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Product Quality and Safety

Efficacy of local *Bacillus thuringiensis* isolates against tomato leaf miner (*Tuta absoluta*) larvae on tomato plants under screenhouse conditions

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Abstract. *The negative impact of chemical pesticides on the environment and the increased resistance of tomato leafminer (Tuta absoluta) field populations to chemical pesticides have promoted research on alternative control measures. Biological control with Bacillus thuringiensis (Bt) may be an alternative, especially against larval instars of T. absoluta. A total of five B. thuringiensis strains were isolated from soil sampled from two different Cow range lands in Zaria, Nigeria; and they were screened for the presence of the cry1 genes using polymerase chain reaction. Of the five isolates, two (40%) showed the presence of the cry1 genes. Results of the bioassay conducted against 2nd instar larvae of T. absoluta at 28±2°C indicated that each of the concentrations (25, 50, 75 and 100 ppm) of the spore crystal mixtures derived from the isolates harbouring cry1 genes caused significant mortality to larvae of T. absoluta after 72 hours in comparison to the control (0 ppm). Probit analysis was used to determine the LC_{50} and LT_{50} values. When the treatments were assessed at 48 and 72 hours, LC_{50} values against larvae were 74.1 and 25.3 ppm for isolate F3, while the LT_{50} values of that same isolate F3 at 100 ppm and 75 ppm were 36.3 and 42.7 hours, respectively. B. thuringiensis strain F2 achieved 68.7% reduction in T. absoluta damage on tomato plants, while B. thuringiensis isolate F3 achieved 71.3% reduction. Therefore, the spore crystal mixture derived from indigenous Bt strains is the candidate to be used for foliar application against T. absoluta and it is recommended into integrated pest control strategies for the management of T. absoluta.*

Keywords: *Bacillus thuringiensis*, biopesticide, *cry1*, pest control, tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the main vegetables in the world. The tomato category is the largest vegetable category in the world, accounting for 16% (Hepsağ and Kizildeniz, 2021). It is a source of many nutrients and its secondary metabolites such as folic acid, potassium, vitamins C and vitamin E, flavonoids, β-carotene and lycopene are essential to the health of humans (Shahzad et al., 2014). Nigeria is the largest tomato producer in sub-Saharan Africa, with an annual output of 1.8 million tons, and it also makes a huge contribution to the production of other major agricultural products (Borisade et al., 2017). The country's agro-ecological regions are diverse, and different regions have environmental advantages in terms of favorable climate and soil adaptability to specific crop production. The northern part of the country is the main producer of tomatoes (Etebu et al., 2013). However, its production is severely affected by the tomato borer *Tuta absoluta* (Meyriek) (Lepidoptera; Gelechiidae) (Youssef and Hassan, 2013).

The tomato leafminer, *Tuta absoluta* is a serious lepidopteran pest. Whether it is outdoor tomatoes or greenhouse tomatoes, it can cause 80-100% damage without effective control measures (Urbaneja et al., 2013). The insects usually deposit eggs on leaves, undersides of stems, and to a lesser extent on fruits. After hatching, the larvae will infiltrate the tomato fruits and leaves. They forage and develop on the leaves, forming mines and galleries. On the leaves, the larvae only feed on the mesophyll, while the epidermis remains intact (Sabbour and Nayera, 2014). *T. absoluta* disease outbreaks initially broke out in two West African countries: Niger and Senegal (Borisade et al., 2017; Huda et al., 2020) and then attacked tomatoes in Nigeria (Borisade et al., 2017). Since the introduction of *T. absoluta* in Nigeria during the 2016 tomato season, it was nicknamed 'Tomato ebola', and appeared in the Northern part of the country; which is the region with the highest tomato production (Borisade et al., 2017). In the first cycle, this pest caused a reduction in tomato production by more than 80%, resulting in a shortage of raw materials for the then newly established Africa's largest tomato processing company (Dangote Farms Tomato Processing Plant in Kano, Nigeria) and a price increase of up to 400% within three months. Therefore, it has become a major threat to the sustainable production of tomatoes in Nigeria (Borisade et al., 2017).

