yield of 3.0–3.5 t ha^{-1}. Our results suggest that the entomopathogens and azadirachtin have the potential for use in integrated pest management of *T. absoluta* in Rwanda, but further studies are needed to incorporate them in the IPM program.

1. Introduction

Control of pests is a pre-requisite for enhanced crop performance and subsequent production as they can inflict severe damage resulting in total destruction (Desneux et al., 2010). Specifically, the tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a major challenge to tomato (*Solanum lycopersicum* L.) production in many parts of the world (Biondi et al., 2018). Following its outbreaks in Rwanda in 2015, FAO (2015) declared this pest among the most important threat.

Tuta absoluta larvae damage all parts of tomato plants, including stems, leaves, flowers, and fruits, resulting in interrupted crop growth and development (Biondi et al., 2018). By feeding within the mesophyll, one larva can make many galleries, moving in and out the leaves (Gözel and Kasap, 2015). Studies reported a positive correlation between leaf and fruit infestations (Cocco et al., 2014). Up to 12 generations of this pest are possible under favourable conditions, which add to its invasive nature (Biondi et al., 2018). In the absence of proper management measures, yield losses inflicted by this pest can reach 100% of the total production (Desneux et al., 2010). Chemical control is the main option used by most African farmers to manage this pest. However, *T. absoluta* management remains a challenge mainly due to its mine-feeding habit, short development cycle, and acquisition of resistance to frequently used pesticides (Roditakis et al., 2013, 2015). This, in addition to the harmful effects of chemicals in the environment, necessitates the need for sustainable alternatives.

Sex pheromones have been widely and successfully used to detect and monitor the population of *T. absoluta* (Megido et al., 2013). In addition pheromone traps have been recommended and used for mass trapping and mating disruption of *T. absoluta* males (Witzgall et al., 2010; Harbi et al., 2012). At high infestation, mating disruption

Abbreviations: EPNs, Entomopathogenic nematodes; EPFs, Entomopathogenic fungi; PEs, Plant extracts; IJs, Infective juveniles.

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technique resulted in reduced pest population, reduced leaf and fruit damages in well isolated greenhouses to prevent entrance of mated females (Vacas et al., 2011; Cocco et al., 2013). Nevertheless, contradicting results have been obtained in greenhouses with lower *T. absoluta* infestation (Vacas et al., 2011) and under open-field conditions (Filho et al., 2000). Since high application rate is required (up to 60 g ha^{-1}), the cost of pheromones limits the wide use of mating disruption technique (Cocco et al., 2013). Furthermore, Megido et al. (2013) reported the limitations of pheromone based strategies against *T. absoluta* because the females can reproduce parthenogenetically.

Different studies showed the possibilities of controlling *T. absoluta* using entomopathogenic fungi (EPFs) (Tadele and Emana, 2017), entomopathogenic nematodes (EPNs) (Van Damme et al., 2016) and insecticides of plant origin (Nilahyane et al., 2012); but there are scarce reports on their use under field conditions. Local isolates of EPNs (Yan et al., 2016) were demonstrated to be effective against white grubs in Rwanda (Kajuga et al., 2018), hence the need to broaden investigations of their efficacy against other economically important pests, including *T. absoluta*. On the other hand, the EPFs *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) and *Metarhizium anisopliae* (Metsch.) Sorok (Ascomycota: Hypocreales) are efficient in killing the host and able to attack all insect developmental stages (Schrank and Vainstein, 2010); however, commercial formulations recommended against this pest are limited (Biondi et al., 2018). Besides, field efficacy of local insecticidal plants like *Tephrosia vogelii* and *Phytolacca dodecandra* against *T. absoluta* also needed to be evaluated because they would be affordable to farmers.

In our previous studies (Ndereyimana et al., 2019a, 2019b; 2019c), laboratory bioassays were carried out in Rwanda to evaluate the potential of three groups of biorational control agents, i.e. local EPNs, commercial formulations of EPFs and local plant extracts against *T. absoluta*. In each of the above groups, some agents demonstrated relatively higher efficacy. Since laboratory relatively high efficacy can only be partly transferred to field conditions (Lacey et al., 2015), field evaluation is mandatory for efficacy confirmation. The objective of the current study was to determine the field efficacy of the entomopathogens and plant extracts on *T. absoluta* infestation in Rwanda.

2. Materials and methods

2.1. Study site and period

Two field trials, denoted as trials one and two, were conducted in a farmer's field located in Rweru Sector, Bugesera District, Eastern Province of Rwanda. GPS coordinates of the location are latitude 02° 32′ 355" S, longitude 030° 26′ 963" E and 1338 m of elevation above sea level. The field was selected in the village where severe *T. absoluta* outbreak was recently recorded, and where many farmers cultivate tomato as a cash crop; thus, there is more concentrated plant host and pest population as compared to other parts of the country. The average annual temperature and rainfall are 21.4 °C and 854 mm, respectively (Kabirigi et al., 2017). Trials one and two were established on 3rd April and 28th June 2019, and ended on 03rd July and 27th September 2019, respectively. Note that similar procedures were followed in both trials.

