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Efficacy of Beneficial Fungi Isolates in *Solanum lycopersicum* L. Protection against Lepidopteran Insects through a Leaf Inoculation Technique

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Abstract

Helicoverpa armigera is a key insect pest of tomatoes reducing drastically yields. The effect of the endophytic colonization of tomato plants by *Beauveria bassiana* using leaf spray as an inoculation method on damage and survival of *H. armigera* was assessed in a screen house. Two *B. bassiana* isolates (Bb 115 and Bb 11) and two tomato varieties (a local variety Tounvi and an improved variety Padma) were included in the study. The adaxial and abaxial leaf surfaces were sprayed at a concentration of 10^7 conidia/ml and 10^9 conidia/ml for each isolate and each of the two tomato varieties. Thirty days after inoculation, five discs of tomato leaf and tomato root were cut for each isolate, each concentration per isolate and for each variety. The samples were incubated at room temperature (28˚C ± 2˚C) and periodically checked for fungal growth. Larval survival was checked and a damage assessment was done on tomato flowers and the leaves. The results show that the lowest Mean Survival Times (MSTs) were recorded on larvae feeding on plants inoculated with Bb 11 (4.2 ± 0.8 days against 11.5 ± 0.2 days for control). Compared to the other treatments, low damage rates of the flowers of the improved variety inoculated with Bb 11 at 10^9 conidia/ml were recorded from the 6th Day After Inoculation (DAI). This rate remains low until the end of treatment. Overall flower damage was lower than leaf damage. The results showed large differences in pathogenicity, with most endophytic isolate belonging to Bb 11 when

inoculated at 10^9 conidia/ml using the leaf spraying technique. Data were discussed with regard to the use of endophytism *B. bassiana* in an integrated tomato pest control approach.

Keywords

Tomato, Insect, *Beauveria bassiana*, Foliar Spray, Endophytic, Pest Management

1. Introduction

With a global production of 177 million tons and an average yield of 37 t/ha [1], the tomato (*Solanum lycopersicum* L) is one of the most nutritionally and economically important crops in the world [2]. In Benin, tomato production is widely established but yields are still low (with an average of 9.5 t/ha) due to biotic pressure from pest [3]. Among the numerous insect pests, the tomato fruit worm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is considered a major pest in Benin due to its direct damage to growing fruit [4]. *Helicoverpa armigera* has also been reported as a major pest of cotton, tomato, sorghum, maize, sunflower, groundnuts, cowpea, and green pepper [5]. The infestation of these crops by *H. armigera* causes heavy yield losses both in quality and quantity, with significant socio-economic impacts [6] [7].

The extreme polyphagy of *H. armigera*, its wide geographic scope, its mobility and ability to migrate and its high fecundity are factors that allow *H. armigera* to adapt to different cropping systems, which greatly contributed to conferring on it the status of major pest [8]. *Helicoverpa armigera* can attack tomato crops from planting to fruit maturity causing heavy damage to growing leaves and fruits [3] [9].

The conventional strategy to manage these pests is based on synthetic pesticides with implications for the economy, human health and the environment. In Benin, these Agrochemicals have been shown to be effective against *Helicoverpa armigera*. These are pyrethroids, cypermethrin, deltamethrin, bifenthrin, and fenvalerate [10] [11]. However, the use of chemical insecticides in the control of *H. armigera* larvae also leads to loss or reduction of biodiversity, pest resistance and toxicity to other non-organisms [12]. Primarily driven by concern about adverse effects of chemical plant protection products on humans and the environment, efforts have been made in recent decades to limit chemical seed treatments by using alternative environmentally sound methods. The alternatives available so far include physical methods, biological control based on the use of microorganisms such as bacteria or fungi and use of natural compounds from plants [13]. Among the most sustainable alternatives, biological control with entomopathogenic organisms ranks first [4] [14] [15]. In particular, entomopathogenic fungi have the advantage of being able to attack several species belonging to different insect orders (Lepidoptera, Coleoptera, Orthoptera, etc.) [16]

[17] [18]. Of these, *Beauveria bassiana* Vuillemin (Ascomycota: Hypocreales) has been recently investigated for its virulence against caterpillars of various crop pests of importance in Benin, including *H. armigera* [4] [19]. In fact, the fungus *B. bassiana* was reported to be a promising option as an entomopathogenic fungal species for the control of *H. armigera*. He can infect all *H. armigera* larvae instars and use several modes of action like infection by conidia and toxins [20]. Besides its direct infection of host stages, the entomopathogen *B. bassiana* has a wide range of host plants in which this fungus can develop endophytically [21] [22]. Therefore, *B. bassiana* has a complex life cycle that can be completed in the soil, in invertebrates, or in plants [23] [24]. Epiphytic and Endophytic microorganisms reside asymptomatically within higher plants, inhabiting leaves, stems and roots without any apparent harm to the plant [25]. Among the modes of action of endophytes, secreting toxic compounds is believed to kill particularly early instars of insect pests, while some of their metabolites can deter insect feeding [24]. Hence, the colonization of plant tissues by *B. bassiana* was reported to provide protection against insect damage and inhibition of insect establishment and development [21] [26].

