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First Report of Field Efficacy and Economic Viability of *Metarhizium anisopliae*-ICIPE 20 for *Tuta absoluta* (Lepidoptera: Gelechiidae) Management on Tomato

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Abstract: Eco-friendly pest control options are highly needed in food crop production systems to mitigate the hazards of synthetic chemical pesticides. Entomopathogenic fungal biopesticides—*Metarhizium anisopliae* strains ICIPE 20 (oil-formulation containing 1.0 × 10^9 conidia/mL) and ICIPE 69 (commercialized biopesticide known as Mazao Campaign®)—were evaluated against *Tuta absoluta* on tomato through inundative foliar spray and compared with the commonly used pesticide Dudu Acelamectin 5% EC (Abamectin 20 g/L + Acetamiprid 3%) and untreated plot. All the treatments were arranged in a randomized complete block design with three replicates. The field experiments were conducted for two consecutive cropping seasons in Mukono district, Uganda. *Tuta absoluta* infestation, injury severity on leaves and fruits, fruit yield loss, marketable fruit yield gain and cost–benefit ratio of the treatments were assessed. The results during both seasons showed a significant lower fruit yield loss in *M. anisopliae* ICIPE 20-treated plots compared to untreated plots, with a marketable fruit yield gain exceeding 22% and a cost–benefit ratio greater than 2.8 (BCR~3). Dudu Acelamectin 5% EC outperformed all the other treatments, but needs to be considered with caution due to its non-target effect and resistance development, whereas *M. anisopliae* ICIPE 69 performed the least well. In addition, the findings showed the high degree of efficacy and economic viability of these biopesticides as a potential *T. absoluta* control option in the field. However, it is important to further explore different formulations of these eco-friendly biopesticides, inoculum delivery approach, application frequency, their effectiveness in different agro-ecological zones and compatibility with commonly used pesticides in tomato production systems for sustainable management of *T. absoluta*.

Keywords: *Metarhizium anisopliae*; biopesticide; entomopathogen; *Tuta absoluta*; fruit yield loss; marketable fruit yield gain; cost–benefit ratio

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is grown and consumed worldwide for its nutritional and health benefits to humans [1,2]. Socioeconomically, the crop is a source of livelihood to many rural, peri-urban and urban farmers. In Africa, tomato yield loss due to biotic stress has been worsened by the invasive tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) [3]. Tomato is the primary host of this pest [4,5] onto which the larvae instars penetrate all aerial parts (stems, leaves, flowers and fruits) during cryptic feeding. This invasive pest from South America [6], if not aptly managed, could cause yield loss as high as 100% in certain situations [7,8]. In addition, it is classified as a quarantine pest which leads to tomato trade restrictions [3], and also lowers tomato fruit market value, increases crop protection costs and consequently upsurges tomato fruit price [9].

Farmers in Africa primarily apply synthetic pesticides to mitigate the impact of *T. absoluta* [3,10], leading to increased high-risk pesticides doses and increased crop protection costs as a result of more frequent spraying upon attack. Moreover, the efficacy of these synthetic pesticides is challenged by the rapid development of resistance [11] and the cryptic feeding behaviour of *T. absoluta* larvae [9]. The use of synthetic pesticides is associated with several untenable hazards to humans and the environment, for instance, suppression of non-target beneficial organisms, environmental pollution due to unbiodegradable constituent compounds, toxicity and poisoning to humans leading to chronic health problems such as asthma, hypertension, reproductive complications and cancer [12,13]. Consequently, management of the tomato leafminer using safer alternative control approaches is preferred [14]. Among the sustainable, one safe option being explored is to develop pest-specific microbial biopesticides from entomopathogens [15–18]. For instance, the fungal-based biopesticide is reported to kill the host insect in 7 to 21 days by contact through a process that starts with viable spores attaching to the cuticle of the insect, germinating and producing a penetrating germ tube and establishing a systemic infection which finally kills the host (https://realipm.com (accessed on 4 July 2019)) [19].

The potential pathogenicity of strains of entomopathogens against *T. absoluta*, mainly under laboratory conditions, has previously been reported, for instance, entomopathogenic bacteria [20,21], entomopathogenic nematodes [22–24] and entomopathogenic fungi (EPF) [25–30]. However, the efficacy results under laboratory conditions may not reflect the ecological host range and virulence of entomopathogens in the field [31–33]. Thus, the identified potent entomopathogens need to be validated in the field before being developed into commercial products, deployed, adopted and integrated into the IPM package for any pest.

Research is underway at the International Centre of Insect Physiology and Ecology (*icipe*) to develop entomopathogenic fungal strains of *Metarhizium anisopliae* (Metch.) into a microbial biopesticide for sustainable *T. absoluta* control [16]. For instance, among the strains evaluated under laboratory conditions, Akutse et al. [30] reported that *M. anisopliae* ICIPE 20 caused the 100% mortality of 4th instar larvae, as well as 87.5% mortality of *T. absoluta* adults. Hence, this isolate was earmarked and suggested to be fielded in efficacy trials—a key step in the development of a biopesticide. Meanwhile, the use of *M. anisopliae* ICIPE 69 (Campaign®) against *T. absoluta* in the field was reported [3]; however, the commercial product is not specifically registered for *T. absoluta* control [16] and field efficacy data are scant. We therefore hypothesized that *M. anisopliae* ICIPE 20 and ICIPE 69 are not effective and economically viable for managing *Tuta absoluta* in the field. Therefore, the purpose of this study was to evaluate the efficacy of *M. anisopliae* ICIPE 20 and ICIPE 69 against *T. absoluta* on tomato in the field. An assessment of *T. absoluta* infestation, crop injury severity on leaves and fruits, fruit yield loss and the economic viability of these candidate biopesticide products under natural infestation in the field was conducted.

2. Materials and Methods

2.1. Experimental Site, Field Preparation and Raising Seedlings

Field experiments were conducted at Mukono Zonal Agricultural Research & Development Institute (Mukono ZARDI), Mukono district, Uganda (0°23′02.3″ N 32°44′03.4″ E), for two cropping seasons: season 1 (April–July 2019) and season 2 (December 2019–March 2020). The experimental field was prepared by slashing, ploughing and harrowing. It was then divided into twelve experimental plots, each measuring 4 × 5 m with inter-plot spaces of 1 m in width.

The tomato seedlings (variety: Rambo F1) were raised in a screen house. The seeds were first sown into a seed tray and managed until germination. Polypots of 5 cm in diameter and 10 cm in height were filled with potting soil that was prepared by mixing sieved forest soil (2 parts) and coarse sand (1 part). The seedlings were pricked-out into polypots and managed up to four weeks, at which time they were transplanted.

2.2. Transplanting and Subsequent Field Management

In each experimental plot, transplanting holes were dug at a spacing of 0.60 m within a row and 0.75 m between rows, resulting into 6 rows with 9 plants per row and a population of 54 plants per plot. The transplanted seedlings were watered whenever necessary by means of a watering can, using water obtained from a fishpond. Weeding was conducted as required, mainly using a hand hoe. Mulching was conducted using dry grass. Staking was conducted using bamboo stems. No fungicides, fertilizer or other pesticides were applied to the experiment during the trials. The experimental field was regularly scouted to ascertain presence of *T. absoluta*, based on visual characteristic injury symptoms on the tomato plants [34–36]. The level of *T. absoluta* infestation and leaf and leaflet damage in experimental plots on the date of commencement of treatment application (prior to treatment) was recorded.