2.2. Plant material and treatments

The study was carried out on tomato (*Solanum lycopersicum* L.), which is the preferred host of *T. absoluta*. The cultivar 'Roma' was selected because it is the mostly cultivated by Rwandan farmers in open fields. Nine treatments (Table 1) were evaluated against *T. absoluta*: two local EPN isolates, two commercial formulations of EPFs, two local plant extracts (PEs), azadirachtin, imidacloprid, and water. The EPNs, EPFs, PEs, and azadirachtin were chosen because they performed well in our previous laboratory bioassays (Ndereyimana et al., 2019a, 2019b; 2019c) and, therefore, suitable to be tested in the field. Imidacloprid, which is widely used against *T. absoluta* in Rwanda, and water were added as positive and negative controls, respectively.

Table 1
Treatments used in the field experiment to control *Tuta absoluta*.

Designation	Treatment description	Type of treatment
T1	*Steinernema* sp. RW14-M-C2a-3	Entomopathogenic nematode
T2	*Steinernema* sp. RW14-M-C2b-1	Entomopathogenic nematode
T3	Metatech®WP (*M. anisopliae*, Strain FCM Ar 23B3, 5×10^9 CFU/g)	Entomopathogenic fungi
T4	Beauvitech® WP (*B. bassiana*, Strain J25, 1×10^{10} CFU/g)	Entomopathogenic fungi
T5	*Tephrosia vogelii*	Local plant extracts
T6	*Phytolacca dodecandra*	Local plant extracts
T7	Azadirachtin 0.03% EC (Nimbecidine)	Botanical insecticide
T8	Imidacloprid (Confidor SL 200)	Neonicotinoid insecticide
T9	Water	-

2.2.1. Entomopathogenic nematodes

The two EPNs used in this study (Table 1) were obtained from the Biological Control Laboratory – EPN Production Facility of Rwanda Agriculture and Animal Resources Development Board (RAB). These EPNs were isolated in 2014 from Musanze District, Northern Province of Rwanda, in a banana field intercropped with sorghum and pumpkin (Yan et al., 2016). To obtain the required number of EPNs for use on experimental plots, they were mass-produced following the *in-vivo* method using *Galleria mellonella* larvae (Kaya and Stock, 1997). Upon harvesting, the infective juveniles (IJs) were rinsed in distilled water and stored at 7 °C for less than 7 days before their use (Mahmoud, 2016).

On the day of application in the field, the EPNs were checked for viability using a stereomicroscope (60 × magnification) after acclimatization for 1 h at room temperature (19 °C). The EPNs were used when more than 90% of IJs were moving actively (Kajuga et al., 2018). After checking their viability, the EPNs were counted and adjusted to the required concentration of 5 x 10^9 IJs ha^{-1} (Gözel and Kasap, 2015; Kamali et al., 2018). The aqueous suspension of IJs for each EPN was then transferred into sponges packed in plastic bags, transported in a cool box to the field and used the same day at dusk (Yan et al., 2016). At the time of application, the sponges containing EPNs were poured in water for the EPNs to get out; and then the required volume for field application was made up by adding water.

2.2.2. Entomopathogenic fungi

The two formulations of EPFs (Table 1) were manufactured by Dudutech Division, Flamingo Horticulture (K) Ltd, Naivasha, Kenya. Before their application in the field, their viability was checked by culturing them on Potato Dextrose Agar media and incubating at 25 °C ± 1 °C for 7 days (Youssef, 2015). The EPFs were observed under light microscopy (× 40 magnification) to ensure that more than 95% of spores had germinated to proceed with them to the field application.

2.2.3. Plant extracts

The two local plant extracts (Table 1) were obtained from the leaves of *T. vogelii* and *P. dodecandra*. These two species were collected from Huye District, Southern Province of Rwanda. Upon collection, the leaves of each species were washed, dried under shade for two weeks and ground into a fine powder using an electric grinder. The obtained powder was packed in biodegradable plastic bags. Before field application, extraction of each plant was carried out by adding 150 g of powder to one litre of boiled water, immediately after its removal from heat, and keeping it for 12 h. After filtration with muslin cloth, the extracts were diluted to one litre each using cold water to give the concentration of 15% w/v.

2.3. Trials set-up and maintenance

Before planting each trial, the field was ploughed twice at 15 days' interval and treated with cow manure at the rate of 20 t ha^{-1}. The experiment was laid out in a randomized complete block design with three replications. Each experimental unit (plot) was 3 m long and 2 m wide to accommodate 24 plants spaced at 0.5 m × 0.5 m. The plots were separated by a 1.5 m wide path to avoid the drifting effect of the treatments. Transplanting was carried out using 30 days old seedlings and the plots were mulched with dry grass. Apart from insecticide application which varied according to the studied treatments, other practices like watering, weeding, pruning to four branches per plant, fertilizer, and fungicide application were carried out uniformly in all plots. The trials relied on natural infestation by *T. absoluta* (Sohrabi et al., 2017) due to its abundance in the area of study.