Despite these advantages, very few studies have been carried out to assess the susceptibility of lepidopteran species to endophytic colonization of tomatoes by *B. bassiana*. In our recent study, we evaluated the endophytic colonization of *B. bassiana* in tomato plants, using a seed coasting method as the fungus conidia naturally live in soil [20]. Indeed, many pathogenic fungi such as *B. bassiana* have been found to enter plant tissues through roots and stomata [27]. With seed coasting, we found higher root colonization by *B. bassiana* compared to leaves and stems [20]. But as *H. armigera* is an above ground insect pest, it was suggested to investigate a spray inoculation technique. This would potentially support designing an effective control strategy based on endophytic colonization of tomatoes by *B. bassiana* for sustainable tomato production in Benin.

2. Material and Methods

2.1. Rearing of *Helicoverpa armigera*

Larvae of *H. armigera* were collected from tomato fields at different localities in Benin and a rearing colony was established in the laboratory using artificial diet [27] (Teakle and Jensen 1985). Experiments were performed at 70% ± 5% relative humidity and 26˚C ± 2˚C, with a photoperiod of 14:10 h. Third instars larvae (L3; 7.4 ± 0.1 days) were used in all bioassays, because at this stage, *H. armigera* cause the greatest damage to host plant [28].

2.2. Fungal Isolates

Two *B. bassiana* isolates Bb11 (endogenous isolate, from Benin*)* and Bb115 (from elsewhere), were obtained from the microbial collection of the International Institute of Tropical Agriculture, IITA-Benin. The two isolates were selected based on their virulence during previous laboratory assays in Benin [29]

[30] [31]. Conidia of the two isolates were obtained from mass culture of the fungus in Petri dishes (9 cm diam) containing Potato Dextrose Agar (PDA). The Petri dishs were sealed with Parafilm. After 15 days of incubation at 26˚C ± 2˚C, conidia suspensions were prepared by scraping conidia from the Petri dishs into a sterile aqueous solution of 0.1% Tween 80 [32]. The conidia suspensions used for the bioassays were adjusted by diluting with 0.1% Tween 80 to get final concentrations of 10^7 conidia/ml and 10^9 conidia/ml.

Conidial germination was tested using a sub-sample of 100 conidia [29]. Conidial viability was assessed prior to bioassays by spreading 0.1 ml of 3×10^6 conidia/ml onto 9 cm Petri dishes containing PDA [33]. Plates were then incubated at 27˚C ± 2˚C and checked 20 hours later under the microscope. Conidia were considered, germinated when the germ tube measured twice the diameter of the conidium. Viability checks were replicated four times.

2.3. Plant Material

The local tomato variety "Tounvi" and an improved variety "Padma" disseminated in Benin by the Benin National Agricultural Research Institute [34] were used for our studies. The improved variety "Padma" originated in Norway and was reported to be resistant to the bacterial wilt caused by *Ralstonia solanacearum* and mosaic virus disease [34]. Both varieties are the most cultivated and consumed in Benin. They are semi-upright with a development cycle lasting 65 - 90 days and 60 - 70 days, and average tomato fruit weights of 24 g and 120 - 130 g for the local and improved varieties, respectively [35]. Seeds were not treated with chemicals prior to bioassays.

2.4. Sowing and Plant Material Preparation

Before sowing, tomato seeds were sterilized by immersing them in 70% ethanol for 2 min, subsequently rinsing them using sterile distilled water, followed by immersion in 0.5% sodium hypochlorite for 1 min, and rinsing again in sterile distilled water. Seeds were placed onto sterile filter paper for drying for 30 min [36], and were subsequently transferred into small plastic pots containing washed sand. The sand was sterilized in an autoclave for 45 min at 121˚C three times with 24 h interval and allowed to cool for 24 h prior to sowing. Three seeds were sown per plastic pot and pots were placed at 27˚C ± 3˚C. Each of the pots contained 3 kg of sterilized soil, collected at the experimental farm. Plants were watered daily, late at night [37]. Growing plants were kept in a greenhouse (26˚C ± 5˚C, 14:10 h photoperiod) and transferred 30 days later into large pots 30 cm height and used for the bioassays.