2.3. Experimental Design and the Treatments

The experiment was laid out in a randomized complete block design (RCBD) with three replicates. The four treatments involved were applied in the evening between 1600 and 1800 HRS (East African Time) at a weekly interval as a foliar spray. During application, separate hand-operated knapsack sprayers were used for the entomopathogenic fungal products and synthetic chemical pesticide to avoid cross contaminations. The treatments were:

i. *Metarhizium anisopliae* isolate ICIPE 20: This was obtained from the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, as dry conidia produced on grain rice. The freshly produced dry conidia had a >95% viability. The haemocytometer quantification method described by Inglis et al. [37] was used to determine the concentration of conidia per gram of the isolate. A conidial suspension was prepared by adding 0.01 g of *M. anisopliae* ICIPE 20 dry conidia to 100 mls of sterile distilled water mixed with Triton X-100 (0.05%) in a conical flask, and vortexed for 5 min at ~700 rpm. From the suspension, 1 mL was pipetted into the improved Neubauer haemocytometer and, thereafter, conidia were counted under a light microscope. The average number of propagules per 'cell' was multiplied by the volume conversion factor (2.5×10^5) to obtain the number of propagules per ml of suspension. The quantity of dry conidia of *M. anisopliae* ICIPE 20 required to provide a concentration of 1.0×10^9 conidia/mL (equivalent to field application rate of the commercial product *M. anisopliae* ICIPE 69) for field application was computed. Subsequently, the procedure described by Ummidi and Vadlamani [38] was followed in the preparation of an oil-in-water formulation of *M. anisopliae* ICIPE 20. For the aqueous formulation, fungal spores were suspended in water containing 0.05% Integra (sticker, Greenlife Crop Protection Africa Ltd, Nairobi, Kenya) with 0.1% nutrient agar, 0.1% glycerine and 0.5% molasses added as protectants and attractants, respectively, whereas in oil formulation, spores were suspended in canola oil with similar proportions of the sticker, nutrient agar, glycerine and molasses, as described above in aqueous formulation. An aqueous *M. anisopliae* ICIPE 20 propagule suspension was prepared and added to a mixture of Triton X-100 (at 1% *v*/*v*) and canola oil (at 1% *v*/*v*). The mixture was then vortexed to obtain a homogenized stable formulation. During application, the oil-in-water formulation of *M. anisopliae* ICIPE 20 was mixed with water at a rate of 10 mL in 20 L of water and the mixture was applied at a rate of 400 mL/Ha.

ii. *Metarhizium anisopliae* isolate ICIPE 69: This is commercially registered as Campaign® and was obtained from Real IPM (U) Ltd., Kampala, Uganda. It is an oil dispersion containing *M. anisopliae* ICIPE 69 at a concentration of 1.0×10^9 cfu/mL, with a preharvest interval (PHI) of 0 day. The product is registered in South Africa for control of mealybugs, thrips and leafminers, whereas in Uganda, it was registered for thrips, fruit flies, and mealybugs [16]. The microbial biopesticide kills the host insect in 7 to 21 days (https://realipm.com (accessed on 4 July 2019)). During application, the oil-in-water formulation of *M. anisopliae* ICIPE 69 was mixed with water at a rate of 10 mL in 20 L of water and the mixture was applied at the rate of 400 mL/Ha.

iii. Dudu Acelamectin (Abamectin 20 g/L + Acetamiprid 3%): This was obtained from Africa One Farmer's Shop, an agro-input shop in Container Village, Kampala, Uganda. Dudu Acelamectin 5% EC is recommended for effective control of leafminers, thrips, mites, beetles, fruit flies and plant bugs. It has the active ingredients Abamectin 20 g/L + Acetamiprid 3%, with PHI of 7 days (http://bukoolachemicals.com (accessed on 16 June 2019)). The recommended mixing of the pesticide is 20–30 mL of Dudu Acelamectin in 20 L of water, a rate equivalent to 400–500 mL/Ha, to be sprayed at an interval of 7–14 days. During application, this pesticide was mixed with water at a rate of 20 mL in 20 L of water and the mixture was applied at a rate of 400 mL/Ha.

iv. Untreated plot: the negative control plots were sprayed with distilled sterile water at a rate of 400 L/Ha.

2.4. Assessing Tuta absoluta Infestation

The scouting of each plot was conducted to identify *T. absoluta* injury on tomato plants. Through visual observation and counting, the total number of plants in each plot and the number of plants with signs of *T. absoluta* injury were recorded prior to treatment and at the start of harvesting (after treatment). All the injured plants were left in the plots (non-destructive sampling). *Tuta absoluta* infestation was computed using Formula (1) [39]:

$$\textit{Tuta absoluta}\text{ infestation} = \frac{\text{Total number of injured plants in a plot}}{\text{Total number of plants in the plot}} \times 100 \tag{1}$$

2.5. Assessing Leaf and Leaflet Damage by Tuta absoluta

The ten innermost plants from each plot were assessed through visual observation to establish the leaves and leaflets injured by *T. absoluta*. On each plant, the total number of leaves and number of injured leaves were recorded. In addition, the total number of leaflets on injured leaves and number of specific leaflets bearing *T. absoluta* injury symptoms were recorded. As a non-destructive sampling approach, all injured leaves and leaflets were left on the plants. The percentages of damaged leaves and leaflets were then computed using Formulas (2) and (3) [39]:

$$\text{Percentage leaf damage} = \frac{\text{Total number of injured leaves on the plant}}{\text{Total number of leaves on the plant}} \times 100 \tag{2}$$

$$\text{Percentage leaflet damage} = \frac{\text{Total number of injured leaflets on the plant}}{\text{Total number of leaflets on the injured leaves}} \times 100 \tag{3}$$

2.6. Assessing Fruit Damage by Tuta absoluta

The ten innermost plants from each plot were assessed through visual observation to establish fruits injured by *T. absoluta*. On each plant, the total number of fruits and the number of injured fruits was recorded (before the start of harvesting). All the injured fruits were left on the plants after assessment. The percentage fruit damage was then computed using Formula (4) [39]:

$$\text{Percentage fruit damage} = \frac{\text{Total number of injured fruits on the plant}}{\text{Total number of fruits on the plant}} \times 100 \tag{4}$$

2.7. Assessing Fruit Yield Loss Due to Tuta absoluta

The procedure followed was similar to the one described by Ghaderi et al. [40]. Mature fruits at the pink stage of ripening were harvested from the ten innermost plants of each plot. At each harvest, visual fruit inspection was conducted to sort injured fruits. The weights of both injured and healthy fruits were measured using a mechanical Salter kitchen weighing scale and recorded. The percentage fruit yield loss of each plot was then computed as per Formula (5):

$$\text{Fruit yield loss } (\%) = \frac{\text{Weight of injured fruits}}{(\text{Weight of healthy fruits} + \text{Weight of injured fruits})} \times 100 \quad (5)$$