2.4. Application of treatments

The application of treatments started one week after transplanting and proceeded on weekly basis until 12 weeks after transplanting. Treatments were applied during the evening hours, slightly before sunset (around 4:30 pm), to avoid the harmful effects of sunlight (Gözel and Kasap, 2015). For each treatment, the spray volume was 1000 L ha^{-1} (Brusselman et al., 2012) using a knapsack sprayer. The dosages used were 5 x 10^9 IJs ha^{-1} for EPNs (Gözel and Kasap, 2015; Kamali et al., 2018), 250 g ha^{-1} for EPFs (as recommended by the manufacturer), 15% w/v for local plant extracts, 5 mL L^{-1} for azadirachtin 0.03% EC and 1 mL L^{-1} of water for imidacloprid. Continuous agitation was done during treatment application to prevent precipitations.

2.5. Data collection and analysis

Data were collected from five plants in the middle of each plot and the averages per plant were computed. Observations started at two weeks after transplanting and continued weekly along 10 weeks after tranplanting. Leaf damage was assessed as the percentage of leaves affected (mined) by *T. absoluta;* while leaflet damage was evaluated as the percentage of leaflets affected by *T. absoluta* on three leaves located in the middle canopy of each plant (Cocco et al., 2014). Total yield (t ha^{-1}) was obtained as an average of fruit weight per plant extrapolated to one hectare; while marketable yield (t ha^{-1}) was calculated by deducting the weight of damaged fruits from the total fruit weight.

Analysis of variance (ANOVA) was carried out to determine the effect of treatments on studied parameters. Normality check was priorly carried out and the appropriate transformation (log or square root) was done to achieve normal distribution and meet the assumptions of ANOVA. The means for statistically different treatments were separated using Tukey's honestly significant difference (HSD) test at $p \leq 0.05$.

3. Results

3.1. Leaf damage

The studied treatments significantly ($p < 0.05$) affected leaf damage by *T. absoluta* in both trials. Leaf damage increased with time and reached the maximum level (100%) in 9–10 weeks after transplanting for all the treatments in trials one and two (Table 2). There was no significant difference in leaf damage among the treatments on weeks two, nine and ten after transplanting in both trials, but also on week eight in trial two. During the other times of observation, the general trend was that the EPNs (*Steinernema* sp. RW14-M-C2a-3 and *Steinernema* sp. RW14-M-C2b-1), EPFs (Metatech®WP: *M. anisopliae*, Strain FCM Ar 23B3, and Beauvitech® WP: *B. bassiana*, Strain J25) and azadirachtin recorded lower leaf damage and were not significantly different from each other in trials one and two.

They all significantly ($p < 0.05$) reduced leaf damage as compared to the controls (imidacloprid and water). Their efficacy was similar to *T. vogelii*, but the later produced significantly higher leaf damage during weeks six and seven in trial one, and during week four in trial two. *Phytolacca dodecandra* was not significantly different from the controls.

3.2. Leaflet damage

There was a significant difference among treatments ($p < 0.05$) in the leaflet damage percentage, except on week two for both trials (Table 3). All entomopathogens (EPNs and EPFs) and azadirachtin recorded lower

Table 2
Leaf damage (%) (mean ± SD) caused by *Tuta absoluta* on tomato cv. Roma crop treated with entomopathogens and plant extracts.

