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Evaluation of Bio-Pesticides against the South American Tomato Leaf Miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in India

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Abstract: *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most economically important pests of tomato worldwide. Despite its global importance, the management of this oligophagous pest has still been a challenging task, due to its high capability to develop resistance against synthetic insecticides. Given the limited studies on the effectiveness of different bio-pesticides in India, the objective of this research was to determine the pathogenicity of different commercial formulations of *Beauveria bassiana*, *Bacillus thuringiensis*, and neem (Azadirachtin), against *T. absoluta*, under laboratory and field conditions. For the *Bacillus thuringiensis* formulations, Green Larvicide® and Delfin® recorded an LC_{50} of 4.10 × 10^9 CFU/mL and 8.06 × 10^6 spores/mg, respectively, while for the *B. bassiana* formulations, Green Beauveria® and BB Power® were 4.473 × 10^7 spores/mL and 1.367 × 10^7 CFU/g, respectively. Furthermore, the results showed high susceptibility to both the commercial neem formulations with Ecotin®, recording an LC_{50} of 91.866 ppm, and Econeem Plus® recording 212.676 ppm. The results from the field conditions at different locations of Andhra Pradesh, India, showed significant differences ($p < 0.001$) for leaf and fruit infestation among the interaction effect of treatments and locations. Bio-pesticides, especially neem and *B. thuringiensis* formulations, reduced *T. absoluta* infestation similarly to the chemical treatment, without affecting the yield. Therefore, bio-pesticides can be considered as safe alternatives to synthetic pesticides, for the management of *T. absoluta*.

Keywords: azadirachtin; *Bacillus thuringiensis*; *Beauveria bassiana*; bioassay; bio-pesticides

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1. Introduction

Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) has become a major food crop in less than a century, mainly due to its outstanding nutritive value, with many health benefits and the ability to produce high yields per unit area [1,2]. The estimated world's production of tomato is about 182 million tons from an area of 4.76 million ha [3], with India being the second largest producer of tomato in the world, next to China. In line with this, tomato is cultivated in an area of about 0.79 million ha, with an annual production of 19.38 million tons, with a yield of 24.65 t/ha [4]. Furthermore, Andhra Pradesh is one of the major tomato growing states in India, where the crop is extensively cultivated in almost all the districts, as a major vegetable crop, with a production of 2.74 million tons in an area of about 61,670 ha [4]. However, tomato production is constrained by diverse biotic and abiotic stresses, including major pests and diseases, which reduce the yield as well as the quality of marketable fruits. In addition, biotic constraints can affect tomato production, with damage and yield losses expected from germination until harvesting stages [5,6].

Currently, one of the most important insect pests constraining tomato production is the new invasive pest, the South American tomato leaf miner, *Tuta absoluta* (Meyrick)

(Lepidoptera: Gelechiidae) [7,8]. *Tuta absoluta* has become the most important pest affecting tomato production in Europe, Africa, and Asia in the recent decade [9], and damage can occur under both greenhouse and open field conditions. *Tuta absoluta* has its origin in South America, but in the last decade it started spreading in Europe, Africa, and Asia [10,11]. In India, *T. absoluta* was first reported infesting tomato fields in 2014 in Maharashtra, and, soon after, it was also recorded in other neighboring states [7]. Due to its high capability to develop resistance to synthetic insecticides, and its concealed feeding behavior, the management of this insect has become a challenging task [12,13]. The continuous application of pesticides against pests and diseases is a common practice with farmers. They are mainly relying in an extensive use of synthetic pesticides, rather than other control methods [5,14]. Unfortunately, *T. absoluta* has developed insecticide resistance relatively fast, against conventional products for its control [15,16]. Hence, the integration of chemical pesticides with more environmentally sound control options, including the use of cultural, mechanical, and biological control components, becomes highly imperative, as the continued use of chemical pesticides could also harm the beneficial insects in the field, and lead to a high intake of chemical residues during food consumption, as well as contaminating soil and water sources [17,18].

The development of control strategies that integrated the use of bio-insecticides as an environmentally sound alternative to chemical pesticides is a highly needed priority within an integrated pest management approach, due to their low persistence in the environment, and their biodegradability [11,19]. The application of different bio-insecticides, such as *Bacillus thuringiensis* (Bt) and Azadirachtin (*Azadirachta indica*), on *T. absoluta* has previously shown a larval mortality of 67.29 and 66.40%, respectively, under field conditions in Iran [20]. In line with this, novel bio-pesticides, such as Prev-Am®, composed of orange oil, salt borax, and biodegradable surfactants, were also effective against *T. absoluta*, and were selective to its natural enemies, *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) in France [21]. Different studies also found that the use of entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, caused a 46–75% reduction in the *T. absoluta* population compared to the untreated plants, under semi-field conditions in Egypt [22,23]. Thus, bio-pesticides were shown to be effective in reducing *T. absoluta* in different geographical regions, with varying climatic conditions. Due to the lack of information available on the effectiveness of different bio-pesticides in India, this study was conducted to generate the evidence and fundamental knowledge of suitable bio-pesticides that could be included as effective components in integrated pest management (IPM) packages for *T. absoluta*. In addition, this study compares different bio-pesticides against a highly used conventional chemical pesticide, to provide information on the yield and reduction in damage, comparing both approaches.

2. Materials and Methods

2.1. Insect Colony

Tuta absoluta colony was established in July 2017 in the World Vegetable Center facilities at the South Asia Regional Office, located at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) campus, in Hyderabad, India (latitude: 17.5102841; longitude: 78.2748131). *Tuta absoluta* adults were initially collected from fields that had not been sprayed with any bio-pesticides. In addition, *T. absoluta* larvae had no contact with any pesticides in the rearing facilities. The larvae were fed with tomato plants that were kept in separate cages (70 × 60 cm). The dead/dry plants were continuously replaced with new ones for continuous supply of diet to the larvae. Adults were provided with ad libitum feeding substrate that consisted of 10% (w/v) sugar solution dispensed on cotton wool placed inside the cages. Adults were allowed to mate in cages, and after egg laying, the eggs were collected in nylon nets. After hatching, neonate larvae were transferred to new cages with healthy tomato plants as feeding substrate for multiplication and the remaining larvae were used for bioassays.

2.2. Bio-Pesticides

The bio-pesticides used in the study consisted of *Bacillus thuringiensis* var. *kurstaki*-based formulations, as follows: (i) Delfin® (Margo Biocontrols Private Limited, Bengaluru, Karnataka, India) and (ii) Green Larvicide® (Greenlife Biotech Laboratory, Coimbatore, Tamil Nadu, India); the following neem oil formulations: (iii) Ecotin® and (iv) Econeem Plus® (Margo Biocontrols Private Limited, Bengaluru, Karnataka, India); the following *Beauveria bassiana*-based formulations: (v) Green Beauveria® (Greenlife Biotech Laboratory, Coimbatore, Tamil Nadu, India) and (vi) BB Power® (KN Biosciences (India) Pvt. Ltd., Hyderabad, Telangana, India). In addition, the pesticide, chlorantraniliprole (Coragen®) was used as chemical treatment control for comparison (Table 1).

Table 1. List of bio-pesticides and chemical pesticide used against *Tuta absoluta*.

Bio-Pesticides	Trade Name	Dosage [a]	Source of Bio-Pesticides
Bacillus thuringiensis kurstaki (2×10^{11} CFU/mL)	Green Larvicide®	5 mL/L of water.	Greenlife Biotech Laboratory, Coimbatore, Tamil Nadu, India
Bacillus thuringiensis kurstaki (6×10^{7} spores/mg)	Delfin® WG	1 gm/L of water	Margo Biocontrols Private Limited, Bangalore, Karnataka, India
Beauveria bassiana (2×10^{8} spores/mL)	Green Beauveria®	10 mL/L of water	Greenlife Biotech Laboratory, Coimbatore, Tamil Nadu, India
Beauveria bassiana (1×10^{8} CFU/gram)	BB Power®	5 gm/L of water	K N Biosciences (India) Pvt. Ltd., Hyderabad, Telangana, India
Azadirachtin 10,000 ppm	Econeem Plus ®	3 mL/L of water	Margo Biocontrols Private Limited, Bangalore, Karnataka, India
Azadirachtin 50,000 ppm	Ecotin®	1 mL/L of water	Margo Biocontrols Private Limited, Bangalore, Karnataka, India
Chlorantraniliprole	Coragen 20 SC	0.4 mL/L of water	Agriplex Private Limited, Bangalore, Karnataka, India

[a] Recommended dosage for each commercial formulation.