2.8. Assessing the Economic Viability of Treatments

2.8.1. Marketable Fruit Yield in Treated Plots Compared to Untreated Plot

The cumulative weight of healthy (marketable) fruits harvested from the ten innermost plants of each plot was recorded. This weight was used to compute marketable fruits weight per plant, then extrapolated to per plot and eventually marketable fruit yield (MFY) per hectare was computed as described by Shabozoi et al. [41]. The untreated plot was used as a standard for comparison with performance of treatments. The cumulative MFY in the treated plots above the untreated plots was considered as MFY gain, the percentage of which was computed using Formula (6) [42]:

$$\text{MFY gain } (\%) = \frac{\text{MFY in the treated plot } - \text{ MFY in untreated plot}}{\text{MFY in untreated plot}} \times 100 \quad (6)$$

2.8.2. Cost–Benefit Analysis

The cost of the pesticide, pesticide application equipment, labour for pesticide application and labour for harvesting the additional yield of the treated plot above the yield recorded from the untreated control plot were totalled. This total represented the season crop protection cost for each experimental plot, which was extrapolated to cost per hectare. The cost of each unit of *M. anisopliae* ICIPE 20 was equated to the price of each unit of *M. anisopliae* ICIPE 69 (UG Shs. 15,000 (USD 4.05) per 20 mL sachet). The pesticide application equipment bought at UG Shs. 150,000 (USD 40.5) was costed at UG Shs. 50,000 (USD 13.5) based on depreciation over an estimated 3-year lifespan. The costs for other items were taken as per the prevailing market prices. Labour for pesticide application per spray per hectare was fixed at UG Shs. 125,000 (USD 33.78). The harvesting of additional yield from treated plots above the yield from the untreated plot was fixed at an estimated average of UG Shs. 100,000 (USD 27.03) per tonne.

To compute the revenue per hectare for each experimental plot, the average farm-gate price of tomato fruits was fixed at UG Shs. 1200 (USD 0.32) per kilogram [43]. Then, price per kilogram of tomato fruits was multiplied by MFY (kg) per hectare. The revenue from the untreated plot was deducted from that of each treated plot to obtain the benefit (value of yield of treated plot above value of the yield of untreated plot) for the respective treatments, following the approach described by Shabozoi et al. [41]. Thereafter, the cost–benefit ratio (BCR) of each treatment was calculated using Formula (7) [44]:

$$\text{Cost–benefit ratio} = \frac{\text{Benefit of the treatment}}{\text{Treatment's total crop protection cost}} \quad (7)$$

2.9. Data Analysis

The data on *T. absoluta* infestation, percentage damage of leaves and leaflets within each experimental plot prior to treatment and after treatment were subjected to a *t*-test. To compare treatments' *T. absoluta* infestation, damage of leaves, leaflets and fruits, fruit yield loss and marketable fruit yield, the data were subjected to analysis of variance (ANOVA). The differences in means were separated using Fisher's protected least significant difference (LSD) at 5% probability. The analyses were conducted using GenStat computer software (12th Edition for Windows, VSN International Ltd., Hemel Hempstead, UK). The data on MFY gain due to treatment application were expressed as percentage [42], whereas the BCR of each treatment was evaluated using the rule for BCR [45].

3. Results

3.1. *Tuta absoluta* Infestation in the Experimental Field

Prior to treatment, *T. absoluta* infestation ranged from 29.58 ± 3.24 to 31.61 ± 1.25% and 27.03 ± 7.69 to 40.85 ± 3.48%, in season 1 and season 2, respectively. There was no significant difference in *T. absoluta* infestation among the treatment plots during both season 1 ($F_{3,6}$ = 0.14, p = 0.932) and season 2 ($F_{3,6}$ = 2.77, p = 0.133) (Table 1).

Table 1. *Tuta absoluta* mean infestation (±SE) during season 1 (April–July 2019) and season 2 (December 2019–March 2020).

Treatment/Season	Mean ± SE (%)		*t*-Value	*p*-Value (df = 2)
	Prior to Treatment	After Treatment		
Season 1				
Untreated plot	30.01 ± 6.06 a	36.88 ± 2.88 b	−1.36	0.308
Dudu Acelamectin	29.58 ± 3.24 a	20.05 ± 2.00 a	5.01	0.038
M. anisopliae ICIPE 69	31.61 ± 1.25 a	32.64 ± 1.82 b	−0.34	0.763
M. anisopliae ICIPE 20	30.74 ± 2.29 a	32.47 ± 1.18 b	−0.63	0.592
p-value (df = 3)	0.932	0.010		
Season 2				
Untreated plot	33.92 ± 1.17 a	30.94 ± 1.74 a	1.15	0.370
Dudu Acelamectin	39.79 ± 6.83 a	18.51 ± 4.50 a	3.54	0.071
M. anisopliae ICIPE 69	27.03 ± 7.69 a	22.12 ± 4.58 a	1.45	0.283
M. anisopliae ICIPE 20	40.85 ± 3.48 a	29.51 ± 1.89 a	3.43	0.076
p-value (df = 3)	0.133	0.088		

df = degrees of freedom. SE = standard error. In a season, means with the same letter in a column are not significantly different by Fisher's protected LSD test (p = 0.05).

After treatment, the results showed the highest rise in *T. absoluta* infestation (though not significant) within untreated plots (*t* test: t_2 = −1.36, p = 0.308), followed by *M. anisopliae* ICIPE 20 (*t* test: t_2 = −0.63, p = 0.592) and *M. anisopliae* ICIPE 69 (*t* test: t_2 = −0.34, p = 0.763) treated plots, during season 1. However, a significant (*t* test: t_2 = 5.01, p = 0.038) reduction in *T. absoluta* infestation within Dudu Acelamectin-treated plots was observed (Table 1). During season 2, results showed a general reduction in *T. absoluta* infestation (though not significant) which was greatest within Dudu Acelamectin-treated plots, followed by *M. anisopliae* ICIPE 20, *M. anisopliae* ICIPE 69 and the lowest in untreated plots (Table 1). When the treatments were compared, the results showed a significant difference in *T. absoluta* infestation during season 1 ($F_{3,6}$ = 9.72, p = 0.010), but not in season 2 ($F_{3,6}$ = 3.53, p = 0.088) (Table 1). The highest *T. absoluta* infestation was observed in untreated plots and the lowest in Dudu Acelamectin-treated plots during both seasons 1 and 2 (Table 1).

3.2. Leaf Damage by *Tuta absoluta*

Prior to treatment, mean leaf damage by *T. absoluta* ranged from 4.94 ± 1.79 to 7.94 ± 2.54% and 7.30 ± 1.52 to 12.84 ± 6.21%, during season 1 and season 2, respectively. There was no significant difference in the level of leaf damage among the experimental plots during season 1 ($F_{3,6}$ = 1.59, p = 0.288) and season 2 ($F_{3,6}$ = 0.65, p = 0.611) (Table 2).

