

REVIEW ARTICLE

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Endophytic colonization of tomato plants by *Beauveria bassiana* Vuillemin (Ascomycota: Hypocreales) and leaf damage in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae

Joelle Toffa^{1,2*}, Yeyinou Laura Estelle Loko¹, Ouorou Kobi Douro Kpindou², Karim Zanzana², Josky Adikpeto¹, Yasmine Gbenontin¹, Augustin Koudamiloro² and Appolinaire Adandonon³

Abstract

Background: The endophytic capacity of *Beauveria bassiana* Vuillemin isolates in 2 tomato varieties and their effects on damage and survival of the tomato fruit worm *Helicoverpa armigera* Hubner larvae were studied. The bioassays consisted of sowing seeds of 2 tomato cultivars soaked for 24 h in *B. bassiana* conidial suspension at the concentration of 1×10^7 and 1×10^9 conidia/ml for the isolates Bb 115 and Bb 11, respectively. Ten leaf, stem, and root segments were cut and incubated for assessing the endophytic growth of the fungus. Percentage of leaf consumption and pathogenicity of *B. bassiana* on *H. armigera* larvae were estimated.

Main body: The fungus *B. bassiana* developed endophytically in the 2 tomato varieties and was detected in tomato leaf, stem, and root. However, higher colonization rates were observed in roots than in leaves and stems. The *B. bassiana* isolate Bb 115 had a greater negative effect on the mean survival times (MSTs) of *H. armigera* larvae and on leaf consumption for local and improved tomato varieties. In fact, the lowest MSTs were recorded at the concentration of 1×10^9 conidia/ml for Bb 115 in 1.5 ± 0.2 days, i.e., 7 days less than the surviving larvae of the control group, which MSTs were 8.4 ± 0.9 days. Consumed leaf areas by larvae averaged ($89.17 \pm 10.33 \text{ mm}^2$) at a fungal concentration of 1×10^9 conidia/ml for Bb115. It was the best compared to that of untreated control ($820.3 \pm 92.77 \text{ mm}^2$). The colonization rate of the different plant parts increased with conidia concentration in both tomatoes varieties.

Conclusion: This study reported the effect of endophytic colonization of tomato by *B. bassiana* on the survival of *H. armigera* larvae and showed that the isolates Bb 115 and Bb 11 could be considered as useful microorganisms for the integrated control of *H. armigera*.

Keywords: Endophytes, Entomopathogenic fungi, *Beauveria bassiana*, Tomato, *Helicoverpa armigera*, Pest management

* Correspondence: joelletoffa@gmail.com

¹Laboratoire d'Entomologie Appliquée, Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), B.P. 14, Dassa, Republic of Benin

²International Institute of Tropical Agriculture (IITA), 08 B.P. 0932. Tri Postal, Cotonou, Republic of Benin

Full list of author information is available at the end of the article



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Background

The tomato (*Solanum lycopersicum* L.) is one of the most important and consumed vegetables in the world. In Benin, yields are low and variable due to insect pests attack, with an average of 9.533 kg per hectare (Assogba Komlan et al. 2016). Tomato producers are facing a significant pressure from insect pests, particularly the damage caused by the tomato fruit worm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), the major threat to tomato crops throughout the growing season due to its direct damage to the fruits resulting in yield losses ranging from 20 to 60% (Herrero et al. 2018). Management of the pest depends mainly on using synthetic pyrethroids' application. Moreover, the use of such chemical compounds led to many side effects such as human hazards, toxic residues in food, insect pests' resistance, environment pollution, and loss of biodiversity. Therefore, there is an urgent need to develop an alternative safe control method. Among the most sustainable alternatives, biological control with the entomopathogenic stands out. The fungus *Beauveria bassiana* (Ascomycota: Hypocreales) is one of the most widely used entomopathogens in the biological control of pests through the direct application of conidia suspension (Douro Kpindou et al. 2012). *B. bassiana* is a fungal species with an extremely broad host spectrum. It is a well-known, naturally occurring and environmentally safe biological control agent (Prasad and Syed 2010). Similar to other species of fungi, *B. bassiana* could endophytically colonize plant tissues and negatively affect herbivore species that feed on them. Endophytic microorganisms reside asymptotically within higher plants, inhabiting leaves, stems, and roots without any apparent harm to the plant. Endophytic fungi are important because they produce secondary metabolites with a range of potential uses in the agricultural and the pharmaceutical industry (Selim et al. 2012).