2.3. Laboratory Bioassays

Five concentrations of each bio-pesticide were used in the preliminary range-finding tests. Five-to-six concentrations of each bio-pesticide, which could cause 10–90% mortality according to the preliminary range-finding tests (Table 2), and an untreated control, were included in each bioassay.

Table 2. Concentration of bio-pesticides used in the bioassays against *Tuta absoluta* larvae.

Bio-Pesticide	Concentration
Green Larvicide (*Bacillus thuringiensis kurstaki*) (2×10^{11} CFU/mL)	1×10^{7}, 1×10^{8}, 1×10^{9}, 1×10^{10}, 1×10^{11}
Delfin (*Bacillus thuringiensis kurstaki*) (6×10^{7} spores/mg)	6×10^{6}, 1.8×10^{7}, 3×10^{7}, 4.2×10^{7}, 6×10^{7}
Green Beauveria (*Beuveria bassiana*) (2×10^{8} spores/mL)	1×10^{7}, 2×10^{7}, 4×10^{7}, 6×10^{7}, 8×10^{7}
BB Power (*Beauveria bassiana*) (1×10^{8} CFU/g)	1×10^{6}, 1×10^{7}, 2×10^{7}, 4×10^{7}, 5×10^{7}
Econeem Plus—Azadirachtin 10,000 ppm	50, 500, 1000, 1500, 2000, 2500
Ecotin—Azadirachtin 50,000 ppm	50, 500, 1000, 2500, 5000

The leaf dip [24] method was used for the bioassays. In brief, tomato leaves were treated with the bio-pesticide solution for 5 s (s) with gentle agitation. The treated leaves were removed and placed on a mesh surface to dry for two hours. At least 10 leaves were placed in each cup (11 × 4 cm). Once the treated materials dried, they were transferred into the cups with their stalks inserted into the solidified agar. Ten second instar larvae were added to each container, and five cups (replications) were maintained for each concentration. Thus, a total of 50 larvae were used per concentration. Then each cup was sealed with a lid and stored in an area where they were not exposed to direct sunlight or extremes of temperature. A mean temperature of 27 ± 3 °C and relative humidity of 70 ± 10% were maintained. The larval mortality was recorded for five days at 24 h intervals. The mortality data from fifth day were used for the analysis. The results were expressed as percentage mortality, correcting for "untreated" (control) mortalities using Abbott's formula [25]. Assays that recorded more than 10% mortality in untreated control were eliminated. At each assessment, larvae were classified as either of the following: (a) unaffected, giving a normal response (such as taking a coordinated step) when gently stimulated using a fine hairbrush or (b) dead or affected, the latter giving no response to stimulation using a fine hairbrush or showing abnormal growth.

2.4. Field Experiments

Based on the bioassay experiments, the bio-pesticides were then evaluated under field conditions and natural infestation levels for their efficacy against *T. absoluta*. The experiment was conducted in two different seasons (May–August 2018, and September–December 2018) and in three locations (Chittoor district, Andhra Pradesh, India viz., Nimmanapalle (latitude: 13.551; longitude: 78.588), Madanapalle (latitude: 13.534; longitude: 78.481), and Kurbalakota (latitude: 13.655; longitude: 78.475). The locations represent the area, where tomato is cultivated throughout the year and the crop is subjected to natural infestation of *T. absoluta*.

2.5. Treatment and Data Collection

Six field trials (i.e., three trials in each season and one trial per block) were conducted during 2018 to assess the efficacy of various bio-pesticides against *T. absoluta* on tomatoes. Eight treatments, which included Delfin®, Green Larvicide®, Ecotin®, Econeem Plus®, Green Beauveria®, BB Power®, a chemical pesticide (chlorantraniliprole (Coragen® 18.5% SC)), and an untreated control were used in each trial. Coragen was chosen as a 'positive' control to compare with the bio-pesticides, because it is widely used by the tomato growers in the study sites. Each treatment had three replications and each replication was imposed on a 4 m × 5 m plot following a randomized complete block design (RCBD). To prevent damage from other pests, seedlings were raised in a nursery using a protective structure. Twenty-day-old seedlings were transplanted in the field plots in all locations. The natural pest incidences were monitored right after transplanting until the final harvest. The bio-pesticide and chemical pesticide treatments were initiated from the second week and continued at 10–12 day intervals until the final harvest. For all the treatments, the percentage of infestation was calculated in ten randomly selected plants for each replication by counting the total number of leaves and fruits, and the number of leaves and fruits with *T. absoluta* infestation after every application. During each harvest, the fruits were categorized as damaged or undamaged, and the marketable and non-marketable yield was calculated. The yield recorded during each harvest was pooled for the entire season and the total fruit yield was derived for each replication. This was further used for estimating the total yield in tons per hectare for each replication.

2.6. Data Analysis for Field Experiments

The lethal concentrations causing 50% mortality (LC_{50}), its 95% fiducial limits (FL) and the slope value of probit line were assessed using the statistical program LdP line (Ehab Mostafa Bakr, Cairo, Egypt). Data on leaf and fruit infestation by *T. absoluta* and tomato yield were analyzed using combined analysis approach of several experiments [26,27]. Preliminary analysis of variance (ANOVA) was completed for each individual analysis (each location in each season); experimental errors were examined for heterogeneity and Shapiro–Wilkinson test for normality was performed in each individual analysis. The data were then analyzed using ANOVA with the procedure Proc GLM of SAS version

9.1 (SAS Institute, Cary, NC, USA). Each season/location was considered as a particular environment for the combined analysis. The significant differences were identified and means were separated by Tukey's HSD test (differences were considered significant at $\alpha = 0.05$). Data on leaf and fruit infestation were arcsine square root transformed (*asin* (*sqrt* (*x*)) before analysis. Non-transformed data are presented in the Results section.

3. Results

3.1. Laboratory Bioassays

Among the *B. thuringiensis* commercial formulations tested, Delfin® was more lethal to *T. absoluta* than Green Larvicide®, based on the lower LC_{50} values (Table 3). Regarding the neem-based formulations, although the toxicity of Ecotin® (azadirachtin 50,000 ppm (5%) EC) was higher than Econeem Plus® (azadirachtin 10,000 ppm (1%) EC), they did not differ significantly, because of their overlapping fiducial limits for LC_{50} values (Table 3). Based on the LC_{50} values of the *B. bassiana*-based formulations, the bio-pesticide BB Power® was more lethal than Green Beauveria® (Table 3).

Table 3. Toxicity of bio-pesticides to *Tuta absoluta* larvae ($n = 100$ individuals).

Bio-Pesticide	LC_{50}	Fiducial Limit	Slope	X^2
Green Larvicide (*Bacillus thuringiensis kurstaki*) (2×10^{11} CFU/mL)	4.10×10^9	1.67×10^9–1.25×10^{10}	0.293 ± 0.042	1.054
Delfin (*Bacillus thuringiensis kurstaki*) (6×10^7 spores/mg)	8.06×10^6	6.35×10^6–9.68×10^6	2.163 ± 0.197	2.368
Green Beauveria (*Beuveria bassiana*) (2×10^8 spores/mL)	4.473×10^7	3.648×10^7–5.638×10^7	1.361 ± 0.185	1.756
BB Power (*Beauveria bassiana*) (1×10^8 CFU/g)	1.367×10^7	8.597×10^6–2.158×10^7	0.595 ± 0.098	6.05
Econeem Plus–Azadirachtin 10,000 ppm	212.676	118.352–319.565	0.703 ± 0.094	8.855
Ecotin–Azadirachtin 50,000 ppm	91.866	14.163–220.892	0.373 ± 0.084	6.959

3.2. Field Experiments

Interaction effects between the season, as well as location and treatments, were observed for the leaf and fruit infestation (Table 4). More specifically, all the bio-pesticides (except Green Beauveria®, especially in the second season) significantly reduced the tomato leaf infestation, and were on par with the chemical pesticide chlorantraniliprole 18.5% SC at both seasons in Kurbalakota and Madanapalle (Figure 1). Only the neem formulations in the first season, and Ecotin® and *B. thuringiensis* formulations in the second season were similar to the chemical pesticide, in their efficacy against *T. absoluta* in Nimmanapalle. The untreated control plots showed the highest leaf infestation across seasons and locations, and differed significantly from the bio-pesticides and the chemical treatment (Figure 1). Tomato fruit infestation by *T. absoluta* did not differ significantly among the treatments in Kurbalakota in the first season, but all the bio-pesticides in Madanapalle and the neem formulations in Nimmanapalle were on par with the chemical pesticide (Figure 2). However, all the bio-pesticides (except Green Beauveria® in Madanapalle) significantly reduced the tomato fruit infestation, and were on par with the chemical pesticide chlorantraniliprole 18.5% SC in the second season in the three locations (Figure 2).