	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT	10 WAT
TRIAL ONE									
T1	3.5 ± 3.1 a	7.5 ± 4.0 b	16.2 ± 1.7 d	36.2 ± 3.7 c	51.9 ± 2.3 c	71.0 ± 0.8 d	83.3 ± 2.2 d	97.9 ± 2.2 a	100.0 ± 0.0 a
T2	3.6 ± 3.1 a	7.9 ± 0.3 ab	17.7 ± 0.5 cd	38.5 ± 2.0 cb	57.2 ± 3.4 c	70.9 ± 1.2 d	83.9 ± 1.5 d	97.9 ± 2.1 a	99.3 ± 1.2 a
T3	3.5 ± 3.1 a	7.3 ± 0.2 ab	16.1 ± 2.2 d	39.0 ± 1.5 cb	52.8 ± 3.1 c	73.4 ± 2.3 d	86.2 ± 1.4 cd	97.9 ± 0.1 a	100.0 ± 0.0 a
T4	3.7 ± 3.2 a	10.0 ± 1.5 ab	19.5 ± 1.1bcd	37.2 ± 2.8 cb	59.5 ± 4.6 bc	75.1 ± 3.1 cd	86.9 ± 3.8 cd	98.6 ± 1.2 a	100.0 ± 0.0 a
T5	5.4 ± 0.6 a	12.3 ± 5.5 ab	22.2 ± 1.9 bc	44.2 ± 2.3 b	68.9 ± 0.9 ab	80.8 ± 1.8 bc	89.0 ± 2.9 bcd	98.6 ± 1.3 a	100.0 ± 0.0 a
T6	5.3 ± 5.3 a	13.2 ± 5.1 ab	24.9 ± 2.9 ab	63.0 ± 2.7 a	74.8 ± 1.7 a	85.4 ± 1.6 ab	92.7 ± 1.7 abc	99.3 ± 1.2 a	100.0 ± 0.0 a
T7	3.4 ± 2.9 a	8.4 ± 1.6 ab	17.5 ± 2.7 cd	39.0 ± 1.0 cb	55.1 ± 3.3 c	71.5 ± 1.7 d	83.0 ± 1.9 d	97.4 ± 1.0 a	99.4 ± 1.1 a
T8	3.2 ± 2.8 a	14.0 ± 3.1 ab	24.3 ± 1.9 ab	61.4 ± 3.5 a	75.3 ± 5.6 a	87.6 ± 2.1 a	94.4 ± 2.5 ab	99.3 ± 1.2 a	100.0 ± 0.0 a
T9	3.5 ± 3.1 a	18.2 ± 3.5 a	30.2 ± 1.2 a	71.9 ± 2.2 a	81.1 ± 2.9 a	91.9 ± 2.0 a	97.1 ± 2.5 a	99.3 ± 1.2 a	100.0 ± 0.0 a
CV	51.63	14.49	3.49	1.56	1.35	0.60	2.84	1.49	0.56
P	0.9963	0.0355	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6473	0.5934
TRIAL TWO									
T1	6.9 ± 3.1 a	11.4 ± 4.1 b	18.9 ± 6.7 c	45.2 ± 6.5 b	63.9 ± 3.0 d	81.9 ± 0.3 c	98.3 ± 2.6 a	100 ± 0.0 a	100 ± 0.0 a
T2	7.0 ± 2.6 a	14.7 ± 3.9 ab	21.8 ± 3.4 c	42.0 ± 6.8 b	66.2 ± 2.3 d	83.2 ± 1.3 c	98.4 ± 1.6 a	100 ± 0.0 a	100 ± 0.0 a
T3	7.0 ± 2.6 a	18.0 ± 3.5 ab	20.3 ± 4.0 c	47.9 ± 2.2 b	70.5 ± 1.6 cd	83.3 ± 1.2 c	100.0 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
T4	5.5 ± 0.4 a	15.1 ± 4.3 ab	23.9 ± 5.6 bc	47.3 ± 4.1 b	72.9 ± 5.3 bcd	83.9 ± 0.2 c	99.5 ± 0.9 a	100 ± 0.0 a	100 ± 0.0 a
T5	6.9 ± 3.2 a	21.0 ± 2.2 ab	44.6 ± 6.5 ab	56.0 ± 7.5 b	71.5 ± 4.4 cd	88.3 ± 3.8 bc	100.0 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
T6	7.0 ± 3.0 a	27.1 ± 3.1 a	53.9 ± 1.7 a	80.1 ± 2.1 a	85.2 ± 4.1 ab	95.2 ± 6.3 ab	100.0 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
T7	6.9 ± 2.7 a	17.0 ± 7.0 ab	23.4 ± 8.5 c	46.3 ± 7.4 b	67.5 ± 2.3 d	83.2 ± 2.5 c	96.8 ± 2.9 a	100 ± 0.0 a	100 ± 0.0 a
T8	7.2 ± 2.8 a	28.2 ± 8.2 a	53.2 ± 3.2 a	78.5 ± 3.7 a	83.3 ± 8.6 abc	91.8 ± 2.7 ab	100.0 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
T9	6.9 ± 3.1 a	29.3 ± 7.5 a	60.9 ± 5.9 a	84.9 ± 2.6 a	83.8 ± 3.7 a	96.6 ± 3.2 a	100.0 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
CV	20.62	9.73	6.66	2.85	1.39	3.05	1.38	0	0
p	0.9992	0.0070	<.0001	<.0001	<.0001	<.0001	0.1050	-	-

T1: *Steinernema* sp. RW14-M-C2a-3, T2: *Steinernema* sp. RW14-M-C2b-1, T3: Metatech®WP (*Metarhizium anisopliae*, Strain FCM Ar 23B3), T4: Beauvitech® WP (*Beauveria bassiana*, Strain J25), T5: *Tephrosia vogelii, T6: Phytolacca dodecandra*, T7: azadirachtin 0.03% EC, T8: imidacloprid, T9: water; Means followed by the same letter (s) are not significantly different according to Tukey's test ($p \leq 0.05$).

Table 3
Leaflet damage (%) (mean ± SD) caused by *Tuta absoluta* on tomato cv. Roma crop treated with entomopathogens and plant extracts.