2.5. Evaluation of *B. bassiana* as an Endophyte of Tomato Plants

Fifteen tomato plant were inoculated with Bb 115 or Bb 11 with leaf spray method as described by Qayyum *et al.* [38] and Kasambala *et al.* [39]. The adaxial and abaxial leaf surfaces were sprayed at a concentration of 10^7 conidia/ml and

10^9 conidia/ml for each isolate and each of the two tomato varieties (local and improved). During the inoculation, the non-inoculated plant organs (stems) and the soil were covered with aluminum foil to avoid exposure to run-off of the suspension. Then, the inoculated leaf area was covered using transparent plastic sheet for 24 h to promote fungal growth. A total of fifteen tomato plants were inoculated per treatment and non-inoculated control plants were sprayed using sterile water with 0.10% Tween 80. The plants for each treatment are protected by cages covered with ventilated netting. Of the fifteen plants for each treatment, ten plants were selected to release the larvae and the remaining five were used to test for the presence of the fungus on PDA.

The endophytic colonization of tomato plants by *B. bassiana* was checked two weeks after inoculation by sampling leaves and roots. Thus, five leaves and roots were sampled randomly from tomato plants that had been inoculated with different concentrations of the two *B. bassiana* isolates. Samples were transferred to the laboratory, and then cut in pieces with a sterilized knife in laminar flow chamber. Five pieces of each tissue were first put in 0.5% sodium hypochlorite for 3 min, then immersed in 70% ethanol for 2 min, dried and placed on PDA in Petri dishes (9 cm diam.). The samples were incubated at room temperature (28 ± 2˚C) and periodically (everyday) checked for fungal growth. Five discs of tomato leaf and root were cut for each isolate, each concentration per isolate and for each variety. Five leaves and roots discs were cut in the control treatments (not inoculated). Thus, the presence or absence of *B. bassiana* on the leaf and root sections was recorded after 14 days at 25˚C [38] [40] based on its morphological characteristics. For each plant organ, percent colonization was calculated as number of sections exhibiting *B. bassiana* out growth over the total number of sections [41].

2.6. Effect of Endophytic Colonization of Tomato by *B. bassiana* on the Survival of *H. armigera* Larvae

A batch of tomato plants inoculated with *B. bassiana* suspension as described above was kept for assessing the effect on survival *H. armigera* larvae. Healthy third instar larvae were transferred onto leaves of inoculated plants [42]. Each treatment consisted of 10 pots, with two larvae per pot, replicated three times for each of the two tomato varieties. Larval survival was checked daily for twelve days [43].

2.7. Assessment of Damage of the Plant Tissues

Damage assessment was done on the flowers and the leaves. In fact, damage to leaves and flowers by *H. armigera* larvae was assessed six times (2th, 4th, 6th, 8th, 10th, 12th DAI). For this observation, ten flowers and/or ten leaves per plant on five plants/treatment randomly selected were collected for evaluation at the laboratory [4] [18]. The presence of *H. armigera* larvae was checked and their damage was assessed.

2.8. Data Analysis

Survival of *H. armigera* larvae and their percent damage to leaves vs flowers were compared using a general linear model (GLM) procedure in SAS (SAS 2002-2008)[1] followed by the test of Student-Newman-Keuls. The proportion of tomato leaf and root colonized by *B. bassiana* in inoculated and control (non-inoculated) plants were compared using SAS. Percent data were transformed [Arcsin (square (p))] prior to the analysis. Mean Survival Times (MSTs) and survival curves for inoculated and non-inoculated plants were obtained through Kaplan–Meier analysis using MedCal software version 17.

3. Results

3.1. Detection of the Endophytic Colonization of Tomato Leaves and Roots by *B. bassiana*

Both fungal isolates tested were able to colonize the leaves, regardless of the tomato varieties. However, higher leaf colonization rates were observed in the improved variety, when tomato plants were inoculated with the isolate Bb 11 at 10^9 conidia/ml compared to isolate Bb 115 ($df = 1$, $F = 111.342$, $P \leq 0.000$). Likewise, significant differences occurred between fungal concentrations in Bb 11 while this was not the case for Bb 115 (Figure 1) (fungal isolate $F = 28.56$, $P < 0.01$; variety used: $F = 172.31$, $P < 0.01$, fungus × variety used: $F = 2.75$, $P = 0.02$). On the other hand, in the local variety, significant differences were obtained between Bb 115 concentrations but not between those of Bb 11. No fungal growth was detected in non-inoculated controls.