Regarding the yield, only individual factors for location and treatment are presented here, since the interaction effect was not statistically significant (Table 4; Figures 3 and 4). Madanapalle and Nimmanapalle recorded significantly higher yields during the second season (September–December), whereas Kurbalakota had the highest tomato yield during the first season (May–August). The lowest yield from the study was observed in Madanapalle, during the first season (May–August) (Figure 3). In addition, the highest yields were observed in the Coragen, Ecotin, and Econeem treatments, with no significant differences among them (Figure 4). The yields on these three treatments ranged from 27 to 29 t/ha and were almost double the yield observed in the untreated control plots. Intermediate yields (ranged 22–25 t/ha) were observed for the remaining bio-pesticides, and they differed significantly from the untreated control treatment (Figure 4).

Table 4. Combined analyses for leaf and fruit infestation by *Tuta absoluta* and yield in tomato for six locations (2 years, three provinces) in Andhra Pradesh, India during two growing seasons (May–August 2018, September–December 2018) of bio-pesticides to *Tuta absoluta* larvae (n = 100 individuals).

Source	df	Leaf Infestation (%)		Fruit Infestation (%)		Yield	
		F	Pr > F	F	Pr > F	F	Pr > F
Location	5	56.39	<0.0001	47.70	<0.0001	252.9	<0.0001
Block	12	0.71	0.7435	0.86	0.5902	0.98	0.4719
Treatment	7	205.69	<0.0001	113.85	<0.0001	68.87	<0.0001
LocationXTreatment	35	3.93	<0.0001	5.86	<0.0001	1.48	0.0756

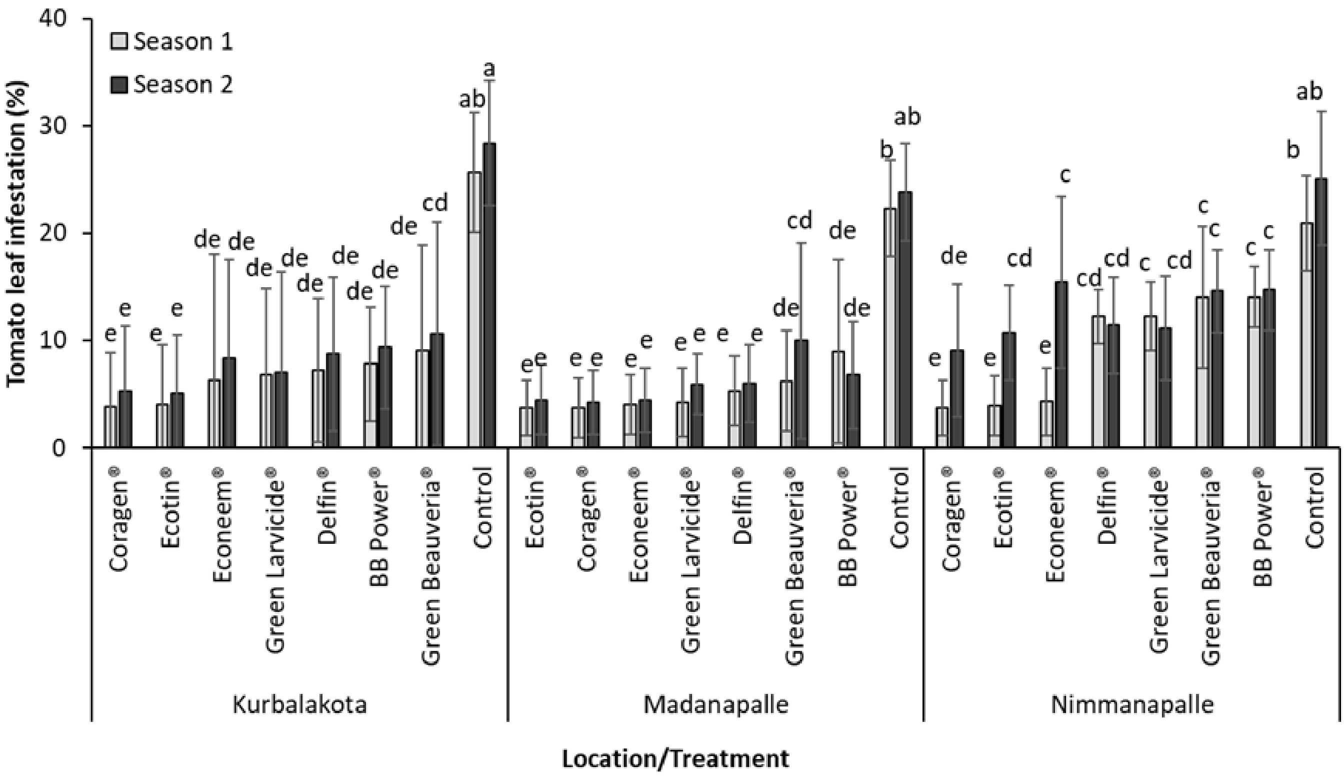

Figure 1. Mean (±SE) tomato leaf infestation percentage in three different locations of Andhra Pradesh, India and the following two growing seasons: season one: May–August 2018; season two: September–December 2018. Means represent average of ten replications for each treatment and three blocks (n = 30). *Beauveria bassiana*-based formulations are as follows: Green Beauveria® and BB Power®; *Bacillus thuringiensis*-based formulations are as follows: Green Larvicide® and Delfin®; neem-based formulations are as follows: Econeem® and Ecotin®; chemical pesticide is the following: Coragen®. Mean(s) followed by same letter(s) are not significantly different based on Tukey's HSD (p = 0.05).