After treatment, season 1 results showed a significant rise in leaf damage by *T. absoluta* within untreated plots (*t* test: t_2 = −12.86, p = 0.006). The rise in leaf damage by *T. absoluta* was not significant within the plots treated with *M. anisopliae* ICIPE 20 (*t* test: t_2 = 1.04, p = 0.406) and *M. anisopliae* ICIPE 69 (*t* test: t_2 = −0.95, p = 0.442), with the former showing the least. On the other hand, reduced leaf damage by *T. absoluta* (though not significant) was observed within Dudu Acelamectin-treated plots (Table 2). During season 2, the results showed reduction in leaf damage by *T. absoluta* (though not significant) which was greatest within Dudu Acelamectin, followed by *M. anisopliae* ICIPE 69 and lowest in *M. anisopliae* ICIPE 20-treated plots. The untreated plots showed increased leaf damage by *T. absoluta*, though this was not significant (Table 2). When the treatments were compared, the results showed a significant difference in leaf damage by *T. absoluta* during season 1 ($F_{3,6}$ = 98.60, $p < 0.001$). Leaf damage levels were significantly lower in Dudu Acelamectin, *M. anisopliae*

ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots than untreated plots (Table 2). During season 2, there was no significant difference observed ($F_{3,6}$ = 3.70, p = 0.081). The highest leaf damage by *T. absoluta* was observed in untreated plots, whereas the lowest was in Dudu Acelamectin-treated plots (Table 2).

Table 2. Mean leaf damage (±SE) by *Tuta absoluta* during season 1 (April–July 2019) and season 2 (December 2019–March 2020).

Treatment/Season	Mean ± SE (%)		*t*-Value	*p*-Value (df = 2)
	Prior to Treatment	After Treatment		
Season 1				
Untreated plot	4.94 ± 1.79 a	18.58 ± 2.39 d	−12.86	0.006
Dudu Acelamectin	7.94 ± 2.54 a	6.06 ± 1.46 a	2.50	0.130
M. anisopliae ICIPE 69	6.91 ± 1.78 a	12.76 ± 1.49 c	−0.95	0.442
M. anisopliae ICIPE 20	7.82 ± 1.46 a	8.78 ± 2.39 b	1.04	0.406
p-value (df = 3)	0.288	<0.001		
Season 2				
Untreated plot	7.30 ± 1.52 a	11.36 ± 2.68 a	−1.85	0.205
Dudu Acelamectin	10.46 ± 4.26 a	4.86 ± 1.56 a	1.80	0.214
M. anisopliae ICIPE 69	12.84 ± 6.21 a	8.96 ± 2.77 a	0.79	0.513
M. anisopliae ICIPE 20	8.12 ± 5.34 a	6.96 ± 2.32 a	0.22	0.846
p-value (df = 3)	0.611	0.081		

df = degrees of freedom. SE = standard error. In a season, means with the same letter in a column are not significantly different by Fisher's protected LSD test (p = 0.05).

3.3. Leaflet Damage by Tuta absoluta

Prior to treatment, mean leaflet damage was not significantly different among the various plots during season 1 ($F_{3,6}$ = 1.12, p = 0.414) and season 2 ($F_{3,6}$ = 1.03, p = 0.445). The level of leaflet damage ranged from 15.07 ± 1.77 to 18.78 ± 1.28%, and 19.81 ± 10.27 to 34.40 ± 2.39%, in season 1 and season 2, respectively (Table 3).

Table 3. Mean leaflet damage (±SE) by *Tuta absoluta* during season 1 (April–July 2019) and season 2 (December 2019–March 2020).

Treatment/Season	Mean ± SE (%)		*t*-Value	*p*-Value (df = 2)
	Prior to Treatment	After Treatment		
Season 1				
Untreated plot	15.07 ± 1.77 a	28.84 ± 1.62 c	−3.62	0.069
Dudu Acelamectin	15.50 ± 1.93 a	15.54 ± 0.65 a	0.60	0.612
M. anisopliae ICIPE 69	18.78 ± 1.28 a	23.94 ± 1.32 b	−2.35	0.143
M. anisopliae ICIPE 20	17.06 ± 0.33 a	20.93 ± 1.50 b	−3.50	0.073
p-value (df = 3)	0.414	0.002		
Season 2				
Untreated plot	21.16 ± 2.13 a	24.17 ± 5.17 a	−0.99	0.427
Dudu Acelamectin	34.40 ± 2.39 a	14.40 ± 2.04 a	6.14	0.026
M. anisopliae ICIPE 69	19.81 ± 10.27 a	17.33 ± 3.54 a	0.36	0.752
M. anisopliae ICIPE 20	22.25 ± 5.66 a	15.12 ± 3.19 a	2.48	0.131
p-value (df = 3)	0.445	0.238		

df = degrees of freedom. SE = standard error. In a season, means with the same letter in a column are not significantly different by Fisher's protected LSD test (p = 0.05).

After treatment, the season 1 results showed general rise of leaflet damage by *T. absoluta* (though not significant) which was lowest within Dudu Acelamectin, followed by *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots, and greatest within untreated plots (Table 3). During season 2, the results showed reduction in leaflet damage by *T. absoluta* (though not significant) which was greater within *M. anisopliae* ICIPE 20 compared to *M. anisopliae* ICIPE 69-treated plots. Dudu Acelamectin-treated plots showed a significant reduction in leaflet damage (*t* test: t_2 = 6.14, p = 0.026), whereas a rise of leaflet damage by *T. absoluta* (though not significant) was observed within untreated plots (Table 3). When the treatments were compared, the results showed a significant difference in leaflet damage by *T. absoluta* during season 1 ($F_{3,6}$ = 17.08, p = 0.002). Leaflet damage levels were significantly lower in Dudu Acelamectin, *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots than untreated plots (Table 3). During season 2, there was no significant difference observed ($F_{3,6}$ = 1.86, p = 0.238). However, leaflet damage by *T. absoluta* was lowest in Dudu Acelamectin-treated plots, followed by *M. anisopliae* ICIPE 20 and highest in untreated plots (Table 3).

3.4. Fruit Damage by Tuta absoluta

The results showed a significant difference in fruit damage by *T. absoluta* during season 1 ($F_{3,6}$ = 5.17, p = 0.042). Fruit damage was lowest in Dudu Acelamectin-treated plots and highest in untreated plots. Significantly lower fruit damage was observed in plots treated with Dudu Acelamectin and *M. anisopliae* ICIPE 20 compared to untreated plots (Table 4). During season 2, there was no significant differences observed ($F_{3,6}$ = 1.36, p = 0.341). However, fruit damage level was lowest in Dudu Acelamectin-treated plots followed by *M. anisopliae* ICIPE 20, *M. anisopliae* ICIPE 69 and highest in untreated plots (Table 4).

Table 4. Fruit damage, fruit yield loss, marketable fruit yield (MFY) and MFY gain during season 1 (April–July 2019) and season 2 (December 2019–March 2020).