Synthetic pesticides partially solve the threat of *T. absoluta*, they also cause many problems because they are non-biodegradable, pollute the environment and leave residues in

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the agricultural products (Bhattacharjee and Dey, 2014). They also affect public health and continuous application can lead to resistance build-up among the pests and pathogens (Stangarlin et al., 2011; Engindeniz and Cosar, 2013; Wagnitz, 2014). Therefore, continuous efforts are being made to promote the use of microbial pesticides, and one such alternative is the use of *Bacillus thuringiensis (Bt)*.

B. thuringiensis is a soil bacterium that can produce selective toxic proteins in the form of crystals within the cell to fight insect pests (Hernández-Rodríguez and Ferré, 2009; Ibrahim et al., 2010; Schünemann et al., 2014). This protein inclusion is the active ingredient in Bt products. An effective tool for evaluating the effectiveness of crystals on pests is to identify genes encoding insecticidal toxins (Konecka et al., 2012). The *cry1* gene specifically encodes a protein that is active against *Lepidoptera* pests (Van Frankenhuyzen, 2009). The greatest success of microbial pesticides comes from the use of commercial formulations of Bt. These are the most successful biological pest control products in the world. 95% of the microbial pesticides sold are of this type of bacterial preparation, with estimated annual sales of $100 million (Schünemann et al., 2014). However, little is known about its effects when applied against *T. absoluta* larvae on tomato plants under screenhouse conditions. This study was conducted to isolate strains of *Bacillus thuringiensis* from various soil types in Zaria, Nigeria, to detect *cry1* gene in the isolates using PCR and to evaluate the biocontrol potentials of the isolates against *Tuta absoluta* larvae under screenhouse conditions

Material and method

Collection of soil samples

Soil samples were collected from the Zango and Shika Cow rangeland of Zaria, for isolation of *Bacillus thuringiensis (Bt)*. One soil sample of 5 g was collected from surface to a depth of 10 cm from different spots in each sampling site and pooled them to get composite soil sample. The collected samples were placed in sterile polythene bags, labelled and brought to the Environmental Research Laboratory, Department of Microbiology, Ahmadu Bello University (ABU), Zaria for isolation of *B. thuringiensis* from the samples.

Isolation of Bacillus thuringiensis

The isolation from soil was carried out by the sodium acetate selection method (Travers et al., 1987). One gram of soil sample was added to 10 mL of sterilized Luria Broth (Tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) buffered with 0.25 M sodium acetate (pH 6.8) in 125 mL conical flask and was incubated for four hours at 30°C with shaking at 200 rpm in incubating shaker. After incubation, aliquots of one mL were heated to 80°C for 3 minutes and tenfold of serial dilutions up to10^{-3} were prepared and one mL of suspension from each test tube was transferred in sterilized petri-plate. The sterilized Luria Agar medium was poured and mixed with aliquot gently. The plates were incubated at 30°C for 24 to 48 hr. The colonies formed were picked up based on their morphological similarities with those of reference *B. thuringiensis*. Selected colonies were purified by repeated streaking on T3 medium (Tryptone 3 g/L, yeast extract 1.5 g/L, Peptone 2 g/L, $MnCl_2$ 0.005 g/L, Sodium phosphate 0.05M and Agar 15 g/L) and then stored at 4°C for further studies.

Characterisation of Bacillus thuringiensis isolates

The morphological characterization of the isolates was observed following Gram staining and spore staining procedures. The isolates were subjected to several biochemical tests: catalase, acid and gas production, starch hydrolysis, citrate utilization, Methyl red-Voges Proskaeur, nitrate reduction, oxidase, casein hydrolysis, and esterase activity (Pagare et al., 2015).

Molecular detection of the cry1 gene using the *cry gene-specific primers*

DNA extraction was carried out using Qiagen DNAeasy extraction kit (Jiangsu Mole Bioscience Co., Ltd, China) according to the manufacturer's instructions. The PCR conditions for the amplification of the *cry1*gene were according to Ben-Dov et al. (1997).