Leaf inoculation with *B. bassiana* incited colonization of roots of both tomato varieties, regardless of isolate. In the local variety, low root colonization rates

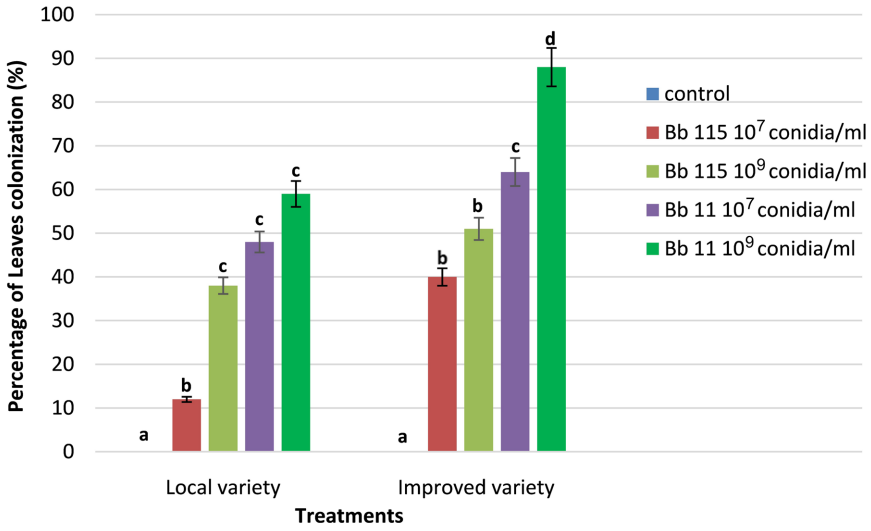

Figure 1. Mean (±standard deviation) colonization (%) rate of tomato leaf 15 days after inoculation with *Beauveria bassiana*, using leaf spraying method. Bars with different letters indicate significant differences after ANOVA followed by Tukey's test ($p < 0.05$).

[1]**SAS Institute Inc (2003)** SAS® 9.2 2003. Qualification Tools User's Guide. SAS Institute Inc., Cary.

(≤40.0%) were observed irrespective of isolate and concentration, with Bb 115 at 10^7 conidia/ml being the lowest (12.5% of roots colonized) (Figure 2). In the improved variety, the highest root colonization rate (86% of roots colonized) was obtained in Bb 11 at 10^9 conidia/ml (df = 23, F = 22.412, P ≤ 0.000).

3.2. Damage Assessment

The percent of damaged leaves of non-inoculated plants was significantly higher than that from inoculated plants (df = 1, F = 101.38, P ≤ 0.000). Leaf damage was lower on plants inoculated with Bb 11 compared to that observed when plants were inoculated with isolate Bb 115, regardless of tomato variety (Table 1). This trend was confirmed during several days after inoculation and the highest leaf damage rate was recorded in non-inoculated control (Table 1). Comparison between varieties did not reveal any significant differences (F = 2.39, P < 0.1467).

The overall damage to flowers was lower than that observed on leaves during the experimental period. The number of flowers damaged by *H. armigera* larvae increased during the twelve days of observation, regardless of tomato varieties. No damage was recorded during the first four days after inoculation (DAI) in improved variety inoculated with Bb 11 at 10^9 conidia/ml, and flower damage remained low after 8th DAI (Table 2). No significant difference was observed between tomato varieties for flower damages (Table 2).

Mean Survival Times (MSTs) of the *H. armigera* larvae

A progressive decrease in the Mean Survival Times (MSTs) of *H. armigera* larvae was observed from control plants to inoculated plants at 10^9 conidia/ml in