Treatment/Season	Mean ± SE (%)		MFY (ton/ha)	MFY Gain [1] (%)
	Fruit Damage	Fruit Yield Loss		
Season 1				
Untreated plot	26.48 ± 4.13 b	43.41± 2.63 b	4.81± 0.71 a	-
Dudu Acelamectin	10.87 ± 1.62 a	6.73 ± 3.64 a	11.07± 1.18 a	130.15
M. anisopliae ICIPE 69	18.84 ± 2.61 ab	18.41 ± 2.94 a	7.47± 1.94 a	55.30
M. anisopliae ICIPE 20	13.92 ± 1.89 a	10.48 ± 4.92 a	8.28± 1.72 a	72.14
p-value (df = 3)	0.042	0.001	0.173	
Season 2				
Untreated plot	6.03 ± 2.21 a	13.01 ± 0.47 c	11.04± 2.86 a	-
Dudu Acelamectin	2.81 ± 0.61 a	2.82 ± 0.48 a	15.59± 1.06 a	41.21
M. anisopliae ICIPE 69	4.47 ± 1.41 a	6.58 ± 1.14 b	12.79± 1.38 a	15.85
M. anisopliae ICIPE 20	3.21 ± 1.06 a	4.90 ± 0.95 b	13.47± 2.25 a	22.01
p-value (df = 3)	0.341	<0.001	0.536	

df = degrees of freedom. SE = standard error. In a season, means with the same letter in a column are not significantly different by Fisher's protected LSD test (p = 0.05). [1] Equation (6).

3.5. Fruit Yield Loss Due to Tuta absoluta

The results showed significant differences in fruit yield loss due *T. absoluta* during both season 1 ($F_{3,6}$ = 22.38, p = 0.001) and season 2 ($F_{3,6}$ = 68.81, p < 0.001). During season 1, fruit yield loss was significantly lower in Dudu Acelamectin, *M. anisopliae* ICIPE 20-and *M. anisopliae* ICIPE 69-treated plots compared to untreated plots (Table 4). During season 2, significantly higher fruit yield loss was observed in untreated plots compared to other treatments. In addition, fruit yield loss in Dudu Acelamectin-treated plots was significantly lower compared to *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots (Table 4).

3.6. Marketable Yield Gain Due to Treatments for Managing Tuta absoluta on Tomato in the Field

The results showed greater marketable fruit yield (MFY) in all treated plots compared to untreated plots during both season 1 and season 2 (Table 4). During season 1, the least well performing *M. anisopliae* ICIPE 69-treated plots showed 2.66 ton/ha above the untreated plots, whereas the best performing Dudu Acelamectin-treated plots showed an excess of 6.26 ton/ha. Accordingly, overall MFY gain exceeded 55% during season 1, lowest in *M. anisopliae* ICIPE 69-treated plots and highest in Dudu Acelamectin-treated plots (Table 4). During season 2, a similar trend of treatment performance was observed, with overall MFY gain exceeding 15%. The least well performing *M. anisopliae* ICIPE 69-treated plots showed 1.75 ton/ha above the untreated plots, whereas the best performing Dudu Acelamectin-treated plots showed an excess of 4.55 ton/ha (Table 4).

3.7. Cost–Benefit Ratio of the Treatments for Managing Tuta absoluta on Tomato in the Field

The results showed that revenue from MFY per hectare was lowest in untreated plots compared to other treatments during both season 1 and season 2. During season 1, the revenue was highest in Dudu Acelamectin-treated plots, followed by *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots, respectively. Accordingly, the benefit of treatment application was greatest in Dudu Acelamectin-treated plots, followed by *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots, respectively (Table 5). A similar trend of treatment performance was observed during season 2 (Table 5). The total crop protection cost was highest in *M. anisopliae* ICIPE 20-treated plots, followed by *M. anisopliae* ICIPE 69, and lowest in Dudu Acelamectin-treated plots, during both season 1 and season 2 (Table 5). Concomitantly, the highest BCR was observed in Dudu Acelamectin-treated plots, followed by *M. anisopliae* ICIPE 20-treated plots, and lowest in *M. anisopliae* ICIPE 69-treated plots, during both season 1 and season 2 (Table 5).

Table 5. Revenue, benefit, crop protection cost and cost–benefit ratio (BCR) per hectare for the treatments during season 1 (April–July 2019) and season 2 (December 2019–March 2020).

Treatment/Season	Revenue/ha (USD)	Benefit/ha [1] (USD)	Crop Protection Cost/ha (USD)				BCR [2]
			Pesticide	Labour	Sprayer	Total	
Season 1							
Untreated plot	1560.00	-	-	-	-	-	-
Dudu Acelamectin	3590.27	2030.27	17.84	196.21	13.51	227.56	8.92
M. anisopliae ICIPE 69	2422.70	862.70	121.62	115.94	13.51	251.07	3.43
M. anisopliae ICIPE 20	2685.41	1125.41	121.62	125.67	13.51	260.80	4.31
Season 2							
Untreated plot	3580.54	-	-	-	-	-	-
Dudu Acelamectin	5056.22	1475.68	17.84	202.97	13.51	234.32	6.30
M. anisopliae ICIPE 69	4148.11	567.57	121.62	129.73	13.51	264.86	2.14
M. anisopliae ICIPE 20	4368.65	788.11	121.62	142.70	13.51	277.83	2.84

[1] (Revenue from each treatment minus revenue from untreated plot). [2] Equation (7).

4. Discussion

The results of both season 1 and season 2 generally demonstrated a degree of restriction of *T. absoluta* infestation and injury severity on leaves and fruits where *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 were applied. Similar findings have also been reported by El-Aassar et al. [46] in Egypt where the biopesticides Biovar® and Bioranza® with *Beauveria bassiana* (Balsamo.) and *M. anisopliae* as active ingredients, respectively, were found to be efficient against *T. absoluta* larvae and larval infestation in the field. In addition, this study found significantly lower leaf damage, leaflet damage and fruit damage during season 1 in *M. anisopliae* ICIPE 20-treated plots compared to untreated plots, as reported by Shiberu and Getu [47] when using *B. bassiana* to tackle the pest in Ethiopia. In fact, untreated plots generally showed highest *T. absoluta* infestation, leaf damage and leaflet and fruit damage in both season 1 and 2. Interestingly, tomato fruit yield loss was significantly lower in plots treated with *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 than that of untreated plots during both seasons. This phenomenon seems to point towards a level of field efficacy of these fungal entomopathogens for managing *T. absoluta* on tomato, as also reported by previous studies [46,47]. Although there exists scant information on the field efficacy of fungal entomopathogens against *T. absoluta*, the findings of this study seem to concur with previous field studies of: (i) El-Aassar et al. [46], where reduction in tomato leaf area infestation by application of *M. anisopliae* (Bioranza®) was reported, and (ii) Shiberu and

Getu [47], which reported a reduction in tomato fruit yield loss due to application of *M. anisopliae*.