PCR reactions were carried out in 25 µL reaction mixture containing 8 µL template DNA, 150 mM dNTPs, 20 pM of each of the four primers (Table 1) and 0.5U of *Taq* DNA polymerase. Amplification of the c*ry1* gene was carried out with the program: 1 initial denaturing cycle at 94ºC for 3 minutes, 35 cycles containing: denaturing at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension for 1 minute at 72ºC and then the reaction being terminated by a final extension for 10 minutes at 72ºC.

The *cry1* gene bands were visualized through agarose gel electrophoresis. An aliquot (15 µL) of each amplification product was loaded onto 1.5% agarose gel and run in TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) at 100 volts for 1 hour. The gels were stained with ethidium bromide and documented with a 100 bp molecular weight marker.

Table 1. Primer sets to be used in the amplification of the *cry1* gene

Gene	Primer Sequence (5'-3')	Product size (bp)
cry1	F CATGATTCATGCGGCAGATAAAC	274
	R TTGTGACACTTCTGCTTCCCATT	

Source: Ben-Dov et al. (1997)

Generation of spore crystal mixtures

Spore crystal mixtures of *Bacillus thuringiensis* were generated as described by Şahin et al. (2018) with some modifications. Single colonies from overnight LB plates were inoculated into 10 mL of T3 sporulation medium and cultured for 5 days at 28ºC on a rotatory shaker at 250 rpm. Spore crystal mixtures were harvested by centrifugation at 4000xg for 25 minutes. The pellets were resuspended in 5 mL distilled water and centrifuged again (4000xg for 10 minutes). This process was repeated twice. Then, the pellets of the spore crystal mixtures were resuspended in 5 mL distilled water and kept at -4°C until required.

Infesting tomato plants by Tuta absoluta's second instar larvae in screenhouse.

Experiments were conducted at the screenhouse of Ahmadu Bello University, Zaria from July to November 2020 in cages that were placed in the screenhouse at an average temperature of 28±2°C.

Tomato seeds (Roman VF variety) were purchased from Institute of Agricultural Research (IAR), ABU Zaria. Seedlings of the specified tomato were initially raised in nursery beds, in a screenhouse of Department of Microbiology, ABU Zaria, using a mixture of loam soil and composed manure. After two weeks, seedlings were transplanted into portable plastic pots (50 cm in diameter and 12 cm in depth) each containing 4 kg of the soil mixture (one seedling per pot). The potted plants were placed in the screenhouse to prevent them from attack by herbivore pests. Watering was done regularly to prevent wilting (Legwaila et al., 2015).

Tuta absoluta larvae were collected from tomato farmlands in Zaria, Nigeria using a glass jar with perforated lids and were taken to Entomology laboratory, department of Crop protection, Ahmadu Bello University Zaria to separate second instar larvae from the larvae collected. Tomato seedlings at the five-leaf stage were infested for 24 hours with the *T. absoluta* larvae confirmed. Pots of tomato plants were placed in each of the cages. Each cage was 120 cm long, 100 cm wide, and 50 cm high, and was covered with clear lumite netting to prevent pest infestation from natural populations. Every cage has a door with a metal sleeve that was used during watering of plants, artificial infestation of the plants, the application of sprays, and the removal of plants during pest larva assessments.

Bioassay and assessment of larval mortality and plant damage under screenhouse conditions

Crude harvested spore crystal mixture was used to spray the larvae to determine the toxicity of the mixture. Treatment was carried out after infesting tomato plants with *T. absoluta* for 24 hours. Three treatments consisting of two Bt concentrations (50 and 100 ppm) and distilled water (control) were used for each isolate confirmed by PCR possessing the *cry1* gene (100 ppm concentration was achieved by addition of 10 ml of distilled water to 1ml on spore crystal while 50 ppm concentration was achieved by addition of 10ml of water to 0.5ml of spore crystal mixture). Each seedling was sprayed separately. The bioassay was carried out in triplicates. Each pot bore a label, which indicates the treatment and its date of application.

A small hand-held trigger sprayer that produced a fine spray of a relatively narrow range of droplet sizes was used to apply the spray solutions. The bioassay was conducted on second instar larvae. The larvae mortality was assessed at intervals of 24, 48, and 72 hours after treatment. Any larvae that did not show signs of life after prodding with a needle was considered as dead (Legwaila et al., 2015) and the number of dead larvae was counted.