	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT	10 WAT
TRIAL ONE									
T1	2.9 ± 2.6 a	4.2 ± 0.4 b	8.5 ± 0.6 d	13.4 ± 0.2 c	18.4 ± 1.4 c	26.1 ± 2.5 d	37.6 ± 2.8 d	48.8 ± 2.2 d	59.7 ± 4.8 c
T2	3.0 ± 2.6 a	4.7 ± 0.5 ab	8.9 ± 0.6 d	14.3 ± 1.0 c	20.1 ± 2.2 c	27.9 ± 3.8 cd	38.7 ± 1.4 d	51.5 ± 1.4 bcd	64.1 ± 2.6 c
T3	2.9 ± 2.6 a	5.5 ± 1.2 ab	9.4 ± 0.7 d	13.7 ± 0.5 c	19.3 ± 1.2 c	26.9 ± 3.0 d	38.5 ± 2.2 d	54.9 ± 0.8 bc	63.5 ± 0.7 c
T4	2.2 ± 3.8 a	5.8 ± 0.8 ab	9.3 ± 0.6 d	15.0 ± 1.1 c	21.1 ± 0.8 c	26.4 ± 0.3 d	39.5 ± 1.7 d	54.5 ± 3.6 bcd	66.0 ± 4.4 c
T5	3.0 ± 1.5 a	5.8 ± 0.8 ab	11.2 ± 0.8 c	18.0 ± 0.8 b	27.0 ± 1.1 b	35.7 ± 2.4 bc	49.3 ± 3.1 c	56.9 ± 2.8 b	71.0 ± 3.2 bc
T6	2.8 ± 2.6 a	7.8 ± 0.9 a	14.4 ± 0.5 b	20.2 ± 0.7 b	26.7 ± 1.6 b	39.6 ± 1.9 ab	60.2 ± 2.7 ab	71.6 ± 1.0 a	81.1 ± 1.5 ab
T7	2.4 ± 2.3 a	4.5 ± 1.3 b	8.2 ± 0.8 d	13.4 ± 0.3 c	21.0 ± 2.2 c	27.7 ± 4.0 cd	38.5 ± 2.0 d	49.8 ± 2.8 cd	63.7 ± 5.7 c
T8	2.7 ± 2.3 a	7.1 ± 1.4 ab	12.7 ± 0.5 cb	20.5 ± 1.3 b	28.6 ± 2.6 ab	37.9 ± 2.8 ab	58.5 ± 3.7 bc	70.6 ± 0.1 a	80.0 ± 4.9 ab
T9	2.6 ± 2.5 a	8.2 ± 2.1 a	17.5 ± 0.7 a	26.9 ± 1.0 a	35.0 ± 1.3 a	47.5 ± 4.6 a	70.0 ± 5.7 a	76.4 ± 2.6 a	90.4 ± 7.2 a
CV	14.4	11.05	2.39	1.75	2.25	2.77	1.60	1.00	1.51
p	0.9997	0.0042	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TRIAL TWO									
T1	2.2 ± 0.2 a	3.9 ± 0.9 bc	6.4 ± 1.4 b	17.7 ± 2.3 c	21.1 ± 2.8 c	28.8 ± 1.6 c	41.8 ± 3.3 c	55.7 ± 2.6 c	68.0 ± 1.5 b
T2	2.1 ± 0.2 a	4.0 ± 0.3 bc	7.0 ± 0.9 b	17.0 ± 1.3 c	21.2 ± 1.1 c	30.1 ± 3.5 c	44.5 ± 1.5 bc	57.4 ± 3.0 c	70.0 ± 2.1 b
T3	1.9 ± 0.3 a	4.7 ± 0.4 abc	6.8 ± 0.2 b	16.7 ± 0.9 c	22.7 ± 3.2 c	32.5 ± 0.7 bc	43.1 ± 0.5 bc	57.0 ± 1.7 c	70.8 ± 1.7 b
T4	2.0 ± 0.2 a	5.0 ± 0.9 abc	6.7 ± 1.2 b	18.8 ± 2.7 bc	23.0 ± 2.7 c	32.4 ± 1.8 bc	44.8 ± 3.9 bc	59.0 ± 2.4 bc	71.3 ± 3.6 b
T5	2.1 ± 0.3 a	3.7 ± 1.0 c	6.9 ± 0.4 b	24.8 ± 1.1 ab	28.4 ± 1.4 cb	37.8 ± 2.6 b	50.1 ± 3.4 b	64.0 ± 1.3 b	74.7 ± 2.6 b
T6	2.0 ± 0.2 a	5.6 ± 0.8 ab	14.5 ± 1.5 a	28.6 ± 2.5 a	35.0 ± 3.3 ab	54.2 ± 1.8 a	71.4 ± 4.1 a	82.4 ± 1.6 a	92.4 ± 2.4 a
T7	2.1 ± 0.2 a	3.6 ± 0.2 c	6.7 ± 0.2 b	17.3 ± 1.9 c	21.9 ± 3.1 c	30.3 ± 1.8 c	43.4 ± 3.4 bc	56.0 ± 1.5 c	70.1 ± 0.6 b
T8	2.3 ± 0.7 a	5.3 ± 0.6 abc	15.2 ± 0.2 a	27.1 ± 2.1 a	36.0 ± 3.0 ab	53.1 ± 1.8 a	70.8 ± 1.1 a	81.7 ± 2.0 a	92.1 ± 3.0 a
T9	2.1 ± 0.7 a	6.5 ± 0.6 a	19.1 ± 0.8 a	31.2 ± 2.7 a	42.6 ± 0.8 a	56.4 ± 2.2 a	73.8 ± 1.3 a	84.8 ± 3.8 a	95.6 ± 3.8 a
CV	9.67	9.97	5.11	3.30	3.23	1.76	1.40	0.89	0.75
p	0.9708	0.0013	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