Our results further showed a marketable fruit yield gain in *M. anisopliae* ICIPE 20- and *M. anisopliae* ICIPE 69-treated plots. This is an indication of improved marketable fruit yield through the application of the fungal entomopathogens compared to untreated plots. The higher marketable fruit yield in the treated plots compared to the untreated plots seems to imply a level of suppression of the activity of *T. absoluta.* As a result, there was better photosynthesis, growth, development, flower and fruit retention by tomato plants, and hence better yields, and also less damaged fruits in the treated plots. In fact, the fruit yield gain can be a desirable efficacy parameter in the evaluation of these *T. absoluta* management products [48]. These findings seem to concur with field studies of Ndereyimana et al. [49] and Shiberu and Getu [47], that reported improved tomato productivity in plots treated with *M. anisopliae* compared to the untreated control. Alongside the marketable fruit yield gain, the cost–benefit ratio (BCR) of applying *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 exceeded 1, during both season 1 and 2. The BCR > 1 is among the indicators of economic viability of pest control measures. In spite of the shortage of data on the BCR of fungal entomopathogens against *T. absoluta* on tomato, the findings of this study seem to corroborate those on other lepidopteran pests where BCR values > 1 were reported such as (i) the use of *M. anisopliae* against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) [50] and (ii) the use *B. bassiana* against *H. armigera* [51]. Our results further corroborate previous studies on non-lepidopteran pests including (i) the use of *B. bassiana* and *Lecanicillium lecanii* against the sucking pests of green gram [52], (ii) the use of *B. bassiana* against *Clavigralla gibbosa* Spinola (Hemiptera: Coreidae) [53] and (iii) the use of *B. bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* against groundnuts pests [54].

The findings generally indicated that Dudu Acelamectin performed better than *M. anisopliae* ICIPE 20, which also performed better than *M. anisopliae* ICIPE 69 for all parameters assessed. The observed outperformance trend for Dudu Acelamectin was probably due to the quicker action and broad-spectrum nature of this synthetic pesticide (http://bukoolachemicals.com (accessed on 16 June 2019)). Furthermore, the performance of the fungal entomopathogens may probably be attributed to their slow infection mechanism as compared to synthetic pesticide [55]; the dosage used may not be the most ideal and their vulnerability to field weather conditions [33,56]. The better performance of *M. anisopliae* ICIPE 20 compared to *M. anisopliae* ICIPE 69 could be related to its high efficacy under laboratory conditions where mortality rates of 87.5 and 100% were recorded among *T. absoluta* adults and fourth instar larvae, respectively [30]. In addition, the lower performance of *M. anisopliae* ICIPE 69 compared to ICIPE 20 may be attributed to the fact that the former could be more effective in controlling leafminers other than *T. absoluta* [16].

Furthermore, although the synthetic pesticide Dudu Acelamectin consistently outperformed the biopesticide products (*M. anisopliae* ICIPE 20 and ICIPE 69) does not necessarily imply that it is the best option for managing *T. absoluta*. It is globally acknowledged that synthetic pesticides are a great danger to biodiversity as they kill non-target beneficial organisms and cause environmental pollution including toxicity and poisoning to humans which can lead to chronic health problems [12,13]. Therefore, in spite of the lower performance of *M. anisopliae* ICIPE 20 and ICIPE 69 compared to Dudu Acelamectin, the entomopathogenic fungal biopesticides are generally associated with plenty of non-monetary benefits. For instance, they are not toxic and poisonous to humans [57], are harmless to beneficial organisms such as pollinators [58], leave no toxins in the environment [59], leave no toxic residues in the food product [60] and the pest cannot develop resistance against them [16]. In addition, there is no risk of an alarming rise in the EPF inoculum levels in agricultural fields when applications are stopped [61], therefore strengthening the safety advantages of biopesticides as nature-based solutions to the environment, human and biodiversity health [62]. These attributes eventually make the fungal biopesticides more appealing in food crop farming systems. However, the study efficacy results reported herein might not be conclusive, as they are based on one agro-ecological zone, on small scale, using a single dosage and formulation of candidate entomopathogenic fungal biopesticide products.

5. Conclusions

The findings could be a promising milestone for the candidate entomopathogenic fungal biopesticides for managing *T. absoluta* on tomato sustainably in the field. The biopesticide products showed better results compared to untreated plot, with *M. anisopliae* isolate ICIPE 20 being found to be more efficacious than ICIPE 69, and with a BCR of 4.31. In addition, the marketable fruit yield gain and the cost–benefit ratio (BCR) of applying *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 exceeded 1 during both cropping seasons. Considering the BCR > 1 and safety of these tested bioproducts, which are among the key indicators of the economic viability of pest control measures, *M. anisopliae* ICIPE 20 could be developed as a potential biopesticide for the sustainable management of *T. absoluta*. However, further studies are warranted before they are developed into commercial products, and deployed and promoted for safer control of *T. absoluta* in tomato production systems. Therefore, further studies are recommended to assess *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 at different dosages and formulations, application frequency, different agro-ecological zones, large scale and also assess their compatibility with the pesticides commonly used in tomato production. In addition, despite the good performance of Dudu Acelamectin, this synthetic pesticide should be used with caution for *T. absoluta* management because of its toxicity, causing an increase in environmental pollution, and development of pest-resistant pest populations, as well as the effects on non-target organisms, especially pollinators and natural enemies, and the cost implications.