The plant damage assessments in each treatment were conducted 7 days after the infestation of tomato plants by *T. absoluta* larvae. The total number of leaves per plant was recorded, the number of leaves with damage symptoms (presence of windows) (Legwaila et al., 2015) was counted, and the results were used to calculate the percentage of damaged leaves per plant. The plant damage assessments were also carried out in triplicates.

Data analysis

Results were presented in tables, graphs and charts where applicable. Probit analysis (Semiz, 2017) was used to analyze the mortality results. The percentage mortality data was corrected for percentage control mortality using Abbott's formula (Abbott, 1925) before being transformed to probits, while the concentrations and time were transformed to $\log_{10}(x+1)$ before analysis. LC_{50} and LT_{50} values were computed by probit analysis. The results on percentage plant damage were transformed to arcsines before analysis in order to achieve normalization of data. Using the SAS JMP Pro 14 statistical package, two-way analysis of variance (ANOVA) was used to analyze the mean toxicity of the crystal spore mixture produced by each isolate and one-way ANOVA was used to analyze the mean data of the plant damage assessment. Averages were separated using Tukey's Honestly significant difference test (Zar, 1984) where significant effects were found.

Results

Occurrence of Bacillus thuringiensis in the study sites

The colonial and microscopic morphology, as well as the biochemical characteristics of the isolates showing the expected characteristics of *Bacillus thuringiensis* are as shown in Table 2.

Table 2. Microscopic, colony morphology and biochemical characterization of isolates *B. thuringiensis*

	F1	F2	F3	F4	F5
Colour/Margin	Creamy white/Wavy	Creamy white/Wavy	Creamy white/Wavy	Creamy white/Wavy	Creamy white/Wavy
Gram reaction/Shape of cells	+/R	+/R	+/R	+/R	+/R
Presence of endospore	+	+	+	+	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Utilization of citrate	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Oxidase	+	+	+	+	+
Methyl red	-	-	-	-	-
Voges proskauer	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+
Esterase activity	-	-	-	-	-
Acid and gas production	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+

F1 and F2 = Isolates from Zango Cow rangeland; F3-F5 = Isolates from Shika Cow rangeland; (+) = positive reaction, (-) = negative reaction, R = rod.

The cry1 gene profile of the isolates

The five isolates identified using colonial, microscopic and biochemical characterizations were screened for the presence of the *cry1* gene using specific primers by PCR. The amplicons of the *cry1* gene (274 bp) were detected in two of the strains of *Bacillus thuringiensis* (Figure 1).

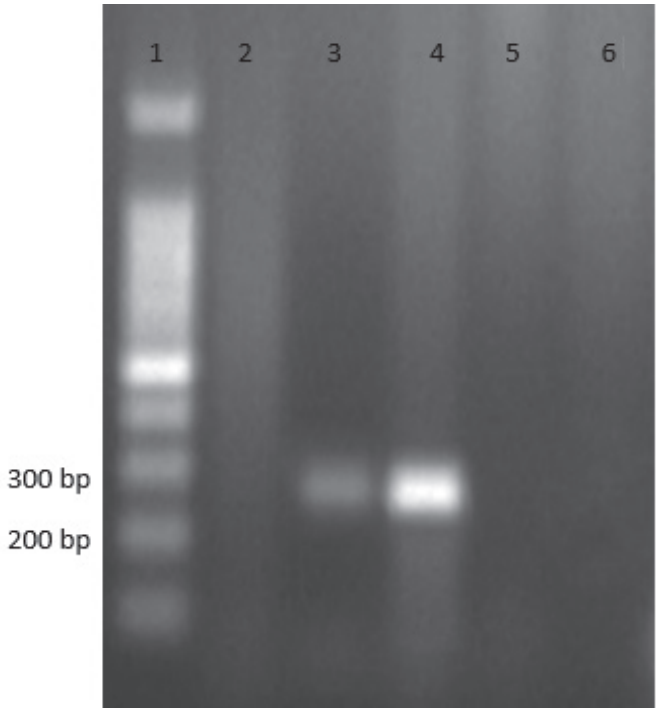

Figure 1. Amplification of the *cry1* gene (274 bp) in *B. thuringiensis* isolated from soil samples (Lane 1: DNA ladder (100bp); Lanes 2 and 3: *Bt* isolated from Zango Cow rangeland /F1 and F2, respectively/; Lanes 4 - 6: *Bt* isolated from Shika Cow rangeland /F3, F4 and F5, respectively/)