An endophytic fungus forms a mutually beneficial symbiotic relationship with the plant species. It lives within the tissues of the plant without causing disease; on the contrary, it stimulates its defenses and the plant in return acts as a host. Metabolites produced by some endophytic fungi, as *B. bassiana*, have been reported to influence the reduction of insect infestations on their host plants (Jaber Lara and Ownley 2017). The insecticidal action of endophytic *B. bassiana* has also contributed to the management of lepidopteran pests. This is most likely due to plant systemic resistance, elicited by these fungi against insect herbivores. Induced systemic resistance (ISR) is an important mechanism by which the whole plant is primed for enhanced defense against a broad range of insect pests (Pieterse et al. 2014). The efficiency with which *B. bassiana* can colonize and induce defense responses in tomatoes to repel pests is still

unknown in Benin. However, investigations should continue within the framework of the biological control of insect pests using endophytic fungi.

Thus, it was important to study the endophytic character of isolates *B. bassiana* Bb115 and Bb 11 on 2 different local and improved varieties of tomato, the most consumed in Benin. The aim of the study was to evaluate the endophytic colonization of *B. bassiana* in tomato plants and their effect on *H. armigera* survival.

Main text

Methods

Plant material

The local tomato variety "Tounvi" and the improved variety "Padma" were used during the bioassays. Both varieties are semi-erect, with a development cycle that elapses from 65 to 90 and 60 to 70 days for local and improved varieties, respectively. The average weights of a tomato fruit is 24 g and 120–130 g for local and improved varieties, in that order. Tounvi and Padma are the 2 most produced and marketed varieties in Benin according to their agronomic quality, their color, and resistance to pests such as *H. armigera* (Assogba Komlan et al. 2016).

Rearing of *Helicoverpa armigera*

A rearing colony of *H. armigera* was established at the laboratory by caterpillars collected from tomato fields at different localities in major tomato production areas in Benin. The larvae were placed in plastic containers (6.5 × 19.5 cm) and reared on an artificial diet under controlled conditions (70 ± 5% RH, 26 ± 2 °C, with a photoperiod of L: D 12:12) until pupation (Douro Kpindou et al. 2012). In order to prevent cannibalism, third-instar caterpillars were transferred individually in Petri dishes provided with artificial diet. The artificial diet consisted of bean flour, beer yeast, methylparaben, ascorbic and sorbic acids, streptomycin, formaldehyde, vitamin complex, agar, and distilled water. Diet was replaced every 2 days in order to avoid desiccation; moistened filter paper was placed in each Petri dish (Barrionuevo et al. 2012). Pupae were collected and placed in polypropylene containers (6 × 12 cm) until adult emergence. Folded paper was placed inside the cages for egg deposition. Collected eggs were kept until hatching, and then the larvae were reared as described above. Third-instar larvae (L3; 7.4 ± 0.1 days) were used in all bioassays.

Source and production of the entomopathogenic fungi

The isolates Bb 11 and Bb 115 were obtained from the entomopathogenic fungi (EPF) collection of the Applied Entomology Laboratory and were selected in previous studies for their pathogenicity towards *H. armigera* (Douro Kpindou et al. 2012). The endophytic characters

of the isolates remained to be demonstrated on different varieties of tomato most consumed in Benin.

Conidia from the 2 fungal isolates were picked from the stock culture and placed onto standard Potato Dextrose Agar (PDA) in Petri dishes ($\varnothing = 9$ cm) (Becton, Dickinson and Company; Sparks, MD 21152 USA) for subculture and incubated for 14 days at $26 \pm 2^\circ\text{C}$ and a photoperiod of 14:10 h (L: D). Then, conidia of each isolate were harvested by scraping them from the PDA, using a sterilized scalpel and suspended in 0.01% (w / v) Tween 80°. Conidial concentrations were estimated, using a Neubauer hemocytometer and adjusted to 1×10^7 conidia/ml and 1×10^9 conidia/ml for isolates (Posada and Vega 2005).

The concentration of conidia to be used was calculated as follows:

$$C' = (C_o \times V_o) / (V_o + V')$$

where C' = concentration to be tested, C_o = concentration of initial conidial suspension, V_o = volume needed, and V' = volume to be added.

The viability of conidia after 24 h incubation on PDA was $89 \pm 3.7\%$ and $92 \pm 1.5\%$ for Bb 11 and Bb 115, respectively.