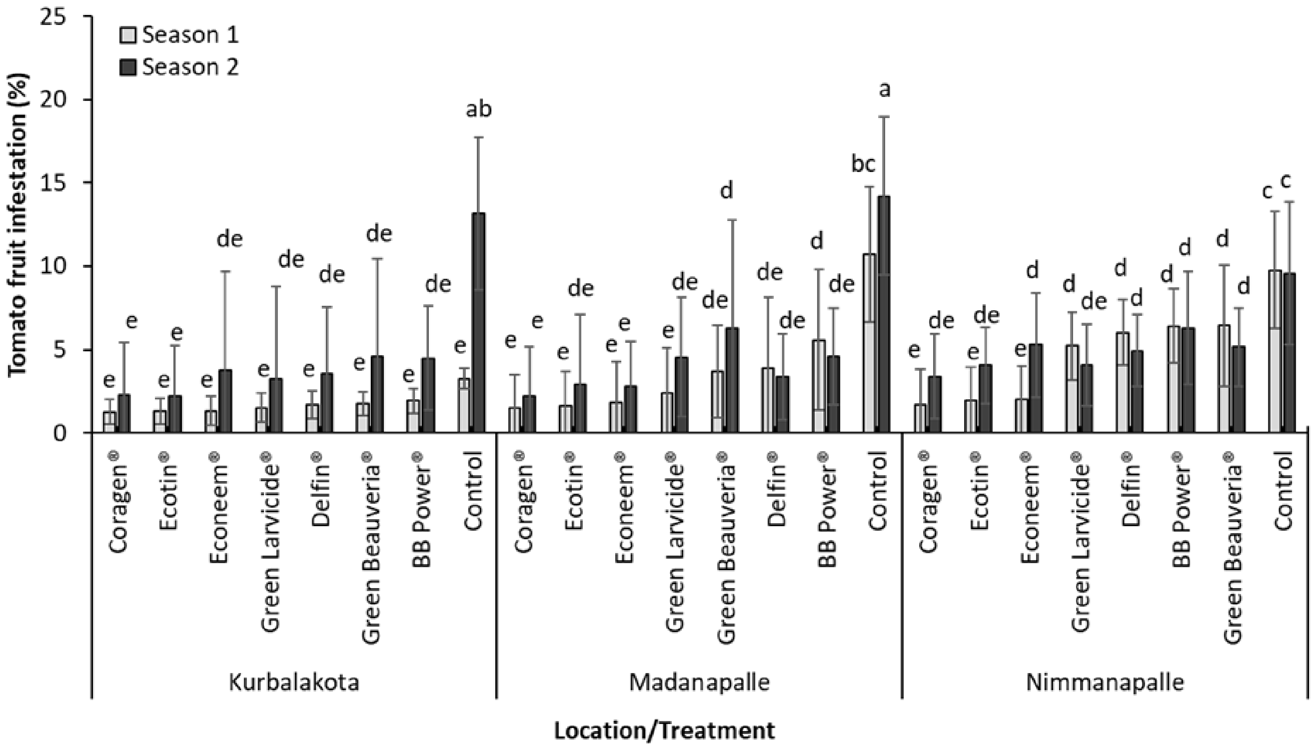

Figure 2. Mean (±SE) tomato fruit infestation percentage in three different locations of Andhra Pradesh, India and two growing seasons (May–August 2018, September–December 2018). Means represent average of ten replications for each treatment and three blocks (n = 30). *Beauveria bassiana*-based formulations are as follows: Green Beauveria® and BB Power®; *Bacillus thuringiensis*-based formulations are as follows: Green Larvicide® and Delfin®; neem-based formulations are as follows: Econeem® and Ecotin®; chemical pesticide is as follows: Coragen®. Mean(s) followed by same letter(s) are not significantly different based on Tukey's HSD (p = 0.05).

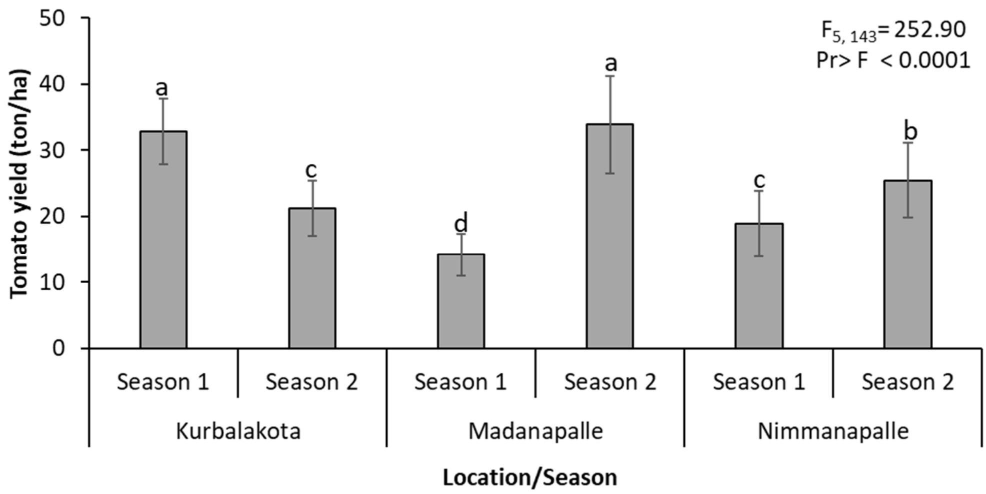

Figure 3. Mean (±SE) tomato yield in three different locations of Andhra Pradesh, India and two growing seasons (May–August 2018, September–December 2018). Means represent average of three replications and eight treatments (n = 24) in each location. Mean(s) followed by same letter(s) are not significantly different based on Tukey's HSD (p = 0.05).

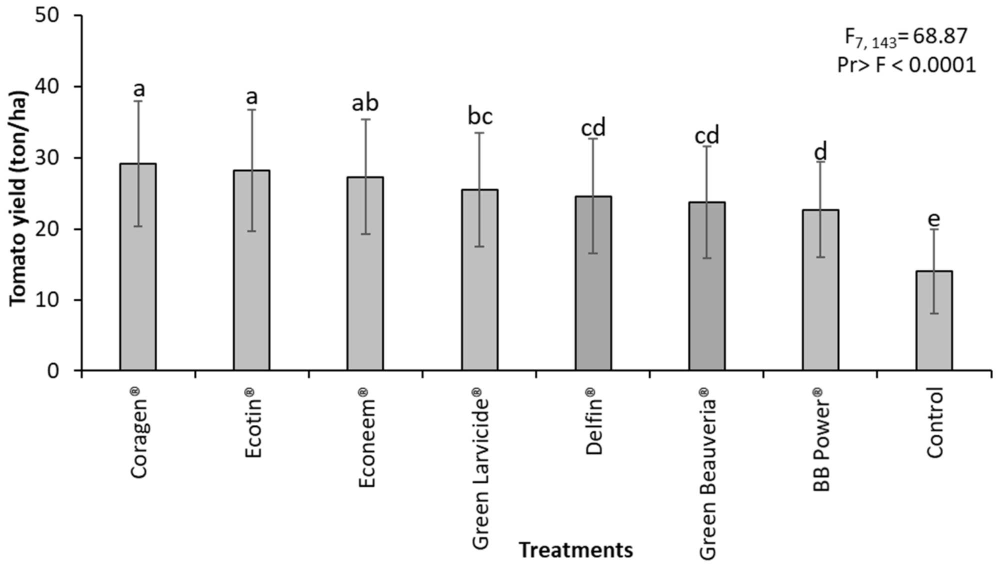

Figure 4. Mean (±SE) tomato yield in eight different treatments across three locations of Andhra Pradesh, India, two growing seasons (May–August 2018, September–December 2018) and three replications (*n* = 18). *Beauveria bassiana*-based formulations are as follows: Green Beauveria® and BB Power®; *Bacillus thuringiensis*-based formulations are as follows: Green Larvicide® and Delfin®; neem-based formulations are as follows: Econeem® and Ecotin®; chemical pesticide is as follows: Coragen®. Mean(s) followed by same letter(s) are not significantly different based on Tukey's HSD (*p* = 0.05).

4. Discussion

Multi-location bio-pesticide trials on tomato in the current study demonstrated the effectiveness of bio-pesticides against the tomato leaf miner *T. absoluta*, which is the new invasive pest threatening tomato production in India. Both the neem and *B. thuringiensis* formulations significantly reduced leaf damage by *T. absoluta* in Kurbalakota and Madanapalle, and were on par with the conventional chemical pesticide. Similarly, these bio-pesticides reduced the fruit damage by *T. absoluta* equivalently to the chemical pesticide. However, only the neem formulations were on par with the chemical pesticide, in reducing the leaf (in the first season) and fruit (in both seasons) damages by *T. absoluta* in Nimmanapalle. The efficacy of the *B. bassiana*-based formulations varied across the locations and seasons, but they reduced the *T. absoluta* infestation significantly lower than in the untreated control plots. Furthermore, the results from the bioassay experiments showed high efficacy of different bio-pesticides against second instar larvae. The findings were similar to Jallow et al. (2019) [28], who reported that when second instar larvae were exposed to tomato leaf discs that were treated with azadirachtin (3 g/L), *B. thuringiensis* (0.5 g/L), or *B. bassiana* (1.5 g/L), 70–86%, 55–65%, and 45.5–58.5% mortality was observed, respectively. Similarly, in the field experiments conducted, the effect of different bio-pesticides (*B. thuringiensis*, *B. bassiana*, and neem formulations) on the larvae of *T. absoluta* also showed highly significant control of the pest. According to these experiments, a high percentage of damage reduction was observed in all the bio-pesticides tested, viz., commercial Btk, *B. bassiana*, and neem formulations, and the above findings were in conformity with the findings of Rodriguez et al. (2006) [29], Gonzalez-Cabrera et al. (2011) [30], and Pires et al. (2010) [31]. Highly invasive pests, such as *T. absoluta*, require the availability of sustainable management strategies, since the need of environmentally safe insecticides is being encouraged to replace the hazardous chemical pesticides, to prevent the spread into new areas, and to keep the pest population density below the economic threshold level [32].