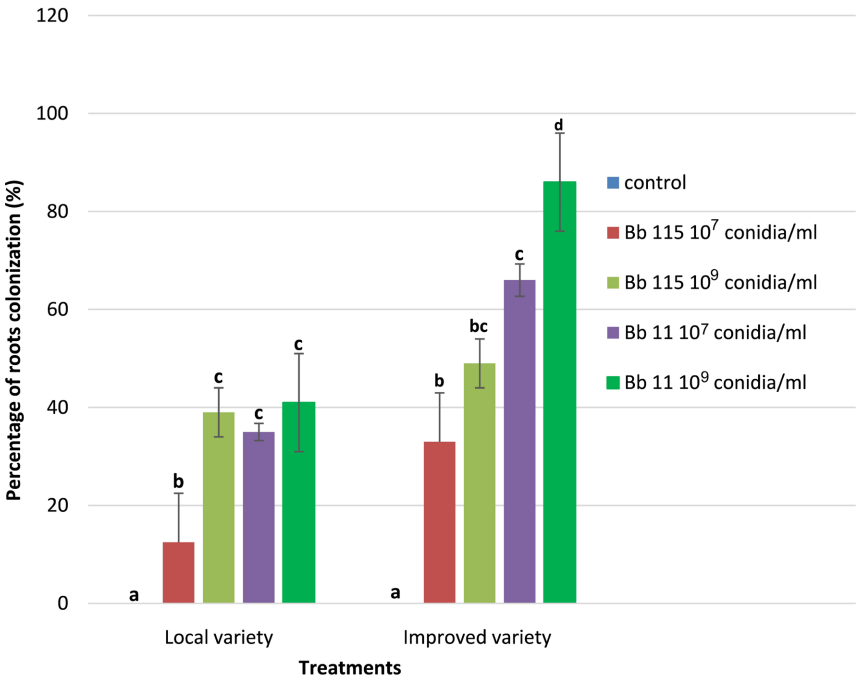

Figure 2. Mean (±standard deviation) colonization (%) of tomato root 15 days after inoculation with *Beauveria bassiana*, using leaf spraying method. Bars with different letters indicate significant differences after ANOVA followed by Tukey's test (p < 0.05).

Table 1. Damage of leaves (Average ± Standard error) by *H. armigera* larvae.

Varieties	Treatments	conidia/ml	Days After Inoculation (DAI)					
			2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI
Improved variety Padma	Control	0	10.3 ± 1.8a	19.7 ± 2.7a	24.1 ± 3.2a	33.1 ± 6.2a	30.5 ± 3.4a	26.0 ± 5.0a
	Bb11	10^7	4.4 ± 1.5c	6.3 ± 1.0c	9.7 ± 3.5c	6.1 ± 3.4c	5.9 ± 3.0c	7.3 ± 2.6bc
		10^9	3.2 ± 0.8c	4.9 ± 1.8c	7.1 ± 2.6c	2.8 ± 4.2c	2.5 ± 5.0c	3.4 ± 5.5c
	Bb115	10^7	6.5 ± 1.8b	9.2 ± 2.6b	15.9 ± 4.8b	12.7 ± 7.0b	9.0 ± 7.1b	7.0 ± 7.1bc
Local variety Tounvi	Control	0	11.4 ± 1.3a	21.5 ± 1.9a	23.8 ± 4.1a	28.1 ± 5.2a	32.2 ± 5.9a	24.7 ± 6.3a
	Bb11	10^7	2.1 ± 0.6c	9.1 ± 2.7b	8.7 ± 5.7c	9.9 ± 6.6c	10.1 ± 6.7c	6.4 ± 4.1b
		10^9	1.3 ± 0.8c	7.2 ± 1.6b	5.3 ± 4.5c	7.2 ± 5.4c	7.4 ± 7.1c	5.5 ± 3.2b
	Bb115	10^7	10.0 ± 0.4b	12.1 ± 1.22c	18.7 ± 2.2c	11.3 ± 2.1b	10.0 ± 2.6c	8.0 ± 2.1b
		F		31.17	16.02	20.71	8.14	7.09
		p-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%). Leaves stung, rotten were recorded to estimate damage index.

Table 2. Damage of flowers (Average ± Standard error) by *H. armigera* larvae.