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References

1. Rodrigues, P.H.M.; Kupski, L.; Souza, T.D.D.; Arias, O.L.J.; D'Oca, M.M.; Furlong, E.B. Relations between nutrients and bioactive compounds of commercial tomato varieties by the Principal Component Analysis. *Food Sci. Technol.* **2021**, *42*, 1–8. [CrossRef]
2. Luna-Guevara, M.L.; Jiménez-gonzález, Ó.; Luna-guevara, J.J.; Hernández-carranza, P.; Ochoa-Velasco, C.E. Quality parameters and bioactive compounds of red tomatoes (*Solanum lycopersicum* L.) cv Roma VF at different postharvest conditions. *J. Food Res.* **2014**, *3*, 8–18. [CrossRef]
3. Rwomushana, I.; Chipabika, G.; Tambo, J.; Pratt, C.; Moreno, P.G.; Beale, T.; Lamontagne-Godwin, J.; Makale, F.; Day, R. Evidence Note. Tomato leafminer (*Tuta absoluta*): Impacts and coping strategies for Africa. *CABI Work. Pap.* **2019**, 12. [CrossRef]
4. Sridhar, V.; Nitin, K.S.S.O.N.; Nagaraja, T. Comparative biology of South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on three solanaceous host plants. *Pest Manag. Hortic. Ecosyst.* **2015**, *21*, 159–161.
5. Younes, A.A.; Nawal, Z.M.; Hazem, A.F.; Reham, F. Preference and performance of the tomato leafminer, *Tuta absoluta* (Lepidoptera-Gelechiidae) towards three Solanaceous host plant species. *CPQ Microbiol.* **2018**, *1*, 1–16.
6. Biondi, A.; Guedes, R.N.C.; Wan, F.; Desneux, N. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: Past, present, and future. *Annu. Rev. Entomol.* **2018**, *63*, 239–258. [CrossRef] [PubMed]
7. Desneux, N.; Wajnberg, E.; Wyckhuys, K.A.G.; Burgio, G.; Arpaia, S.; Narváez-Vasquez, A.C.; González-Cabrera, J.; Ruescas, D.C.; Tabone, E.; Frandon, J.; et al. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *J. Pest Sci.* **2010**, *83*, 197–215. [CrossRef]
8. Mansour, R.; Brévault, T.; Chailleux, A.; Cherif, A.; Grissa-Lebdi, K.; Haddi, K.; Mohamed, S.A.; Nofemela, R.S.; Oke, A.; Sylla, S.; et al. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomol. Gen.* **2018**, *38*, 83–112. [CrossRef]
9. Retta, A.; Berhe, D. Tomato leafminer–*Tuta absoluta* (Meyrick), a devastating pest of tomatoes in the highlands of Northern Ethiopia: A call for attention and action. *Res. J. Agric. Environ. Manag.* **2015**, *4*, 264–269.
10. Aigbedion-atalor, P.O.; Hill, M.P.; Zalucki, M.P.; Obala, F.; Idriss, G.E.; Midingoyi, S.K.; Chidege, M.; Ekesi, S.; Mohamed, S.A. The South America tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), spreads its wings in Eastern Africa: Distribution and socioeconomic impacts. *J. Econ. Entomol.* **2019**, *112*, 2797–2807. [CrossRef]
11. Guedes, R.N.C.; Roditakis, E.; Campos, M.R.; Haddi, K.; Bielza, P.; Siqueira, H.A.A.; Tsagkarakou, A.; Vontas, J.; Nauen, R. Insecticide resistance in the tomato pinworm *Tuta absoluta*: Patterns, spread, mechanisms, management and outlook. *J. Pest Sci.* **2019**, *92*, 1329–1342. [CrossRef]
12. Aktar, W.; Sengupta, D.; Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip Toxicol.* **2009**, *2*, 1–12. [CrossRef] [PubMed]
13. Kim, K.H.; Kabir, E.; Jahan, S.A. Exposure to pesticides and the associated human health effects. *Sci. Total Environ.* **2017**, *575*, 525–535. [CrossRef] [PubMed]
14. Aynalem, B. Tomato leafminer [(*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)] and its current ecofriendly management strategies: A review. *J. Agric. Biotechnol. Sustain. Dev.* **2018**, *10*, 11–24. [CrossRef]
15. Koul, O. Microbial biopesticides: Opportunities and challenges. *Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2011**, *6*, 1–26. [CrossRef]
16. Akutse, K.S.; Subramanian, S.; Maniania, N.; Dubois, T.; Ekesi, S. Biopesticide research and product development in Africa for sustainable agriculture and food security–Experiences from the International Centre of Insect Physiology and Ecology (*icipe*). *Front. Sustain. Food Syst.* **2020**, *4*, 1–14. [CrossRef]
17. Tarusikirwa, V.L.; Machekano, H.; Mutamiswa, R.; Chidawanyika, F.; Nyamukondiwa, C. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on the "offensive" in Africa: Prospects for integrated management initiatives. *Insects* **2020**, *11*, 764. [CrossRef]
18. Erasmus, R.; van den Berg, J.; du Plessis, H. Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) Pupae to Soil Applied Entomopathogenic Fungal Biopesticides. *Insects* **2021**, *12*, 515. [CrossRef]
19. Murtaza, G.; Naeem, M.; Manzoor, S.; Khan, H.A.; Eed, E.M.; Majeed, W.; Makki, H.A.; Ramzan, U.; Ummara, U.E. Biological control potential of entomopathogenic fungal strains against peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *PeerJ* **2022**, *10*, e13316. [CrossRef]
20. Gonzalez-Cabrera, J.; Molla, O.; Monton, H.; Urbaneja, A. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioControl* **2011**, *56*, 71–80. [CrossRef]
21. Alsaedi, G.; Ashouri, A.; Talaei-hassanloui, R. Evaluation of *Bacillus thuringiensis* to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. *Agric. Sci.* **2017**, *8*, 591–599. [CrossRef]
22. Batalla-carrera, L.; Morton, A.; Garcia-del-Pino, F. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* **2010**, *55*, 523–530. [CrossRef]
23. Kamali, S.; Karimi, J.; Koppenhöfer, A.M. New insight into the management of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *Biol. Microb. Control* **2018**, *111*, 112–119. [CrossRef]
24. Ndereyimana, A.; Nyalala, S.; Murerwa, P.; Gaidashova, S. Potential of entomopathogenic nematode isolates from Rwanda to control the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt J. Biol. Pest Control* **2019**, *29*, 1–7. [CrossRef]
25. Contreras, J.; Mendoza, J.E.; Martínez-Aguirre, M.R.; García-Vidal, L.; Izquierdo, J.; Bielza, P. Efficacy of entomopathogenic fungus *Metarhizium anisopliae* against *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **2014**, *107*, 121–124. [CrossRef]