The bioassay results also showed that *T. absoluta* was highly susceptible to both the commercial neem formulations tested. A similar susceptibility to neem formulations has been previously reported. Hosseinzadeh et al. (2019) [20] recorded an LC_{50} value of 2572.09 ppm (2423.62–2736.74) for third instar larvae. Similarly, Kona et al. (2014) [33] reported that after four days of application, about 24–26% egg mortality was observed, due to application of different concentrations of neem seed extracts, when compared to the untreated control (5.1%). Further, Abd El-Ghany et al. (2016) [34] reported that the LC_{50} values inside and outside the mines had a two-fold significance with 0.62 and 0.31–1 mL, respectively, for azadirachtin. As previously described for other insect pests, azadirachtin offers different insecticidal effects, including deleterious effects on development, leading to high larval mortality [35], interference with the growth and molting process of insects [36], and it also acts as a feeding and oviposition deterrent [37]. Likewise, it is likely that the susceptibility observed in *T. absoluta* is caused by similar reasons. However, more studies need to be performed to better understand the specific pathways that lead to the susceptibility observed on *T. absoluta* to neem formulations.

Neem formulations showed consistent effects in reducing the leaf damage in all the three experimental locations. The efficacy of both the neem formulations was mostly similar to chlorantraniliprole, in reducing the leaf infestation. The present study was in line with Abd El-Ghany et al. (2018) [22], who reported a 70–83% reduction in the leaf infestation of *T. absoluta*, with the application of azadirachtin. Similarly, Srinivasan and Dilipsundar (2019) [38] also reported the maximum reduction in larval population (65.26%) and a higher fruit yield (17.55 t/ha) with neem oil 3% in Southern India. Neem Azal T/S @ 0.3% showed high effectiveness (>80%) against different larval instars of *T. absoluta* [39]. The extract of *Azadirachta indica* gave about 74% larval mortality within 10 days of its application [40]. Neem has antifeedant and repellent properties, and it was found to be relatively less toxic to beneficial organisms than other harmful chemical pesticides, which made it an eco-friendly and effective substitute for IPM packages [22,41]. The high effectiveness of neem extract was due to its contact, as well as systemic action against the larval stages of *T. absoluta* [34]. *T. absoluta* adults and larvae exhibited egg-laying and walking avoidance, respectively, to azadirachtin [35], which could be due to its masking effect of tomato leaf secondary metabolites that are attractive to the insects. Such avoidance might be useful in "push–pull" strategies, minimizing leaf miner incidence in azadirachtin-sprayed areas and favoring leaf miner dispersal to areas with attractants (such as pheromone traps) or attractive (alternative) host plants. Such a combination of neem and pheromone traps, or trap cropping, should be experimented in future studies. Thus, azadirachtin is potentially useful against *T. absoluta* not only in IPM fields, but also in organic tomato fields, where there is a lack of suitable insecticides for pest management [35].

The results of the current study also confirmed the lethality of *B. thuringiensis* var. *kurstaki* against *T. absoluta* in India. Our results were also supported by previous studies showing the efficacy of different *B. thuringiensis* commercial formulations that were evaluated against *T. absoluta* in several countries worldwide. For instance, Sabbour and Soliman (2014) [42] recorded the LC_{50} value of 90–140 µg/mL when *T. absoluta* was treated with different concentrations of *B. thuringiensis* in Egypt. Similarly, Sabbour and Singer (2016) [43] also recorded an LC_{50} value of 99–115 µg/mL after treatment with *B. thuringiensis*. Various *B. thuringiensis* strains on *T. absoluta* showed an LC_{50} of 55–150 µg/mL [44]. *B. thuringiensis* was undoubtedly one of the most studied bio-pesticides in recent years, since it produced proteins with insecticidal properties (Cry and Cyt toxins) during its sporulation phase, which were toxic to insect larvae, and upon their ingestion, they caused intestinal paralysis and rapid death [45]. The toxicity of *B. thuringiensis* var *kurstaki* against lepidopteran pests was related to the strains and proportion of δ-endotoxins contained in the different commercial formulations. In fact, the larval mortality rate varied with the applied *B. thuringiensis* var *kurstaki* concentrations and the age of the larvae [46]. Thus, the commercial formulations based on *B. thuringiensis* have been used for decades, to manage insect pests, as an alternative to chemicals [43].

B. thuringiensis var. *kurstaki* was also found to be highly effective against *T. absoluta* in field conditions, thereby reducing its damage on leaves and fruits, and the results were consistent with those obtained in the laboratory. Both the commercial *B. thuringiensis* formulations, Delfin® and Green Larvicide®, showed consistent effects in reducing the leaf infestation. The fruit infestation by *T. absoluta*, recorded from *B. thuringiensis*-based formulations-treated plots, was on par with the chlorantraniliprole-treated plots, showing a similar reduction in fruit infestation in both the seasons. The efficacy of *B. thuringiensis* var. *kurstaki* strains in field conditions varied due to environmental factors [47], toxin degradation [48], gut microbiota competition [49], and inactivation by the target organism [50]. It was found that *B. thuringiensis* var. *kurstaki* strains had a specific mode of action that affected the different life stages of *T. absoluta.* In the current study, the efficacy of *B. thuringiensis* formulations was similar to other bio-pesticides, as well as the chemical pesticide, which confirmed that it was not affected by the prevailing environmental conditions in the study locations. Thus, these results were in close conformity with Gonzalez-Cabrera et al. (2011) [30], who reported that commercial formulations based on *B. thuringiensis* could be a good alternative for the successful management of *T. absoluta* in the laboratory, greenhouse, and open field conditions in Spain.

Bue et al. (2012) [51] suggested that the combination of azadirachtin and *B. thuringiensis* was able to reduce the impact of *T. absoluta* on the marketable tomatoes. Similarly, the application of Bt and Neem Azal separately, and in combination, had provided a promising alternative to chemical control [11]. The highest long-term effect on the pest abundance and damage was observed by Nazarpour et al. (2016) [52], in azadirachtin + Bt, which caused a 100% reduction in fruit and foliage damage compared to the untreated control. Since we also recorded better efficacies for the neem and *B. thuringiensis* formulations in the current study, the combined use of those bio-pesticides may be considered for the better control of *T. absoluta* in field conditions. The use of bacterial isolates interfered with egg formation, leading to a reduction in the number of eggs laid by the adults. The phenomenon revealed that some larvae may be slightly infected and require a longer duration to attack the stomach cells, and for further appearance of infection symptoms that would appear later in pupae and adults [53]. Future studies may focus on those sub-lethal effects of *B. thuringiensis* formulations on *T. absoluta* in India.

Regarding *Beauveria*-based formulations, the current findings showed that *T. absoluta* was more susceptible to the talc formulation of *B. bassiana* compared to the liquid formulation. These results were in agreement with those of Shalaby et al. (2013) [54], who reported that different concentrations of *B. bassiana* on *T. absoluta* larvae recorded LC_{50} values of 0.28×10^6 to 0.45×10^6 conidia/mL against early larval instars. Similarly, Klieber and Reineke (2016) [55] also reported high mortality rates and significant reductions in larval longevity, when the larvae were fed for a period of around 15 days, on leaves with *B. bassiana* propagules. At the dosage of 10^8 spores/mL, the highest mortality rate was recorded [56], and the mortality rate of *T. absoluta* and different concentrations of *B. bassiana* had a linear relationship [54]. It was generally observed that the mortality rates increased with an increase in time. Hence, the mortality recorded on the fifth day was used for the estimation of the median lethal concentration. In the current study, it was revealed that there was no immediate effect (within three days of application) of *B. bassiana* on *T. absoluta* larvae, as the establishment of the fungi took a few days. This was in agreement with another study, which demonstrated that the fungal infection was very low in the first 3 days, and started to be significantly different among the treatments from the fourth day, after the application in Rwanda [56]. Similarly, Shiberu and Getu (2018) [40] also recorded that the mortality rate increased up to 78%, after 10 days of treatment in different locations of Ethiopia. Although the disease progression took a few days for the entomopathogenic fungi-based bio-pesticide formulations, they were able to reduce the pest infestation quite significantly in the field conditions. For instance, the application of *B. bassiana* caused a 46–75% reduction in *T. absoluta* infestation on tomatoes, when compared to the untreated plants [22]. Similarly, Abd El-Ghany et al. (2016) [34] also reported that *B. bassiana* was second in its effectiveness, next to spinosad, causing more than 50% larval mortality outside the mines. *B. bassiana* had a high negative impact on the pest oviposition, pupation, and adult emergence in tomato, in addition to its significant reduction in mine formation in the host plants [57]. Thus, *B. bassiana*-based bio-pesticide formulations were shown to be effective in controlling *T. absoluta* on tomatoes. Under the right climatic conditions (temperature and humidity), the fungus starts an initial degradation of the cuticle, by secreting proteases, chitinases, and lipases. Moreover, the fungus overcome the host immune response by suppressing the host defense system, or by developing cryptic growth forms, which are masked from the arthropod defense responses [58].