Larvicidal activity and effect on plant damage of the spore crystal mixture derived from the isolates

Figures 2-7 show a positive linear relationship between the concentration (transformed to $\log_{10}$/x+1/) and mortality (transformed to probits) caused by *B. thuringiensis* isolates F2 and F3. Figures 2 and 5 indicate that *B. thuringiensis* F2 and F3 insecticide did not achieve 50% larval mortality 24 hours after application at all the concentrations. When *B. thuringiensis* isolate F2 was applied at a concentration of 100 ppm, the mortality of larvae of 4.33, 5.00 and 5.47 was achieved at 24, 48 and 72 hours, respectively. The LC_{50} value were 96.7 and 39.8 ppm for isolate F2 (Figures 3 and 4) and 74.1 and 25.30 ppm for isolates F3 (Figures 6 and 7) when assessed 48 and 72 hours, respectively.

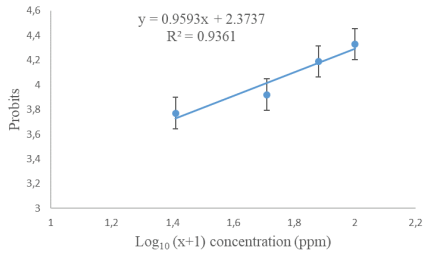

Figure 2. Probit mortality of *T. absoluta* larvae 24 hours after application of different concentration of *B. thuringiensis* F2

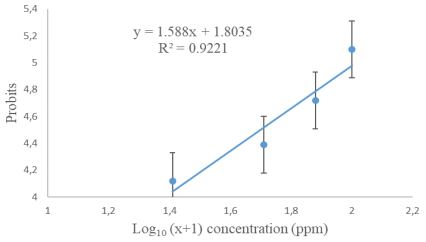

Figure 3. Probit mortality of *T. absoluta* larvae 48 hours after application of different concentration of *B. thuringiensis* F2

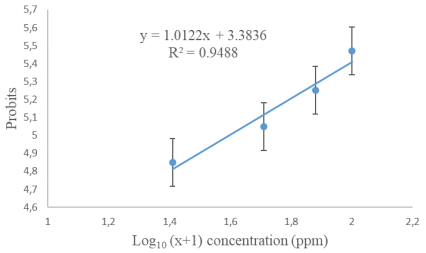

Figure 4. Probit mortality of *T. absoluta* larvae 72 hours after application of different concentration of *B. thuringiensis* F2

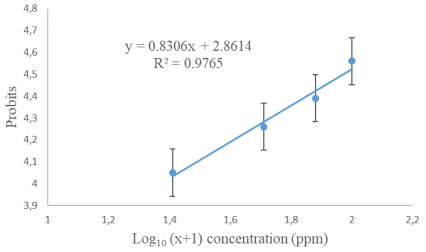

Figure 5. Probit mortality of *T. absoluta* larvae 24 hours after application of different concentration of *B. thuringiensis* F3

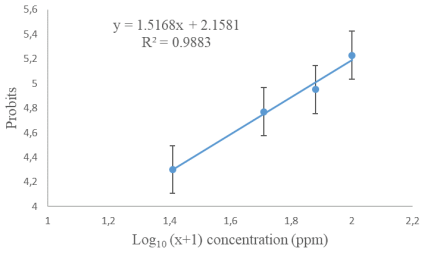

Figure 6. Probit mortality of *T. absoluta* larvae 48 hours after application of different concentration of *B. thuringiensis* F3