Varieties	Treatments	conidia/ml	Days After Inoculation (DAI)					
			2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI
Improved variety Padma	Control	0	12.2 ± 0.1a	9.7 ± 0.6a	11.9 ± 0.4a	10.5 ± 0.7a	9.1 ± 0.2a	9.3 ± 0.5a
	Bb11	10^7	1.0 ± 0.1b	2.4 ± 0.1b	3.1 ± 0.3b	2.7 ± 0.4c	2.2 ± 0.2b	1.3 ± 0.4c
		10^9	0.0 ± 0.0c	0.0 ± 0.0c	0.2 ± 0.1c	1.7 ± 0.1c	1.1 ± 0.4c	1.1 ± 0.7c
	Bb115	10^7	1.9 ± 0.3b	3.6 ± 0.2b	2.7 ± 0.1b	5.7 ± 0.4b	3.7 ± 0.3b	3.1 ± 0.1b
		10^9	0.6 ± 0.1b	3.2 ± 0.1b	1.1 ± 0.6b	4.1 ± 0.3b	2.4 ± 0.4b	3.3 ± 0.3b
Local variety Tounvi	Control	0	11.6 ± 0.2a	7.1 ± 0.7a	9.8 ± 0.9a	7.7 ± 0.6a	11.3 ± 0.6a	13.9 ± 0.8a
	Bb11	10^7	0.7 ± 0.4c	0.5 ± 0.1c	1.3 ± 0.7b	1.8 ± 0.5b	1.9 ± 0.3c	2.6 ± 0.6b
		10^9	0.2 ± 0.3c	1.9 ± 0.3c	0.4 ± 0.4b	1.3 ± 0.4b	1.4 ± 0.7c	1.9 ± 0.8b
	Bb 115	10^7	3.8 ± 0.2b	3.1 ± 0.5b	1.9 ± 0.2b	2.1 ± 0.1b	3.0 ± 0.6b	2.2 ± 0.2b
		F	14.17	9.37	28.00	20.71	45.01	32.03
		p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

In the same column means followed by the same letter are not significantly different (ANOVA followed SNK test at 5%). Flowers stung, rotten were recorded to estimate damage index.

both varieties. This demonstrates that larval lifespan was heavily affected at higher concentrations (10^9 conidia/ml). The lowest larval MST (4.2 ± 0.8 days) was recorded with Bb 11 in the local variety, and was 7 days shorter than that observed in control plants (11.5 ± 0.2 days) (Figure 3). However, no significant difference was observed between Bb 11 and Bb 115 at 10^7 conidia/ml ($P > 0.05$), regardless of tomato varieties. But, H. armigera larvae feeding on plants inoculated with Bb 115 died faster in the improved variety (6.1 ± 0.5 days against 11.0 ± 0.4 days) (Figure 4). Comparison of survival curves showed significant differences

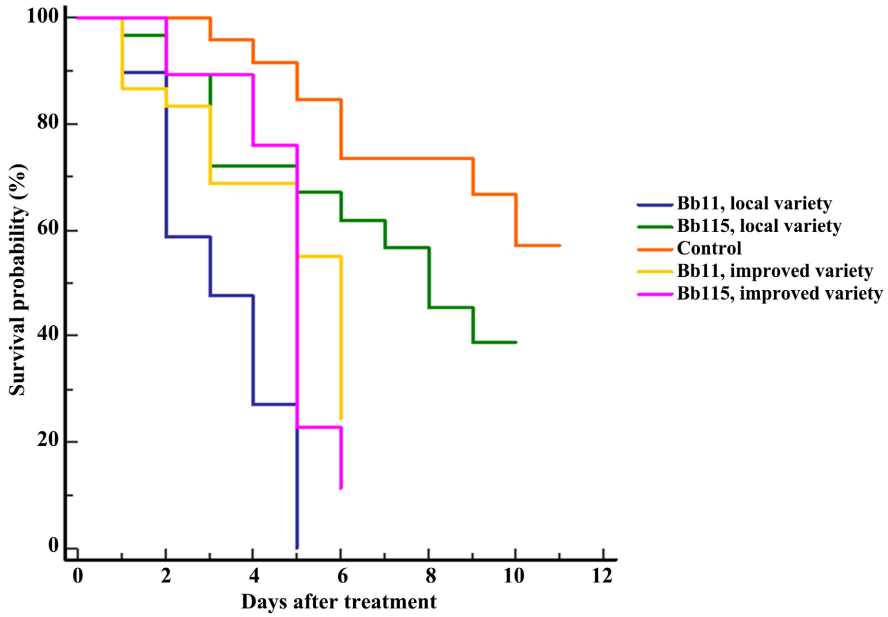

Figure 3. Kaplan-Meier survival plots of *Helicoverpa armigera* larvae, 12 days after inoculation with *Beauveria bassiana* at 10^9 conidia/ml in different tomato varieties. Days after treatment = Days after inoculation.