While most of the biocontrol agents only infected the insects when ingested, even a simple contact with *B. bassiana* was sufficient to cause infection of the susceptible host insect, under suitable environmental conditions and insect physiology [59]. As noted by Neves and Alves (2000), the insect mortality increased due to an increase in the release of toxins or enzymes, triggered by enhanced conidia penetration into the insect cuticle [60]. However, there were multiple factors that caused the variation in the virulence of entomopathogenic strains, including differences in the enzymes and toxins production in conidia germination speed, mechanical activity in the cuticle penetration, and the colonization capacity and cuticle chemical composition of the host insect [61]. The pathogenicity of a particular entomopathogen varied with the strain/isolate and environment [62]. For instance, the efficacy of *B. bassiana* was mainly dependent on environmental factors, such as moisture, temperature, precipitation, and UV radiation [59]. A relative humidity of 100% was highly favorable for mycelial growth and spore germination of *B. bassiana*, in general, but there were certain strains, which germinated at a relative humidity as low as 56.8%, and supported sporulation [59]. Similarly, temperatures above 36 °C did not support the growth of *B. bassiana.* The maximum temperature recorded during the entire experimental period of the current study was 34 °C, during the month of May, with low relative humidity, which was conducive for the fungal growth [63]. Thus, *B. bassiana* was found to be an effective bio-pesticide for managing *T. absoluta* on tomato in Andhra Pradesh conditions, as well as in other locations in India that have similar agro-climatic conditions.

It has been observed that newly invaded areas may have a few natural enemy species, as evidenced for *T. absoluta* in Italy [64], and their effect on the exotic species could be weak. Another point of interest is that indigenous natural enemies need time to colonize, get adapted, and effectively control the exotic species. Hence, the conservation of indigenous natural enemies, also by means of habitat management techniques, should be taken into account when planning an integrated management strategy for *T. absoluta* [64]. As the chemical or biological compounds used against *T. absoluta* could directly or indirectly affect its natural enemies, and evaluating such effects is necessary when choosing the suitable insecticides or bio-pesticides to be included in the pest management programs. The entomopathogenic fungus was reported to be not only safer for the egg parasitoid, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), but also showed a synergistic effect on leaf miner egg mortality [65]. Interestingly, an effective control of *T. absoluta* could also be achieved at its pupal stage, by using *M. anisopliae*. The application of MA-Prep at the recommended dose of 5.58×10^9 viable conidia per liter, against *T. absoluta* at its pupal stage, with irrigation water, could be taken into account as part of an integrated pest management program [66].

Currently, bio-pesticides comprise a small share of the total crop protection market globally, with a value of about USD 3 billion worldwide, accounting for just 5% of the total crop protection market [67,68]. In India, bio-pesticides represent only about 4.2% of the overall pesticide market [69]. Hence, there is an urgent need to increase the production of bio-pesticides for pest management and sustainable agriculture in the country. Recently, phytochemical and natural product studies have led to the discovery of a large number of compounds, with a variety of chemical structures and bioactivities. However, only 12 types of bio-pesticides have been registered hitherto in India. Neem-based pesticides, *B. thuringensis*, nucleopolyhedrovirus (NPV), and *Trichoderma*, are the most commonly produced and used bio-pesticides in India [70]. All these non-chemical approaches could play a significant role in reducing insecticide selection pressure. However, no single approach is a panacea to solve the problem of insecticide resistance. A sustainable integrated resistant management strategy requires the use of insecticides and bio-pesticides with multiple modes of action, applied in space and time (rotations), with as many other approaches as possible [70]. For instance, the continuous application of any *B. thuringiensis* formulation on a large scale could also lead to the development of resistance in insect pests [71]. Therefore, the use of *B. thuringiensis* formulations containing different toxins is recommended, as the evolution of resistance to toxin combinations with different target sites in insect species is minimal [72,73]. In the current study, both the formulations of *B. thuringiensis* are *B. thuringiensis* var *kurstaki*. Hence, formulations based on *B. thuringiensis* var *aizawai* should also be made available in India, so that the tomato growers will have more choices. Moreover, the effect of the IPM package on *T. absoluta* infestation, comprising *B. thuringiensis* var *kurstaki*, *B. bassiana*, neem, and chlorantraniliprole, along with the installation of pheromone traps, was found to be quite promising [74], and it was on par with the farmers' practice of calendar-based application of chemical pesticides in reducing *T. absoluta* infestation, without any compromise in the yield. In conclusion, performing an intervention that includes combined methods of bio-pesticides, chemical pesticide, and mass trapping in the proper period could reduce the infestation rate from 80 to 95% [75]. Hence, bio-pesticides offer a viable alternative to reduce the reliance on chemical pesticides for managing *T. absoluta* on tomatoes in India and other countries in the region.

5. Conclusions

The neem-based formulations and *B. thuringiensis*-based formulations tested in this study reduced tomato leaf and fruit infestation by *T. absoluta* almost similarly to the conventional chemical treatment. The efficacy of *B. bassiana*-based formulations varied across the locations and seasons, but they significantly reduced *T. absoluta* infestation compared to the untreated control plots. Therefore, bio-pesticides can be considered as safe alternatives to synthetic pesticides, for the management of *T. absoluta*. These bio-pesticides are readily available, easily biodegradable, exhibit various modes of action, and have low toxicity to humans and non-target organisms. Moreover, bio-pesticides reduce the risk of resistance development in *T. absoluta*, which is quite common for chemical pesticides. Hence, bio-pesticides could provide efficient, economical, and promising options for managing *T. absoluta* on tomatoes in India.