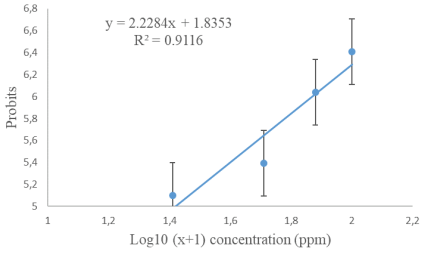

Figure 7. Probit mortality of *T. absoluta* larvae 72 hours after application of different concentration of *B. thuringiensis* F3

Results in Tables 3 and 4 show that both concentration and time significantly affected the average mortality of *T. absoluta* larvae per plant (ANOVA, $p<0.05$). The

interactions were also significant. For isolate F2, the greatest mortality (60 and 68% per treatment) occurred 72 hours after application of 75 and 100 ppm, respectively, while for isolate F3, the greatest mortality (84.6 and 92.3%) occurred 72 hours after application of 75 and 100 ppm, respectively. Of the two *B. thuringiensis* isolates, isolates F3 achieve over 90% larval mortality when applied for 72 hours. The results also show that the least mortality (3.3-16.7% per treatment) occurred in the control treatment throughout the study period for the two isolates F2 and F3. The overall treatment averages show that *B. thuringiensis* isolates F2 and F3 concentrations also had a significant effect on the mortality of larvae. Overall larval mortalities differed significantly from each other and increased in the order 24.6 < 31.1 < 40.0 < 47.7% when treated with 25, 50, 75 and 100 ppm, respectively, for isolate F2. The larval mortalities also differed significantly from each other and increased in the order 32.1 < 43.1 < 53.1 < 61.6% when treated with 25, 50, 75 and 100 ppm, respectively, for isolate F3. The overall exposure period results were also significantly different and increased in the order 20.7 < 36.8 < 61.9 when assessment was done 24, 48 and 72 hours after application of isolate F3. For isolate F2, the overall exposure period results were also significantly different and increased in the order 15.6 < 29.6 < 48.1 when assessment was done 24, 48 and 72 hours.

Table 3. Effect of *B. thuringiensis* F2 spray concentration and period of exposure on *T. absoluta* larval mortality

Period after application (hours)	Control	25 ppm	50 ppm	75 ppm	100 ppm	Overall period averages
24	6.7^{i*}	10.7^{fgi}	14.3^{fg}	21.4^{d}	25.0^{cd}	15.6^{c**}
48	13.3^{fg}	19.2^{de}	27.0^{c}	38.5^{bc}	53.8^{b}	29.6^{b}
72	16.7^{f}	44.0^{bc}	52.0^{b}	60.0^{a}	68.0^{a}	48.1^{a}
Overall treatment averages	12.2^{d***}	24.6^{cd}	31.1^{c}	40^{b}	47.7^{a}	31.1

*Interaction averages in the body of the table followed by the same letters are not significantly different (Tukey's Honestly significant difference test - p<0.05);

**Averages in the column followed by the same letters are not significantly different (Tukey's Honestly significant difference test - p<0.05);

***Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test - p<0.05).

Table 4. Effect of *B. thuringiensis* F3 spray concentration and period of exposure on *T. absoluta* larval mortality

Period after application (hours)	Control	25 ppm	50 ppm	75 ppm	100 ppm	Overall period averages
24	3.3^{f*}	16.7^{cde}	23.3d^{e}	26.7^{d}	33.3^{cd}	20.7^{c**}
48	10.0^{cdef}	25.9^{d}	40.7^{c}	48.1^{c}	59.3^{b}	36.8^{b}
72	13.3^{cde}	53.8^{b}	65.4^{b}	84.6^{a}	92.3^{a}	61.9^{a}
Overall treatment averages	8.9^{c***}	32.1b^{c}	43.1^{b}	53.1^{ab}	61.6^{a}	39.8

*Interaction averages in the body of the table followed by the same letters are not significantly different (Tukey's Honestly significant difference test - p<0.05);
**Averages in the column followed by the same letters are not significantly different (Tukey's Honestly significant difference test - p<0.05);
***Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test - p<0.05).

Table 5 shows that the greater the concentration of *B. thuringiensis* isolates spray, the lesser the LT_{50} required. The highest LT_{50} were at concentration 25 ppm for both isolates which were 106.2 hours and 79.4 hours for isolates F2 and F3, respectively. While the lowest LT_{50} values for *B. thuringiensis* isolates F2 and F3 when assessed were 49.1 and 36.3 hours, respectively, at concentration 100 ppm for both isolates.