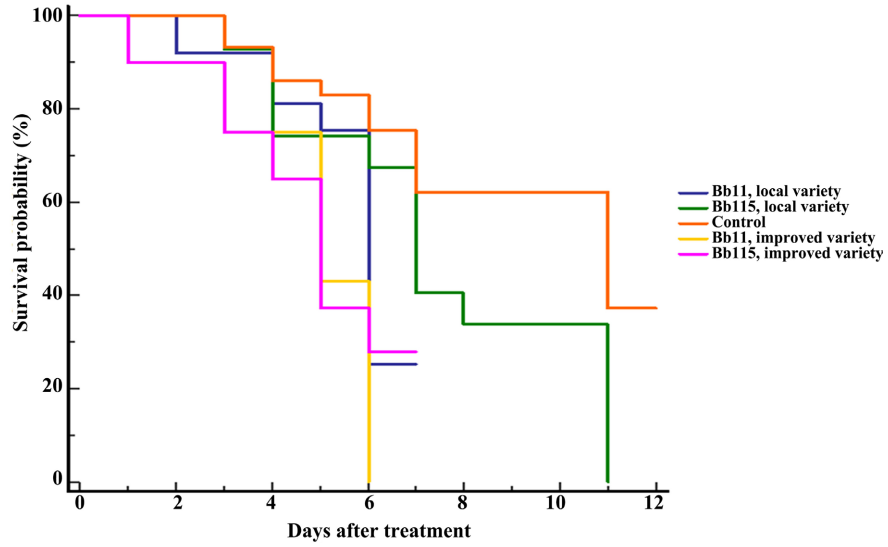

Figure 4. Kaplan-Meier survival plots of *Helicoverpa armigera* larvae, 12 days after inoculation with *Beauveria bassiana* at 10^7 conidia/ml of in different tomato varieties. Days after treatment = Days after inoculation.

between non-inoculated control plants and plants inoculated with *B. bassiana* at 10^7 conidia/ml (chi-squared = 69.178, df = 2, $P < 0.0001$), and at 10^9 conidia/ml (chi-squared = 77.642, df = 2, $P < 0.0001$).

4. Discussion

Our current study assessed the ability of endogenous *B. bassiana* isolates to colonize tomato varieties after a leaf inoculation method. Isolates Bb 11 and Bb 115

were detected in plant tissues sampled from inoculated plants through morphologic and microscopic observations. However, significantly higher leaf and root colonization rates were observed in an improved tomato variety when tomato plants were inoculated with isolate Bb 11 compared to Bb 115 at the concentration of 10^9 conidia/ml. This observation did not confirm our finding in previous study using seed coating method where root colonization was higher in the isolate Bb 115 [19]. The ability of *B. bassiana* to colonize endophytically tomato tissue may depend on the isolate and inoculation method [44]. For instance, Posada *et al.* [44] reported that leaves turned out to be poor entry routes for *B. bassiana* in coffee. Indeed, the limited entry of conidia may be due to the adaxial side of the leaf lacking stomata but provided with cuticular components hindering conidia entry. Moreover, environmental factors such as temperature, relative humidity and UV radiation may affect conidia viability in leaves [42]. In a study on sorghum, Tefera & Vidal [45] found a higher colonization rate in leaves compared to sorghum grain and roots, confirming our current finding with the isolate Bb 11. A very low roots colonization rate recorded in the local variety may be to due to plant regulating defense metabolism, or to interactions between endophytic organisms in plant roots [46] [47] [48].

Endophytic colonization of tomato varieties may therefore be isolate-specific but also depends on the inoculation method and fungal concentration. On the other hand, no significant differences were observed between the two isolates for leaf damage, regardless of tomato variety (**Table 1**). Similar result were found in our previous study even higher leaf colonization rate was observed in the isolate Bb 115 [19]. However, leaf damage was significantly lower when larvae were fed using inoculated plants compared to that obtained on the non-inoculated plants. Similar results were observed by [49] Lopez & Sword in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) fed with leaves of cotton plants inoculated with *B. bassiana.* In the present study, the lowest flower damage was observed in the improved variety with Bb 11 at the concentration of 10^9 conidia/ml (**Table 2**). This suggests that the effect of endophytic colonization of tomato plant varied between plant tissues with specific physiological conditions [41] [50].