T1: *Steinernema* sp. RW14-M-C2a-3, T2: *Steinernema* sp. RW14-M-C2b-1, T3: Metatech®WP (*Metarhizium anisopliae*, Strain FCM Ar 23B3), T4: Beauvitech® WP (*Beauveria bassiana*, Strain J25), T5: *Tephrosia vogelii, T6: Phytolacca dodecandra*, T7: azadirachtin 0.03% EC, T8: imidacloprid, T9: water; Means followed by the same letter (s) are not significantly different according to Tukey's test ($p \leq 0.05$).

leaflet damages which were not significantly different from one another. *Tephrosia vogelii* recorded slightly higher leaflet damage as compared to the entomopathogens and azadirachtin, but it was not significantly different from them or one of them during weeks seven, nine, and ten in trial one, and during all weeks in trial two. Higher leaflet damage was observed in plots treated with *P. dodecandra* and controls (imidacloprid and water), which were not significantly different for most of the weeks after transplanting.

3.3. Total and marketable yields

Higher total and marketable yields were obtained in plots treated with entomopathogens and azadirachtin which were not significantly different from one another in trials one and two. Lower yields were recorded with *T. vogelii* and *P. dodecandra* plants extracts, which were more close to controls (imidacloprid and water) in both trials (Fig. 1).

4. Discussion

The potential of using the studied entomopathogens and plant extracts against *T. absoluta* had been previously obtained in laboratory conditions (Ndereyimana et al., 2019a, 2019b; 2019c); field efficacy confirmation was, therefore, the subsequent step because laboratory efficacy does not always ensure field efficacy (Lacey et al., 2015). The evaluated EPNs, EPFs, and azadirachtin exhibited higher efficacy against *T. absoluta* under the field conditions of Rwanda as they significantly reduced leaf and leaflet damages compared to the controls (imidacloprid and water). Thus, in addition to environmental protection, these biorational control agents can contribute to reducing the population of *T. absoluta*.

Higher field efficacy of EPNs against *T. absoluta* was also obtained by Shams-El-Din et al. (2014), Gözel and Kasap (2015) under field conditions and by Battalla-Carrella et al. (2010) in pot experiments. Moreover, EPNs have already been used on other crops as foliar applications under field conditions against various other insect pests (Mahmoud, 2016). The observed EPNs' efficacy could be linked to their ability to penetrate leaf galleries, formed by *T. absoluta* larvae, where they get protection against harsh environmental conditions (Battalla-Carrella et al., 2010; Kamali et al., 2018). It also seems that *T. absoluta* larvae might have served as ideal hosts upon which infective juveniles (IJs) of the EPNs could multiply while preparing to attack other larvae. The ability of IJs of EPNs to survive and multiply in different hosts was reported by Belien (2018). Furthermore, higher efficacy of EPNs belonging to the *Steinernema* genus could be due to the bacteria associated with their genus (*Xenorhabdus*) and host scavenging behaviour, ambusher strategy, by which they diligently wait for their host (Mahmoud, 2016).

Mahmoud (2016) and Belien (2018) reviewed different formulations that can be used to boost the efficacy of EPNs under field conditions. These include: vermiculite, alginate, clay, polyacrylamide gels, water-dispersible granules, peat, surfactants, polymers, and capsules among others. This means that the EPNs' efficacy obtained in this study can be improved further by adopting a specific formulation. Other factors that could affect the efficacy of EPNs include application equipment, relative humidity or high moisture levels on plants (Mahmoud, 2016).

Similar to the results obtained on the efficacy of the studied EPFs against *T. absoluta*, Tadele and Emana (2017) also reported high efficacy of *B. bassiana* and *M. anisopliae* against *T. absoluta* under laboratory and glasshouse conditions in Ethiopia. High efficacy of *B. bassiana* was also observed against *Stomphastis thraustica*, the leaf miner of the jatropha plant (Moawad et al., 2017). Higher efficacy of *M. anisopliae* compared to chemical pesticides was also reported by Ansari et al. (2007) against pupae of the Western flower thrips (*Frankilinea occidentalis*). According to Klieber and Reineke (2016), *T. absoluta* larvae mortality inflicted by EPFs takes place at late developmental stage, but during this infection stage, the feeding activity of larvae is reduced progressively until death. This is the reason why with the use of EPFs, crop damage can still occur but the long-term effect is expected through reduction of population density (Klieber and Reineke, 2016). A similar situation was observed in this study where leaf and leaflet damages were significantly lower than controls, which suggests that the efficacy obtained with the studied EPFs should not be underrated.