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References

1. Agarwal, S.; Rao, A.O. Tomato lycopene and its role in human health and chronic diseases. *Can. Med. Assoc. J.* **2000**, *163*, 739–744.
2. Raiola, A.; Tenore, G.C.; Barone, A.; Frusciante, L.; Rigano, M.M. Vitamin E content and composition in tomato fruits: Beneficial roles and bio-fortification. *Int. J. Mol. Sci.* **2015**, *16*, 29250–29264. [CrossRef] [PubMed]
3. Food and Agriculture Organization of the United Nations. FAOSTAT Statistical Database. 2018. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 1 November 2019).
4. Government of India. Horticultural Statistics at a Glance. 2018. Available online: http://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf (accessed on 1 November 2019).
5. Arora, S.; Kanojia, A.K.; Kumar, A.; Mogha, N.; Sahu, V. Bio-pesticide formulation to control tomato lepidopteran pest menace. *Asian Agrihist.* **2014**, *18*, 283–293.
6. Mondal, B.; Mondal, P.; Das, A.; Bhattyacharyya, K. Seasonal incidence of different insect pests of tomato (*Lycopersicon esculentum* Mill.) and their correlation with abiotic factor in lateritic zone of West Bengal. *J. Entomol. Zool.* **2019**, *7*, 1426–1430.
7. Shashank, P.R.; Chandrashekar, K.; Meshram, N.M.; Sreedevi, K. Occurrence of *Tuta absoluta* (*Lepidoptera Gelechiidae*), an invasive pest from India. *Indian J. Entomol.* **2015**, *77*, 323. [CrossRef]
8. Sridhar, V.; Chakravarthy, A.K.; Asokan, R.; Vinesh, L.S.; Rebijith, K.B.; Vennila, S. New record of invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*) in India. *Pest Manag. Hort. Ecosyst.* **2014**, *20*, 148–154.
9. Biondi, A.; Guedes, R.N.C.; Wan, F.H.; Desneux, N. Ecology, world- wide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: Past, present, and future. *Annu. Rev. Entomol.* **2018**, *63*, 239–258. [CrossRef]
10. Desneux, N.; Luna, M.G.; Guillemaud, T.; Urbaneja, A. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: The new threat to tomato world production. *J. Pest Sci.* **2011**, *84*, 403–408. [CrossRef]
11. El Hajj, A.K.; Rizk, H.; Gharib, M.; Houssein, M.; Talej, V.; Taha, N.; Aleik, S.; Mousa, Z. Management of *Tuta absoluta* Meyrick (*Lepidoptera Gelechiidae*) using bio pesticides on tomato crop under Greenhouse conditions. *J. Agric. Sci.* **2017**, *9*, 123–129. [CrossRef]
12. Guedes, P.; Picanço, M.C. The tomato borer *Tuta absoluta* in South America: Pest status, management and insecticide resistance. *Bull. OEPP* **2012**, *42*, 211–216. [CrossRef]
13. The Insecticide Resistance Action Committee. *Tuta absoluta*—The Tomato Leaf Miner or Tomato Borer. Recommendations for Sustainable and Effective Resistance Management. 2011. Available online: https://irac-online.org/content/uploads/2009/12/Tuta_brochure_print-version_11Oct11.pdf (accessed on 1 September 2017).
14. Rao, J.U. Andhra Pradesh and Telangana Suffer from High Pesticide Residues. Deccan Chronicle. 2016. Available online: https://www.deccanchronicle.com/nation/current-affairs/181016/andhra-pradesh-and-telangana-suffer-from-high-pesticide-residues.html (accessed on 26 July 2021).
15. Domínguez, A.; López, S.; Bernabé, A.; Guerrero, Á.; Quero, C. Influence of age, host plant and mating status in pheromone production and new insights on perception plasticity in *Tuta absoluta*. *Insects* **2019**, *10*, 256. [CrossRef]
16. Lietti, M.M.M.; Botto, E.; Alzogaray, R.A. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop. Entomol.* **2005**, *34*, 113–119. [CrossRef]
17. Aktar, W.; Sengupta, D.; Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* **2009**, *2*, 1–12. [CrossRef]
18. Landgren, O.; Kyle, R.A.; Hoppin, J.A.; Beane Freeman, L.E.; Cerhan, J.R.; Katzmann, J.A.; Rajkumar, S.V.; Alavanja, M.C. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood* **2009**, *113*, 6386–6391. [CrossRef]
19. Khater, H.M. Prospects of botanical bio-pesticides in insect pest management. *Pharmacologia* **2012**, *3*, 641–656.
20. Hosseinzadeh, A.; Aramideh, S.; Ghassemi-Kahrizeh, A. Efficacy of bio-insecticides on *Tuta absoluta* (Meyrick) (*Lep. Gelechiidae*) in laboratory and field conditions. *AgricEngInt: CIGR J.* **2019**, *21*, 164–170.
21. Soares, M.A.; Campos, M.R.; Passos, L.C.; Carvalho, G.A.; Haro, M.M.; Lavoir, A.V.; Biondi, A.; Zappala, L.; Desneux, N. Botanical insecticide and natural enemies: A potential combination for pest management against *Tuta absoluta*. *J. Pest Sci.* **2019**, *92*, 1433–1443. [CrossRef]
22. Abd El-Ghany, N.M.; Abdel-Razek, A.S.; Djelouah, K.; Moussa, A. Efficacy of bio-rational insecticides against *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*) on tomatoes. *Biosci. Res.* **2018**, *15*, 28–40.
23. Hossain, M.S.; Das, A.K.; Akhter, S.; Mian, M.Y.; Muniappan, R. Development of biorational management for tomato leaf miner, *Tuta absoluta*. *J. Biol. Control* **2019**, *33*, 132–136. [CrossRef]
24. IRAC. *Tuta absoluta* Susceptibility Test Method #022. 2012. Available online: http://www.irac-online.org/methods/tuta-absoluta-larvae/ (accessed on 4 March 2017).
25. Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265–267. [CrossRef]

26. Moore, J.K.; Dixon, P.M. Analysis of combined experiments revisited. *Agron. J.* **2015**, *107*, 763–771. [CrossRef]
27. Peterson, R.G. *Agriculture Field Experiments. Design and Analysis*; Marcel Dekker: New York, NY, USA, 1994.
28. Jallow, M.F.A.; Dahab, A.A.; Albaho, M.S.; Devi, V.Y. Efficacy of some biorational insecticides against *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*) under laboratory and greenhouse conditions in Kuwait. *J. Appl. Entomol.* **2018**, *143*, 187–195. [CrossRef]
29. Rodriguez, M.; Gerding, M.; France, A. Effectivity of entomopathogenic fungus strains on tomato moth *Tuta absoluta* Meyrick (*Lepidoptera Gelechiidae*) larvae. *Agric. Tec.* **2006**, *66*, 159–165.
30. 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]
31. Pires, L.M.; Marques, E.J.; de Olivieira, J.V.; Alves, S.B. Selection of isolates of entomopathogenic fungi for controlling *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*) and their compatibility with insecticides used in tomato crop. *Neotrop. Entomol.* **2010**, *39*, 977–984. [CrossRef] [PubMed]
32. Theoduloz, C.; Vega, A.; Salazar, M.; González, E.; Meza-Basso, L. Expression of a *Bacillus thuringiensis* δ-endotoxin cry1Ab gene in *Bacillus subtilis* and *Bacillus licheniformis* strains that naturally colonize the phylloplane of tomato plants (*Lycopersicon esculentum* Mills). *J. Appl. Microbiol.* **2003**, *94*, 375–381. [CrossRef] [PubMed]
33. Kona, N.E.M.; Taha, A.K.; Mahmoud, M.E.E. Effects of botanical extracts of neem (*Azadirachta indica*) and Jatropha (*Jatropha curcus*) on eggs and larvae of tomato leaf miner, *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*). *Persian Gulf Crop Protec.* **2014**, *3*, 41–46.
34. Abd El-Ghany, N.M.; Abdel-Razek, A.S.; Ebadah, I.M.A.; Mahmoud, Y.A. Evaluation of some microbial agents, natural and chemical compounds for controlling tomato leaf miner, *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*). *J. Plant Prot. Res.* **2016**, *56*, 372–379. [CrossRef]
35. Tome, H.V.V.; Martins, J.C.; Correa, A.S.; Galdino, T.V.S.; Picanco, M.C.; Guedes, R.N.C. Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. *Crop Prot.* **2013**, *46*, 63–69. [CrossRef]
36. Martinez, S.; van Emden, H.F. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (*Lepidoptera Noctuidae*) caused by Azadirachtin. *Neotrop. Entomol.* **2001**, *30*, 113–125. [CrossRef]
37. Berxolli, A.; Shahini, S. Azadirachtin, a useful alternative for controlling *Tuta absoluta* (Myerick). *EJPAS.* **2017**, *5*, 41–45.
38. Srinivasan, G.; Dilipsundar, N. Evaluation of bio-efficacy of botanicals against tomato pinworm, *Tuta absoluta* (Meyrick, 1917). *Pest Manag. Hort. Ecosyst.* **2019**, *25*, 44–50.
39. Yankova, V.; Valchev, N.; Markova, D. Effectiveness of phytopesticide neem azal T/S ®against tomato leaf miner (*Tuta absoluta* Meyrick) in greenhouse tomato. *Bulg. J. Agric. Sci.* **2014**, *20*, 1116–1118.
40. 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**, *07*, 1–8. [CrossRef]
41. Cunha, U.S.; Vendramim, J.D.; Rocha, W.C.; Vieira, P.C. Fractions of *Trichilia pallens* with insecticidal activity against *Tuta absoluta*. *Pesq. agropec. Bras.* **2006**, *41*, 1579–1585. [CrossRef]
42. Sabbour, M.M.; Soliman, N. Evaluations of three *Bacillus thuringiensis* against *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*) in Egypt. *Int. J. Sci. Res.* **2014**, *3*, 2067–2073.
43. Sabbour, M.M.; Singer, S.M. Observations of the effect of two isolated nano *Bacillus thuringiensis* on *Tuta absoluta* infestation under laboratory and field condition. *Res. J. Pharm. Biol. Chem. Sci.* **2016**, *7*, 1891–1897. [CrossRef]
44. Magda, S.; Moharam, M.E. Comparing the effect of seven isolated *Bacillus thuringiensis* against *Tuta absoluta* infesting in laboratory and field condition. *Int. J. Sci. Res.* **2015**, *4*, 458–462. [CrossRef]
45. Rezaei, A.A.; Talaei-Hassanlous, R. The Use of *Bacillus thuringiensis*-based Products in Biocontrol of Tomato Leaf Miner, *Tuta absoluta* (*Lepidoptera Gelechiidae*). *Int. J. Agric. Innov. Res.* **2016**, *4*, 814–817.
46. Harizia, A.; Benguerai, A.; Boukhari, Y. Larvicidal activity of *Bacillus thuringiensis* kurstaki against *Tuta absoluta* (*Lepidoptera Gelechiidae*). *LSJ* **2019**, *20*, 352–362. [CrossRef]
47. Hilbeck, A.; Otto, M. Specificity and combinatorial effects of *Bacillus thuringiensis* Cry toxins in the context of GMO environmental risk assessment. *Front. Environ. Sci.* **2015**, *3*, 71. [CrossRef]
48. Pang, A.S.; Gringorten, J.L. Degradation of *Bacillus thuringiensis* δ-endotoxin in host insect gut juice. *FEMS Microbiol. Lett.* **1998**, *167*, 281–285. [CrossRef]
49. Broderick, N.A.; Robinson, C.J.; McMahon, M.D.; Holt, J.; Handelsman, J.; Raffa, K.F. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biol.* **2009**, 7–11. [CrossRef] [PubMed]
50. Miranda, R.; Zamudio, F.Z.; Bravo, A. Processing of Cry1Ab δ-endotoxin from *Bacillus thuringiensis* by *Manduca sexta* and *Spodoptera frugiperda* midgut proteases: Role in protoxin activation and toxin inactivation. *Insect Biochem. Mol. Biol.* **2001**, *31*, 1155–1163. [CrossRef]
51. Bue, P.L.O.; Abbas, S.; Peri, E.; Colazza, S. Use of biorational insecticides for the control of *Tuta absoluta* (Meyrick) infestations on open field tomato. *New Medit.* **2012**, *4*, 39–41.
52. Nazarpour, L.; Yarahmadi, F.; Saber, M.; Rajabpour, A. Short and long term effects of some bio-insecticides on *Tuta absoluta* Meyrick (*Lepidoptera Gelechiidae*) and its coexisting generalist predators in tomato fields. *J. Crop Prot.* **2016**, *5*, 331–342. [CrossRef]
53. Youssef, N.A.; Hassan, G.M. Bioinsecticide activity of *Bacillus thuringiensis* isolates on tomato borer, *Tuta absoluta* (Meyrick) and their molecular identification. *Afr. J. Biotechnol.* **2013**, *12*, 3699–3709.