Treatment with *B. bassiana* through seed coating

The seeds were sown and monitored under greenhouse conditions ($26 \pm 5^\circ\text{C}$, 14:10 h photoperiod) until use of the tomato plants. On the other hand, before sowing, the seeds were mixed with a suspension of fungal conidia with a fungal inoculum of 1×10^7 conidia/ml and 1×10^9 conidia/ml of each isolate. Then, they were placed on filter paper and kept for 24 h before sowing (Russo et al. 2015). Experiment consisted of 5 treatments: (i) tomato seeds soaked in Bb 11 at 1×10^7 conidia/ml; (ii) tomato seeds soaked in Bb 11 at 1×10^9 conidia/ml; (iii) tomato seeds soaked in Bb 115 at 1×10^7 conidia/ml; (iv) tomato seeds soaked in Bb 115 at 1×10^9 conidia/ml; and (v) untreated control. Seeds were sown in 10 pots for each treatment. All treatments were replicated 3 times for each of the 2 tomato varieties.

Assessment of the endophytic colonization *B. bassiana*

The methods of Arnold et al. (2000) and Kambreakar and Aruna (2018) were used to re-isolate *B. bassiana* from inoculated plant organs. For this purpose, all the glassware were sterilized, using an autoclave at 121°C for 15 min and then kept in a hot air oven at 55°C for 1 h. Then, 10 leaves and roots randomly sampled from inoculated tomato plants were cut in 5 pieces with a sterilized knife under a laminar air flow chamber. The 5 pieces of each organ (leaf, root) variety and 3 replicates per treatment and per variety pieces (3 cm^2) were

sterilized in 0.5% sodium hypochlorite for 3 min, then with 70% ethanol for 2 min, and then washed with sterile water and dried before placing it onto PDA in Petri dishes (9 cm diameter). Petri dishes were incubated at room temperature ($28 \pm 2^\circ\text{C}$) and periodically checked for fungal growth. The purity and sporulation of the culture were checked using a microscope. Colonization of *B. bassiana* was confirmed by microscopic observations (Ma et al. 2008). Petri dishes with *B. bassiana* were counted for each organ per treatment and per variety.

$$\text{Percentage of colonization} = \frac{\text{no. of segments colonized}}{(\text{total no. of plant sampled segments} \times 100)}$$

Percent colonization was determined for different plant organs and the most virulent *B. bassiana* isolate with the highest endophytic colonization was determined.

Assessment of leaf consumption and larvae survival in inoculated and untreated tomato plants

Leaf consumption was assessed by measuring leaf area consumed by each larva in each treatment. The test consisted of feeding 10 third-instar larvae of *H. armigera* for 24 h on tomato leaves sampled from inoculated and untreated tomato plants of both local and improved varieties. Ten discs of tomato leaves (3 cm in diameter) were obtained by cutting sampled leaves and offered each to the 10 *H. armigera* larvae placed in Petri dish (90 mm) (Magrini et al. 2015). The Petri dishes were then incubated for 24 h at 25°C , 60% RH. Thus, using a graduated paper, the consumed area was estimated per treatment and per variety (Russo et al. 2015). Experiments were replicated 3 times. In parallel, larval survival and mortality of *H. armigera* larvae was checked daily per treatment and per variety. Leaves were replaced every 2 days until the 10th day after treatment (Ma et al. 2008).

Data analysis

The percentage of plants colonized by *B. bassiana* was compared using the chi-square test. Data on larval mortality and sporulation rates were processed by analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS (SAS Institute Inc 2003). Percentages were based on the initial number of larvae exposed. In case of significant F values, means were compared by using SNK (Student-Newman-Keuls). The modeling of the time-dose-mortality data was carried out, using the "Cox regression" model (Statistical Package for Social Science (SPSS) Inc. 1989-2003). Data on leaf area consumed by larva was compared by applying ANOVA, followed by SNK.

Results

Endophytic colonization of tomato plants by *B. bassiana*

The endophytic colonization of the tomato organs placed onto PDA showed a whitish color, characteristic of *B. bassiana* mycelium. This colonization was also confirmed by microscopic observations. Thus, the presence of *B. bassiana* was confirmed in leaves, stems, and roots of tomatoes with seeds coasting inoculation method. Regardless to the concentration, the 2 *B. bassiana* isolates were able to colonize endophytically the different organs in both tomato varieties (Fig. 1).