Table 6 shows the effect of different *B. thuringiensis* isolates F2 and F3 concentrations on the tomato leaf damage per treatment. One-way ANOVA shows that the average damage was significantly (ANOVA, p<0.05) affected by the isolates concentrations used. The least damage caused by *T. absoluta* (31.3% per treatment) was observed with application of 100 ppm of *B. thuringiensis* isolate F2, while the greatest damage (64.2%) was observed at 0 ppm (p<0.05). For isolate F3, the least damage (28.7% per treatment) was achieved with application of 100 ppm, while the greatest damage (60.0%) was achieved at 0 ppm (p<0.05).

Table 5. LT_{50} values of *B. thuringiensis* F2 and F3 spray concentrations against 2nd larval instar of *T. absoluta* under screenhouse at 28±2°C

Concentrations (ppm)	F2	F3
	LT_{50}*	LT_{50}*
25	106.2	79.4
50	80.3	56.2
75	62.1	42.7
100	49.1	36.3

*LT_{50} value = time needed (hours) by *B. thuringiensis* F2 and F3 spray concentrations to cause 50% mortality

Table 6. Effect of *B. thuringiensis* F2 spray and F3 concentrations on tomato leaf damage per treatment after 7 days

Treatment averages	Concentrations (ppm)				
	0	25	50	75	100
F2	64.2^{a*}	56.1^{ab}	47.3^{abc}	38.9^{bc}	31.3^{c}
F3	60.0^{a*}	51.7^{ab}	40.4^{abc}	33.8^{bc}	28.7^{c}

*Averages in the row followed by the same letter are not significantly different (Tukey's Honestly significant difference test - $p<0.05$).

Discussion

Nigeria is facing the dual problems of population growth and reduced food production. Therefore, it is necessary to increase the production of food. However, there are many factors that affect the quality and yield of agricultural production. One of these main factors is damage from pest. Insect pests are the main destabilizing factor of crop productivity in every agricultural ecosystem, leading to the loss of major economic crops such as tomato. For decades, the control of pests through toxic chemicals has minimized pest-related crop losses. Unfortunately, the massive and indiscriminate use of these pesticides has caused environmental pollution, harm to human and animal health and adverse effects on non-target beneficial insects. In order to circumvent the many limitations of synthetic pesticides, there is need to consider the use of biological pesticides as one of the most promising source for a more reasonable and safe crops management practices that have the potential to satisfy consumer needs, take care of human health and environmental safety. On this context, this research was conducted to isolate and characterize local strains of *B. thuringiensis* with the *cry1* gene specific primer and bioassay on *T. absoluta* under screenhouse conditions.

In the present study, five *B. thuringiensis* isolates were isolated from the soil. The microscopic, colonial morphology and the biochemical characteristics of the organisms as shown in Table 2 confirmed the characteristics typical of *B. thuringiensis*, being Gram-positive, having a rod shape and subterminal spores position were seen in the isolates. The biochemical characteristics showed the ability to utilize citrate among others. These characteristics are similar to those reported by Ahmed et al. (2015) and Riskuwa-Shehu et al. (2019).

The *cry1* toxins display activity against lepidopteran to which *T. absoluta* belongs, dipteran, and coleopteran pests. Two of the five soil-isolated strains (F2 and F3) had *cry1* gene. This *cry1* gene was noted as the most frequent in *B. thuringiensis* strains by several authors (López-Pazos et al., 2009; Baig and Mehnaz, 2010; Saadaoui et al., 2010; Liang et al., 2011).