54. Shalaby, H.H.; Faragalla, F.H.; El-Saadany, H.M.; Ibrahim, A.A. Efficacy of three entomopathogenic agents for control the tomato borer, *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*). *Nat. Sci.* **2013**, *11*, 63–72.
55. Klieber, J.; Reineke, A. The entomopathogen *Beauveria bassiana* has epiphytic and endophytic activity against the tomato leaf miner *Tuta absoluta*. *J. Appl. Entomol.* **2016**, *140*, 580–589. [CrossRef]
56. Ndereyimana, A.; Nyalala, S.; Murerwa, P.; Gaidashova, S. Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leaf miner, *Tuta absoluta* (Meyrick) (*Lepidoptera Gelechiidae*). *Egypt J. Biol. Pest Co.* **2019**, *29*, 1–5. [CrossRef]
57. Marrone, P.G. The market and potential for biopesticides. In *Biopesticides: State of the Art and Future Opportunities*; Gross, A.D., Coats, J.R., Duke, S.O., Seiber, J.N., Eds.; American Chemical Society: Washington, DC, USA, 2014; pp. 245–258.
58. Schrank, A.; Vainstein, M.H. *Metarhizium anisopliae* enzymes and toxins. *Toxicon* **2010**, *56*, 1267–1274. [CrossRef] [PubMed]
59. Fabrice, D.H.; Elie, D.A.; Kobi, D.O.; Valerien, A.; Thomas, H.A.; Joelle, T.; Maurille, E.I.A.T.; Denis, O.B.; Manuele, T. Toward the efficient use of *Beauveria bassiana* in integrated cotton insect pest management. *J. Cotton Res.* **2020**, *3*, 1–21.
60. Neves, P.J.; Alves, S.B. Selection of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. strains for control of *Cornitermes cumulans* (Kollar). *Braz Arch Biol Technol* **2000**, *43*, 319–328. [CrossRef]
61. Kleespies, R.G.; Zimmermann, G. Effect of additives on the production, viability and virulence of blastospores of *Metarhizium anisopliae*. *Biochem. Sci. Technol.* **1998**, *8*, 207–214. [CrossRef]
62. Borisade, O.A.; Magan, N. Growth and sporulation of entomopathogenic *Beauveria bassiana, Metarhizium anisopliae*, *Isaria farinosa* and *Isaria fumosorosea* strains in relation to water activity and temperature interactions. *Biocontrol Sci. Technol.* **2014**, *24*, 999–1011. [CrossRef]
63. Agbessenou, A.; Akutse, K.S.; Yusuf, A.A.; Ekesi, S.; Subramanian, S.; Khamis, F.M. Endophytic fungi protect tomato and nightshade plants against *Tuta absoluta* (*Lepidoptera Gelechiidae*) through a hidden friendship and cryptic battle. *Sci. Rep.* **2020**, *10*, 1–13.
64. Zappalà, L.; Bernardo, U.; Biondi, A.; Cocco, A.; Deliperi, S.; Delrio, G.; Giorgini, M.; Pedata, P.; Rapisarda, C.; Tropea Garzia, G.; et al. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in southern Italy. *Bull Insectol.* **2012**, *65*, 51–61.
65. Nozad-Bonab, Z.; Hejazi, M.J.; Iranipour, S.; Arzanlou, M.; Biondi, A. Lethal and sublethal effects of synthetic and bio-insecticides on *Trichogramma brassicae* parasitizing *Tuta absoluta*. *PLoS ONE* **2021**, *16*, e243334. [CrossRef] [PubMed]
66. Contreras, J.; Mendoza, J.E.; Martınez-Aguirre, M.R.; Garcıa-Vidal, J.; Izquierdo and Bielza, P. Efficacy of entomopathogenic fungus *Metarhizium anisopliae* against *Tuta absoluta* (*Lepidoptera Gelechiidae*). *J. Eco. Entomol.* **2014**, *107*, 121–124. [CrossRef] [PubMed]
67. Olson, S. An analysis of the biopesticide market now and where is going. *Outlooks Pest Manag.* **2015**, *26*, 203–206. [CrossRef]
68. Das, S.K. Recent development and future of botanical pesticides in India. *Popular Kheti* **2014**, *2*, 93–99.
69. Sharma, K.R.; Raju, S.V.S.; Jaiswal, D.K.; Thakur, S. Biopesticides: An effective tool for insect pest management and current scenario in India. *IJAAS* **2018**, *4*, 59–62.
70. Zhu, F.; Lavine, L.; Neal, S.O.; Lavine, M.; Foss, C.; Walsh, D. Insecticide Resistance and Management Strategies in Urban Ecosystems. *Insects* **2016**, *7*, 2. [CrossRef]
71. Huang, F.; Buschman, L.L.; Higgins, R.A.; McGaughey, W.H. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science* **1999**, *284*, 965–967. [CrossRef] [PubMed]
72. Lee, M.K.; Aguda, R.M.; Cohen, M.B.; Gould, F.L.; Dean, D.H. Determination of binding of *Bacillus thuringiensis* δ-endotoxin receptors to rice stem borer midguts. *Appl. Environ. Microbiol.* **1997**, *63*, 1453–1459. [CrossRef]
73. Srinivasan, R. Susceptibility of legume pod borer (LPB), *Maruca vitrata* to δ-endotoxins of *Bacillus thuringiensis* (Bt) in Taiwan. *J. Invertebr. Pathol.* **2008**, *97*, 79–81. [CrossRef] [PubMed]
74. Buragohain, P.; Saikia, D.K.; Sotelo-Cardona, P.; Ramasamy, S. Development and validation of an integrated pest management strategy against the invasive South American tomato leaf miner, *Tuta absoluta* in South India. *Crop. Prot.* **2021**, *139*, 1–6. [CrossRef]
75. Shahini, S.; Bërxolli, A.; Kokojka, F. Effectiveness of bio-insecticides and mass trapping based on population fluctuations for controlling *Tuta absoluta* under greenhouse conditions in Albania. *Heliyon* **2021**, *7*, e05753. [CrossRef]