Assessment of leaf colonization by *B. bassiana*

The fungus presence was detected in tomato leaves at the 2 concentrations of Bb 11 and Bb 115 isolates. However, the colonization rate for the isolate Bb 115 was more efficient, depending of the inoculation concentration and tomato variety. The highest endophytic colonization rates 91.2 and 74.55% were observed at the local and improved varieties, respectively, at the concentration of 1×10^9 conidia/ml (Fig. 2). After incubation, the lowest colonization for both varieties was registered with the concentration of 1×10^7 conidia/ml of the Bb115 isolate (local variety). For all treatments, colonization of leaves in the local variety Tounvi of 1×10^9 conidia/ml of the Bb 115 was significantly greater than colonization of leaves in the improved variety Padma ($df = 7.81$, $P = 0.0050$) (Fig. 2). Samples from untreated control did not show any fungal colonization. Regarding stem colonization, the fungus was more uniform than for leaves, and the absence of colonization was only recorded by the 10^7 concentration of the Bb 11 strain (Fig. 3). However, non-significant differences were observed between the treatments ($DF = 1$, $P = 0.8266$). Colonization rates on the roots showed strong colonization of the fungal isolates unlike those observed in the leaves and stems.

With the improved variety Padma, the highest colonization rates were registered, where the strain Bb 115 stood out, with 93.51 and 89.3% for concentrations 10^9 and 1×10^7 conidia/ml, respectively. On the other hand, low colonization of roots was observed in the local variety inoculated with the 2 isolates Bb 115 and Bb 11 at 1×10^9 conidia/ml (Fig. 4). However, non-significant difference was observed between the 2 varieties ($DF = 1$, $P = 0.2764$). No endophytic colonization was observed at the control.

Leaf consumption by *H. armigera* and larvae survival

Statistical analysis showed significant differences ($F = 13.66$, $P = 0.0043$) in the consumption of leaves inoculated with the strains of the fungus, which suggests that the endophytic presence of *B. bassiana* reduced the consumption of *H. armigera*. The use of *B. bassiana* as endophytic fungus in tomato induced an overall significant reduction in leaf consumption by *H. armigera* larvae ($F = 76.55$, $P < 0.0001$). However, there was non-significant difference between local and improved varieties. On the other hand, in the improved variety, the average leaf area consumed was $32.08 \pm 7.51 \text{ mm}^2$ for Bb 115 at 1×10^9 conidia/ml against $730.7 \pm 62.41 \text{ mm}^2$ for the untreated control. In the local variety, *H. armigera* larvae consumed an average of $89.17 \pm 10.33 \text{ mm}^2$ of the leaf when plants were inoculated with Bb 115 at 1×10^9 conidia/ml against $820.3 \pm 92.77 \text{ mm}^2$ for untreated control (Fig. 5). The use of *B. bassiana* Bb 115 induced an overall significant reduction in leaf consumption by *H. armigera* larvae ($F = 76.55$, $P < 0.0001$).

Survival of *H. armigera* larvae was affected at the 2 concentrations of both isolates Bb 115 and Bb 11, when offered leaves from inoculated plants in both local and improved varieties. Compared to untreated control, the survival of third-instar larvae of *H. armigera* was significantly affected by the isolate Bb 115 ($F = 11.22$, $P < 0.0001$; $F = 47.13$, $P < 0.0001$; in both local and improved