According to the results in Tables 3 and 4, several points can be observed. Longer exposure time is required to achieve over 60% and over 80% *T. absoluta* larval mortality when *B. thuringiensis* isolates F2 and F3 were used, respectively. Also, higher concentration of the *B. thuringiensis* spore crystal mixture is needed to effectively protect tomato plants from *T. absoluta* damage in a screenhouse. For example, *B. thuringiensis* isolate F3 achieved 84.6 and 92.3% larval mortality when concentrations of 75 and 100 ppm were used, respectively. The main reason for the longer exposure time may be due to the fact that *B. thuringiensis*-affected larvae die of starvation, which may take several days. Due to the fact that *B. thuringiensis* cannot kill rapidly, potential users may mistakenly believe that it is ineffective if treatments are evaluated one or two days after use (Talekar, 1992; Legwaila et al., 2015; Melo et al., 2016; Zhang et al., 2017; Fernández-Chapa et al., 2019). These findings corroborated those obtained by Legwaila et al. (2015) and Fatimah and Hasmiwati (2020). Therefore, the results of this study indicate that in addition to the concentration that should be used for the pest, the information on the pesticide label should also include the exposure time needed to achieve the target pest control level. This information will enable farmers to determine the interval between sprays (Legwaila et al., 2015).

Probit analysis was used to obtain the toxicity value of pesticides to experimental insects. The toxicity value can be expressed by lethal concentration (LC) and lethal time (LT) (Fatimah and Hasmiwati, 2020). Figures 2-7 show that the lines of probit analysis when compared can also provide insight into the relative toxicities of *B. thuringiensis* spore crystal mixtures. The slopes of the probit lines show that the concentration mortality response of *T. absoluta* to the different concentrations of *B. thuringiensis* isolates F2 and F3 indicated a slight variability among the isolates to kill the insects. The lowest LC_{50} (25.3 ppm) was observed for F3 which indicated the comparatively high effectiveness of the isolate. The differences in the insecticidal activities of the two isolates F2 and F3 may be attributed to the difference in the carbohydrate affinity of the cry proteins domain II, which results in different binding specificities with the insect larvae brush border membrane receptors, resulting in differences in the toxicity of the cry proteins (Smedley and Ellar, 1996).

The time mortality response of isolates F2 and F3 against *T. absoluta* larvae as shown in Table 5 and 6 revealed that the isolate F3 had the faster knock down action. The LT_{50} was recorded as 79.4, 56.2, 42.7 and 36.3 h for the concentrations 25, 50, 75 and 100 ppm, respectively. This showed the efficacy of the local strain F3 to cause mortality within a short time. This could be ascribed to the synergistic effect of spores, which was promoted by the activity of the specific crystal protein and leads to the rapid killing effect of the isolate. Aly et al. (1985), Wilson and Benoit (1990) and Borgonie et al. (1995) reported that the spores contaminated the haemocoel through a channel formed in the midgut membrane, and use the nutrients in the haemolymph to reproduce at a faster rate, which may cause bacterial sepsis and the death of larvae within two to three days of ingestion.

From the results in Tables 7 and 8, it can be inferred that in order to determine the efficacy of *B. thuringiensis* isolates in *T. absoluta* management on tomato plants, it is important to not only consider the percentage of pest mortality achieved but also to assess the significant reduction in crop damage caused

by the use of the spore crystal mixture derived from these isolates (Legwaila et al., 2015). It was observed in this work that the higher the concentration used, the lower the damage on the tomato plants after 7 days of the spore crystal mixture application. This observation shows that the concentration of spore crystal mixture and damage of crops by pests are inversely proportional. This finding is in agreement with the plant damage assessment conducted by Legwaila et al. (2015), who reported that at higher concentrations of *Btk* applications, there's a significant reduction in the damage of the cabbage plants used.

Conclusion

This work is the first assessment of the performance of local strains of *B. thruingiensis (Bt)* on *Tuta absoluta* infestation of tomato plants under screenhouse in Nigeria. It can be concluded that if longer time exposure and higher concentrations are allowed, these two isolates F2 and F3 can effectively reduce and control the serious damage caused by the larvae of *T. absoluta* on tomato plants in Nigeria especially, in the Northern parts of the country. In this work, we demonstrated that exposure time is a factor in determining the efficacy of *Bt* spore crystal mixture. Further investigation is required to determine whether factors such as relative humidity, temperature, and adjuvant is viable to increase *Bt* toxin efficacy under screenhouse conditions. Also, since the local strains of *Bt* used in this study exhibited a good activity in the screen house, they should be subjected in open field application to see their effectiveness.

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