The observed efficacy of *B. bassiana* can be explained by its ability to exhibit epiphytic and endophytic activity against various insect pests, including *T. absoluta* (Klieber and Reineke, 2016). Through the endophytic activity, spores also enter in plant tissues and can persist for many

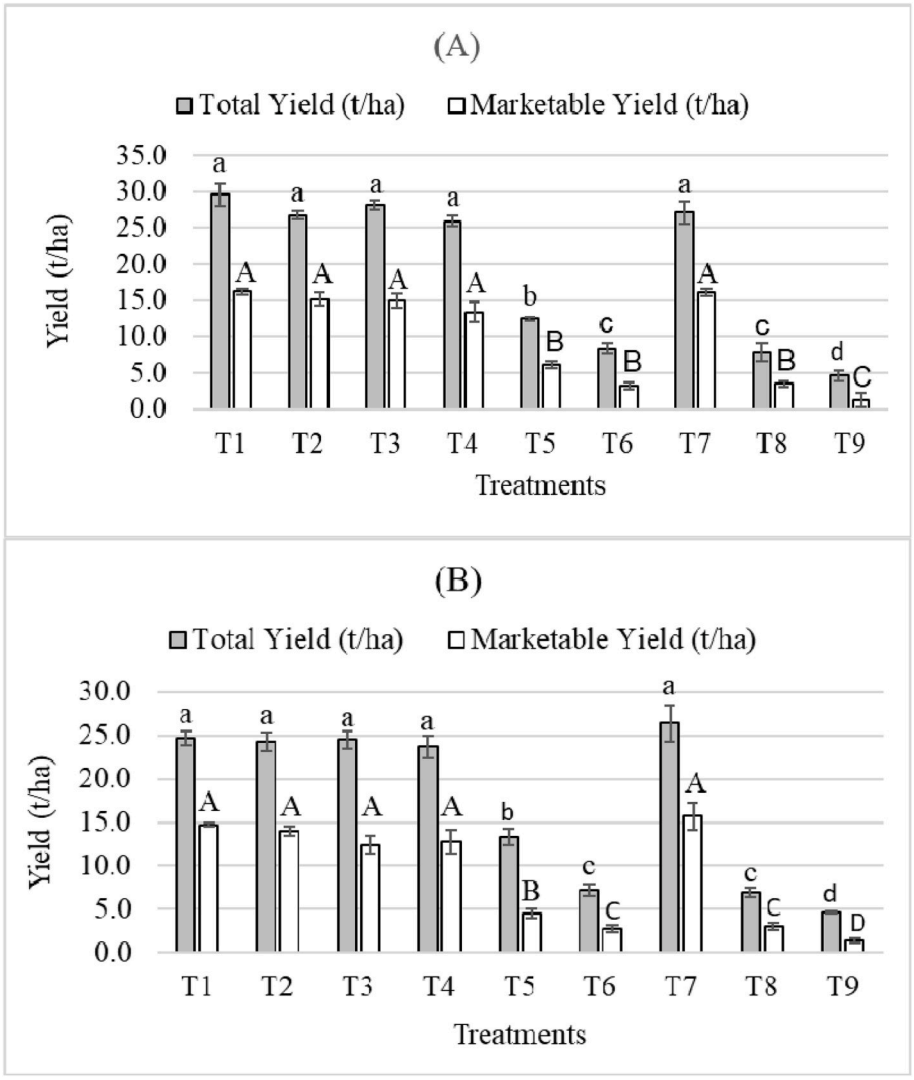

Fig. 1. Total and marketable yield of tomato cv. Roma crop treated with entomopathogens and plant extracts against *Tuta absoluta* in trials one (A) and two (B). T1: *Steinernema* sp. RW14-M-C2a-3, T2: *Steinernema* sp. RW14-M-C2b-1, T3: Metatech®WP (*Metarhizium anisopliae*, Strain FCM Ar 23B3), T4: Beauvitech® WP (*Beauveria bassiana*, Strain J25), T5: *Tephrosia vogelii, T6: Phytolacca dodecandra*, T7: azadirachtin 0.03% EC, T8: imidacloprid, T9: water; Similar letters (lowercase for total yield, uppercase for marketable yield) above the bars indicate non-significant difference according to Tukey's test ($p \leq 0.05$).

months so that the control of pest progenies is guaranteed through the ingestion of spore-colonised tissues by a pest (Allegrucci et al., 2017). The endophytic behaviour of *B. bassiana* is of great importance because it allows for the persistence of fungi propagules that could otherwise be killed by unfavourable environmental factors (Klieber and Reineke, 2016). Virulence of *M. anisopliae* could be explained by presence of numerous proteases (more than 14) used to penetrate the host cuticle (Schrank and Vainstein, 2010). In addition, strains of *M. anisopliae* produce a higher quantity of dextruxins, toxins known for the most virulence factors (Schrank and Vainstein, 2010).