Another exciting finding of this study was the influence of colonization of tomato plants by *B. bassiana* on the Mean Survival Time (MSTs) of *H. armigera*. We observed the lowest MST of *H. armigera* larvae in the local tomato variety with Bb 11 at 10^9 conidia/ml (**Figure 3**). But, significantly reduced MSTs of *H. armigera* larvae were obtained with Bb 115 at 10^7 and 10^9 conidia/ml in our previous study using seed coating as the inoculation technique, regardless of tomato variety [19], Moreover, comparison between survival curves in non-inoculated plants and inoculated plants revealed significant differences, suggesting a reduced effect on the survival of *H. armigera* larvae when fed using inoculated plant tissues. This finding was confirmed by [49], who reported lower survival rates in *H. zea* larvae when fed using tomato plants colonized by *B. bassiana.* The average survival time of *H. armigera* larvae influence the colonization of tomato plants. While there are a number of studies claiming that secondary metabolites

produced by entomopathogenic fungal species might deter consumption by herbivorous insects, other studies attributed the effect of endophytic colonization to an induced systemic response of plant defense conferring resistance to herbivorous insects [22] [51] [52]. Thus, the endophytic colonization of tomato plants by *B. bassiana* could reduce damage of feeding insect pest [53] by affecting their development [21] [54]. The endophytic relationship between an entomopathogenic fungus and a plant opens a new approach for biological control, in particular the application of fungal inoculum on crops. Once established in plants endophytic fungi such as *B. bassiana* may provide protection of crops against various insect pests at lower costs as there is no need to repeat applications during crop growth. But, a number of factors can alter the ability of entomopathogen to endophytically colonize plant species. This includes the entomopathogen strain/isolate, route of entry, inoculation method, environmental compatibility, origin, lifestyle, compatibility to other entomopathogens, responses to plant chemicals and other biotic and abiotic factors [55] [56].

Since various environmental factors affect the virulence of endophytic fungal species, further research should be conducted to better assess the interactions with these factors and the impact of endophytes on the nutritional quality of tomato.

5. Conclusion

This study assessed the effect of endophytic colonization of *B. bassiana* on damage and survival of *H. armigera* larvae using leaf spray inoculation. Higher leaf colonization rates were obtained in an improved tomato variety with the isolate Bb 11 at a concentration of 10^9 conidia/ml. Reduced damage was observed in inoculated plants compared to the non-inoculated ones. However, leaf or flower damage and larval survival may depend on *B. bassiana* isolate, tomato variety, fungal concentration and inoculation methods. Such factors should be considered to develop sound strategies for *H. armigera* management in tomato crops.

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Declarations

Availability of Data and Materials

All data and material are stated in the manuscript.

Conflicts of Interest

The authors declare no competing interests.

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Supplementary Material

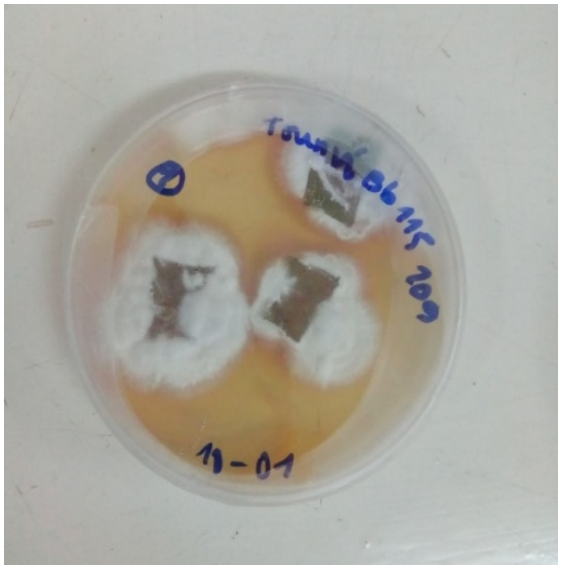

Figure A1. Colonization of different tomato leaves segments by *B. bassiana* after microscopic observation.

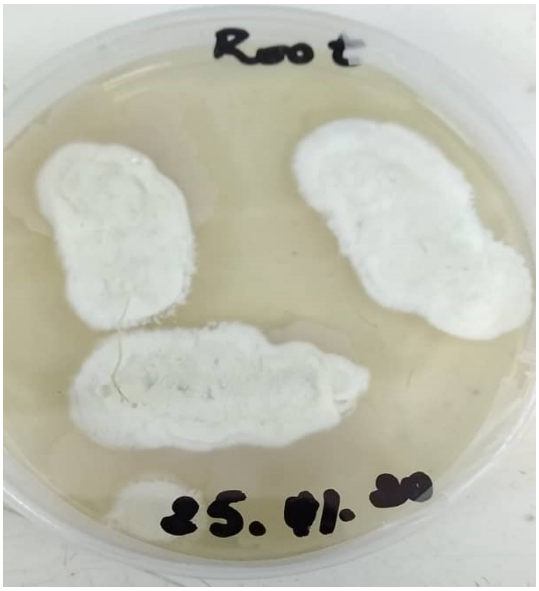

Figure A2. Colonization of different tomato root segments by *B. bassiana* after microscopic observation.