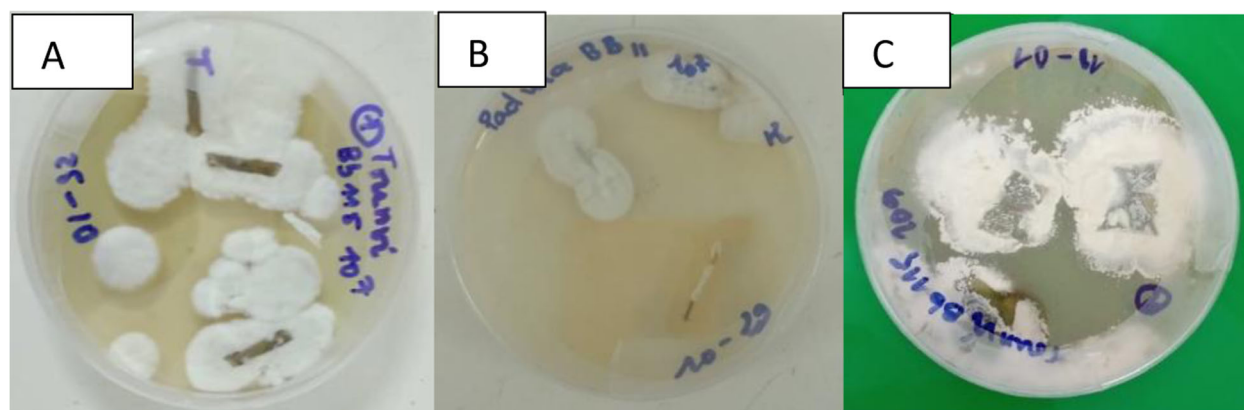


Fig. 1 Colonization of different tomato stem (a), root (b), and leaf (c) segments by *Beauveria bassiana* after microscopic observation

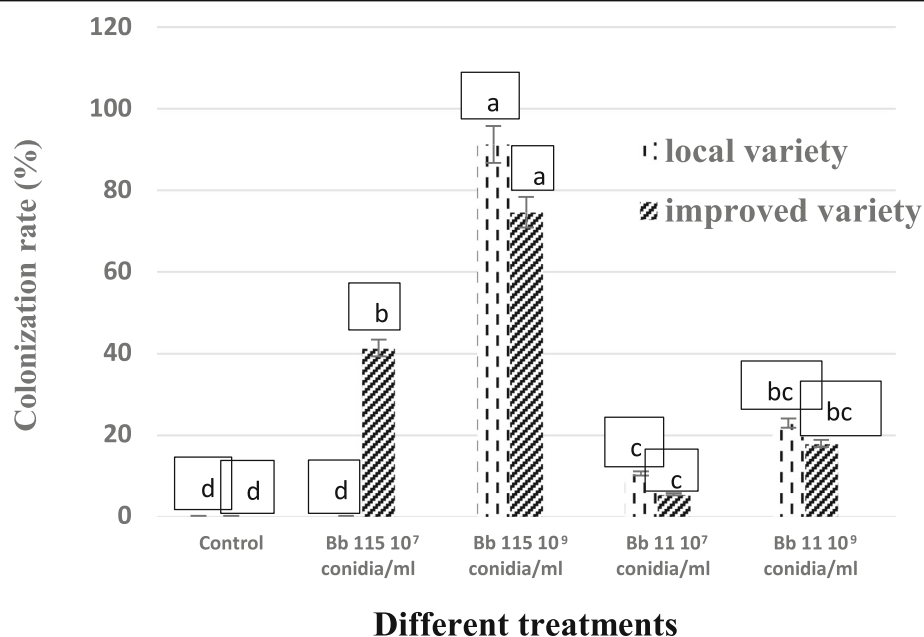


Fig. 2 Leaf colonization rate (%) by *Beauveria bassiana* in two tomato varieties with seed coating inoculation. Histograms with the same letters are not significantly different at the 5% level (SNK). With Bb115 10^7 conidia/ml = *Beauveria bassiana* Bb115 at 10^7 conidia/ml; Bb115 10^9 conidia/ml = *Beauveria bassiana* Bb115 at 10^9 conidia/ml; Bb11 10^7 conidia/ml = *Beauveria bassiana* Bb11 at 10^7 conidia/ml; Bb11 10^9 conidia/ml = *Beauveria bassiana* Bb11 at 10^9 conidia/ml