Possibilities also exist to enhance further the efficacy of EPFs by manipulating the formulations (Murugasridevi et al., 2017). Therefore, more studies are needed with the commercial formulations tested in this study to boost their efficacy. Since the EPFs used are the commercial formulations (Metatech®WP and Beauvitech®WP) currently recommended against other pests, they can be easily registered and used in IPM of *Tuta absoluta* in Rwanda.

Azadirachtin's field efficacy against the Rwandan population of *T. absoluta* under field conditions is now reported for the first time. These findings agree with other researchers (Tomé et al., 2013) who also obtained high efficacy of azadirachtin against *T. absoluta*. Similarly, Nadeem et al. (2015) reported high field efficacy of azadirachtin against jassid (*Amrasca devastans*) and whitefly (*Bemisia tabaci*) which was as good as lambda-cyhalothrin 2.5 EC in Okra. Debashri and Tamal (2012) reported azadirachtin to be also effective against several economically important pests, such as the pod borer *Helicoverpa armigera*, the cabbage aphid *Brevicornye brassicae* and the potato tuber moth *Phthorimaea operculella*. Azadirachtin was reported to act as oviposition-deterrent, repellent, anti-feeding, growth and development inhibitor (Senthil-Nathan, 2013). Tomé et al. (2013) reported the ability of azadirachtin to affect larvae development and compromise their survival, not only in *T. absoluta* but also several other leafminers such as the coffee leafminer *Leucoptera coffeella* and Diptera leafminers (*Liriomyza* spp.). The field efficacy observed in this study could be due to the combination of these modes of action that compromise the overall activity of the pest. Further studies are recommended for incorporation of azadirachtin in IPM of *T. absoluta* in Rwanda.

Tephrosia vogelii showed relatively medium to lower field efficacy compared to the entomopathogens and azadirachtin. This efficacy can be attributed to the presence of rotenoids (Stevenson et al., 2012). Previous studies also reported higher efficacy of some other plant extracts against *T. absoluta*. For instance, leaf extracts of *Thymus vulgaris* L. and seed extracts of *Ricinus communis* L. caused up to 95% and 58% of *T. absoluta* larval mortality, respectively (Nilahyane et al., 2012). The bioactivity of plant extracts against various pests is due to secondary metabolites (Stevenson et al., 2012). The reduced field efficacy of plant extracts used in this study compared to the entomopathogens and azadirachtin may be partly due to the quick degradation of active components when exposed to sunlight (James et al., 2019). Other factors affecting the efficacy of plant extracts, namely extraction method and

concentration, were discussed in our previous paper (Ndereyimana et al., 2019a).

The observed lower efficacy of imidacloprid could be partly due to the ability of *T. absoluta* to develop resistant strains to frequently used pesticides (Roditakis et al., 2015). The resistance of *T. absoluta* was reported against the pesticides belonging to different chemical classes, including organophosphates, pyrethroids, spinosyns, avermectins, cartap, benzoylureas, indoxacarb, and diamides, among others (Guedes et al., 2019). This indicates that relying on synthetic pesticides is not a sustainable solution for the management of *T. absoluta*.

Higher total and marketable yields obtained with application of the entomopathogens and azadirachtin would be explained by lower leaf and leaflet damages observed in these treatments as compared to the plant extracts and controls. By feeding on leaf mesophyll, *T. absoluta* might have negatively affected the process of photosynthesis through which carbohydrates are synthesised. Moreover, fruit boring by larvae of the pest also resulted in direct loss of their marketable yield and this was demonstrated by the efficacy of the treatments vis-à-vis the pest. In line with this, Biondi et al., 2018 also reported reduced tomato crop yield as a result of crop damage by *T. absoluta* and emphasized on the need to keenly select and implement effective control measures against this pest to avoid yield losses which can even reach 100%.

5. Conclusion

The studied entomopathogens and azadirachtin exhibited significant field efficacy against *T. absoluta*. Higher efficacy was obtained with *Steinernema* sp. RW14-M-C2a-3, *Steinernema* sp. RW14-M-C2b-1, Metatech®WP (*Metarhizium anisopliae*, Strain FCM Ar 23B3), Beauvitech® WP (*Beauveria bassiana*, Strain J25) and azadirachtin 0.03% EC compared to plant extracts (*T. vogelii* and *P. dodecandra*). Our results suggest that the entomopathogens and azadirachtin have the potential for use in integrated pest management of *T. absoluta* in Rwanda, but further studies are needed to incorporate them in the IPM program and optimise their use and efficicacy (dose, frequency, combinations with other IPM components).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Assinapol Ndereyimana: Conceptualization, Methodology, Investigation, Resources, Data curation, Software, Formal analysis, Writing - original draft, Funding acquisition, Writing - review & editing. **Samuel Nyalala:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing - review & editing. **Patrick Murerwa:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing. **Svetlana Gaidashova:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing.

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