26. Tadele, S.; Emana, G. Entomopathogenic Effect of *Beauveria bassiana* (Bals.) and *Metarrhizium anisopliae* (Metschn.) on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae under laboratory and glasshouse conditions in Ethiopia. *J. Plant Pathol. Microbiol.* **2017**, *8*, 8–11. [CrossRef]
27. Alikhani, M.; Safavi, S.A.; Iranipour, S. Effect of the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, on demographic fitness of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt J. Biol. Pest Control* **2019**, *29*, 1–7. [CrossRef]
28. Ndereyimana, A.; Nyalala, S.; Murerwa, P.; Gaidashova, S. Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt J. Biol. Pest Control* **2019**, *29*, 23. [CrossRef]
29. Zekeya, N.; Mtambo, M.; Ramasamy, S.; Chacha, M.; Ndakidemi, P.A.; Mbega, E.R. First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania. *Biocontrol Sci. Technol.* **2019**, *29*, 626–637. [CrossRef]
30. Akutse, K.S.; Subramanian, S.; Khamis, F.M.; Ekesi, S.; Mohamed, S.A. Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. *J. Appl. Entomol.* **2020**, *144*, 777–787. [CrossRef]
31. Hajek, A.E.; Goettel, M.S. Guidelines for evaluating effects of entomopathogens on non-target organisms. In *Field Manual of Techniques in Invertebrate Pathology*, 2nd ed.; Lacey, L.A., Kaya, H.K., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 815–833.
32. Wraight, S.P.; Inglis, G.D.; Goettel, M.S. Fungi. In *Field Manual of Techniques in Invertebrate Pathology*, 2nd ed.; Lacey, L.A., Kaya, H.K., Eds.; Springer: Dordrecht, The Netherlands, 2007; pp. 223–248.
33. Jaronski, S.T. Ecological factors in the inundative use of fungal entomopathogens. *BioControl* **2010**, *55*, 159–185. [CrossRef]
34. Sridhar, V.; Chakravarthy, A.K.; Asokan, R.; Vinesh, L.S.; Rebijith, K.B. New record of the invasive South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India. *Pest Manag. Hortic. Ecosyst.* **2014**, *20*, 148–154.
35. Tumuhaise, V.; Khamis, F.M.; Agona, A.; Sseruwu, G.; Mohamed, S.A. First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Uganda. *Int. J. Trop Insect Sci.* **2016**, *36*, 135–139. [CrossRef]
36. Simmons, A.; Wakil, W.; Qayyum, M.; Ramasamy, S.; Kuhar, T.; Philips, C.R. Lepidopterous pests: Biology, ecology, and management. In *Sustainable Management of Arthropod Pests of Tomato*; Wakil, W., Brust, G.E., Perring, T.M., Eds.; Academic Press: London, UK, 2018; pp. 131–152.
37. Inglis, G.D.; Enkerli, J.; Goettel, M.S. Laboratory techniques used for entomopathogenic fungi: Hypocreales. In *Manual of Techniques in Invertebrate Pathology*, 2nd ed.; Lacey, L.A., Ed.; Elsevier Ltd: London, UK, 2012; pp. 189–253.
38. Ummidi, V.R.S.; Vadlamani, P. Preparation and use of oil formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* larvae. *Afr. J. Microbiol. Res.* **2014**, *8*, 1638–1644. [CrossRef]
39. Rasheed, V.A.; Rao, S.K.; Babu, T.R.; Krishna, T.M.; Reddy, B.B.; Naidu, G.M. Infestation of South American tomato leafminer, *Tuta absoluta* (Meyrick) in Chittoor district of Andhra Pradesh, India. *J. Entomol. Zool. Stud.* **2018**, *6*, 2407–2414.
40. Ghaderi, S.; Fathipour, Y.; Asgari, S.; Reddy, G.V.P. Economic injury level and crop loss assessment for *Tuta absoluta* (Lepidoptera: Gelechiidae) on different tomato cultivars. *J. Appl. Entomol.* **2019**, *143*, 493–507. [CrossRef]
41. Shabozoi, N.U.K.; Abro, G.H.; Syed, T.S.; Awan, M.S. Economic appraisal of pest management options in okra. *Pakistan J. Zool.* **2011**, *43*, 869–878.
42. Banerjee, A.; Pal, S. Estimation of crop losses due to major insect pests of field pea in Gangetic plains of West Bengal. *J. Entomol. Zool. Stud.* **2020**, *8*, 1063–1067. Available online: http://www.entomoljournal.com (accessed on 9 July 2021).
43. Dijkxhoorn, Y.; Galen, M.V.; Barungi, J.; Okiira, J.; Gema, J.; Janssen, V. *The Uganda Vegetables and Fruit Sector: Competitiveness, Investment and Trade Options*; Wageningen Economic Research: Wageningen, The Netherlands, 2019; p. 80. [CrossRef]
44. Shiberu, T.; Getu, E. Experimental Analysis of Economic action level of tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on tomato plant under open field. *Adv. Crop Sci. Technol.* **2018**, *6*, 327. [CrossRef]
45. Gayi, D.; Ocen, D.; Lubadde, G.; Serunjogi, L. Efficacy of bio and synthetic pesticides against the American bollworm and their natural enemies on cotton. *Uganda J. Agric. Sci.* **2016**, *17*, 67–81. [CrossRef]
46. El-Aassar, M.R.; Soliman, M.H.A.; Abd-Elaal, A.A. Efficiency of sex pheromone traps and some bio and chemical insecticides against tomato borer larvae, *Tuta absoluta* (Meyrick) and estimate the damages of leaves and fruit tomato plant. *Ann. Agric. Sci.* **2015**, *60*, 153–156. [CrossRef]
47. Shiberu, T.; Getu, E. Evaluation of bio-pesticides on integrated management of tomato leafminer, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera) on tomato crops in Western Shewa of Central Ethiopia. *Entomol. Ornithol. Herpetol.* **2018**, *7*, 1–8. [CrossRef]
48. Food and Agricultural Organisation (FAO). *International Code of Conduct on the Distribution and Use of Pesticides: Guidelines on Efficacy Evaluation for the Registration of Plant Protection Products*; FAO: Rome, Italy, 2006; p. 61. Available online: https://www.fao.org/3/bt474e/BT474E.pdf (accessed on 15 June 2021).
49. Ndereyimana, A.; Nyalala, S.; Murerwa, P.; Gaidashova, S. Growth, yield and fruit quality of tomato under different integrated management options against *Tuta absoluta* Meyrick. *Adv. Hort. Sci.* **2020**, *34*, 123–132. [CrossRef]
50. Sathish, B.N.; Singh, V.V.; Kumar, S.; Kumar, S. Incremental cost-benefit ratio of certain chemical and bio- pesticides against tomato fruit borer, *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) in tomato crop. *Bull. Environ. Pharmacol. Life Sci.* **2018**, *7*, 102–106.
51. Ojha, P.K.; Kumari, R.; Chaudhary, R.S.; Pandey, N.K. Incremental cost-benefit ratio of certain bio- pesticides against *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) in chickpea. *Legum. Res. Int.* **2017**, *42*, 119–126. [CrossRef]

52. Sujatha, B.; Bharpoda, T.M. Bio-efficacy of biopesticides against sucking pests in green gram grown during Kharif. *Int. J. Pure App. Biosci.* **2017**, *5*, 1827–1834. [CrossRef]
53. Narasimhamurthy, G.M.; Keval, R. Field evaluation of some insecticides and bio-pesticide against tur pod bug, *Clavigralla gibbosa* (Spinola) in long duration pigeonpea. *Afr. J. Agric. Res.* **2013**, *8*, 4876–4881. [CrossRef]
54. Sahayaraj, K.; Namachivayam, S.K.R. Field evaluation of three entomopathogenic fungi on groundnut pests. *Tropicultura* **2011**, *29*, 143–147.
55. Maina, U.M.; Galadima, I.B.; Gambo, F.M.; Zakaria, D. A review on the use of entomopathogenic fungi in the management of insect pests of field crops. *J. Entomol. Zool. Stud.* **2018**, *6*, 27–32.
56. Agbessenou, A.; Akutse, K.S.; Yusuf, A.A.; Wekesa, S.W.; Khamis, F.M. Temperature-dependent modelling and spatial prediction reveal suitable geographical areas for deployment of two *Metarhizium anisopliae* isolates for *Tuta absoluta* management. *Sci. Rep.* **2021**, *11*, 23346. [CrossRef]
57. Zimmermann, G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Sci. Technol.* **2007**, *17*, 553–596. [CrossRef]
58. Omuse, E.R.; Niassy, S.; Wagacha, J.M.; Ong'amo, G.O.; Lattorff, H.M.G.; Kiatoko, N.; Mohamed, S.A.; Subramanian, S.; Akutse, K.S.; Dubois, T. Susceptibility of the western honey bee *Apis mellifera* and the African stingless bee *Meliponula ferruginea* (Hymenoptera: Apidae) to the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. *J. Econ. Entomol.* **2021**, *115*, 46–55. [CrossRef] [PubMed]
59. Zimmermann, G. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Sci. Technol.* **2007**, *17*, 879–920. [CrossRef]
60. Skinner, M.; Parker, B.L.; Kim, J.S. Role of entomopathogenic fungi in integrated pest management. In *Integrated Pest Management: Current Concepts and Ecological Perspectives*; Abrol, D.P., Ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2014; pp. 169–192. [CrossRef]
61. Scheepmaker, J.W.A.; Butt, T.M. Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. *Biocontrol Sci. Technol.* **2010**, *20*, 503–552. [CrossRef]
62. Curl, C.L.; Spivak, M.; Phinney, R.; Montrose, L. Synthetic pesticides and health in vulnerable populations: Agricultural workers. *Curr. Environ. Health Rep.* **2020**, *7*, 13–29. [CrossRef]