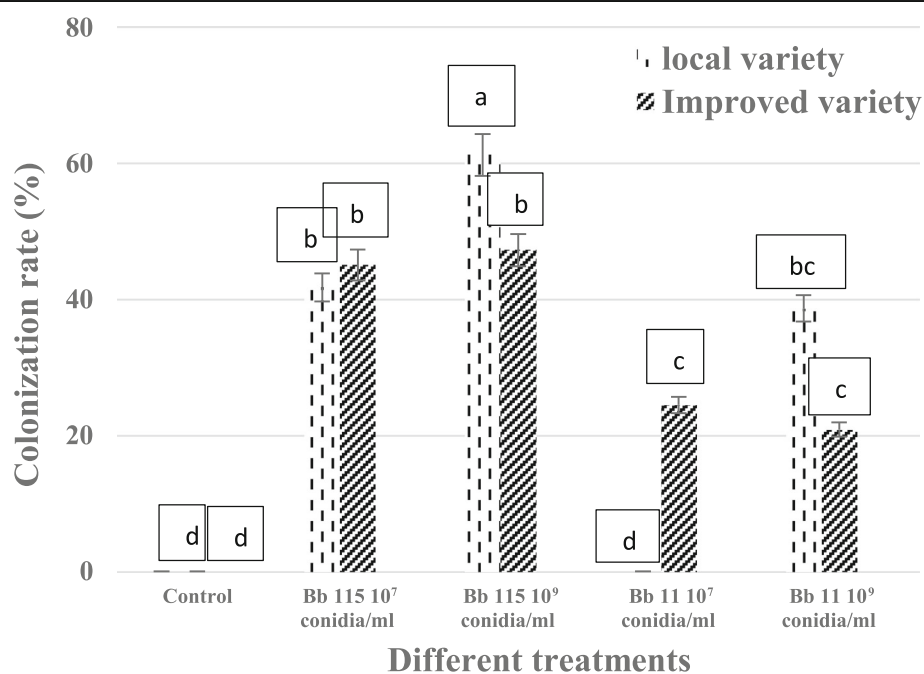


Fig. 3 Stem colonization rate (%) by *Beauveria bassiana* in two tomato varieties with seed coating inoculation. Histograms with the same letters are not significantly different at the 5% level (SNK). With Bb115 10^7 conidia/ml = *Beauveria bassiana* Bb115 at 10^7 conidia/ml; Bb115 10^9 conidia/ml = *Beauveria bassiana* Bb115 at 10^9 conidia/ml; Bb11 10^7 conidia/ml = *Beauveria bassiana* Bb11 at 10^7 conidia/ml; Bb11 10^9 conidia/ml = *Beauveria bassiana* Bb11 at 10^9 conidia/ml

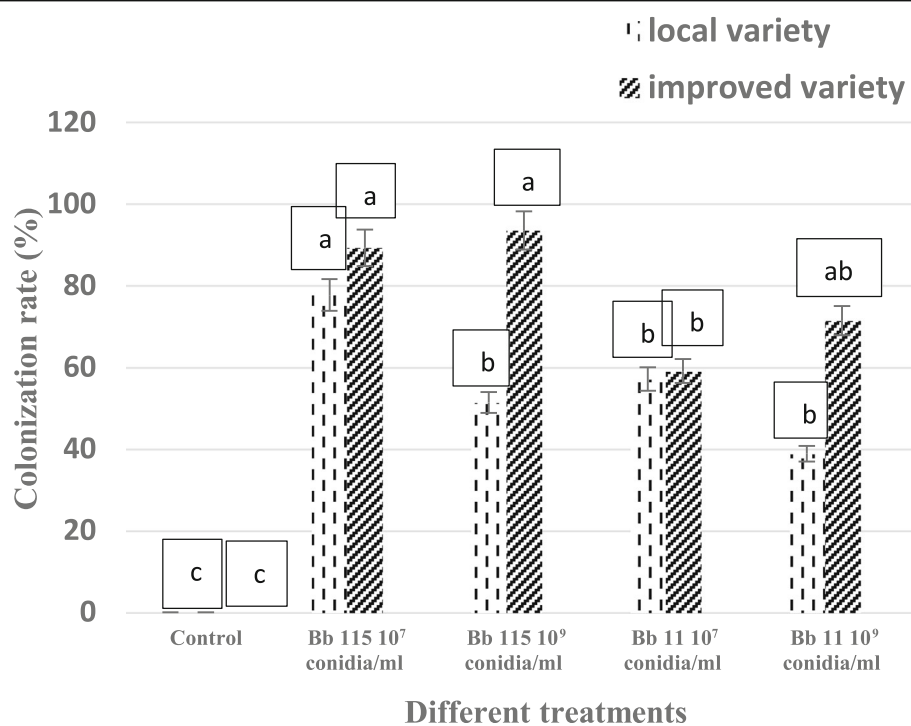


Fig. 4 Root colonization rate (%) by *Beauveria bassiana* in two tomato varieties with seed coating inoculation. Histograms with the same letters are not significantly different at the 5% level (SNK). With Bb115 10^7 conidia/ml = *Beauveria bassiana* Bb115 at 10^7 conidia/ml; Bb115 10^9 conidia/ml = *Beauveria bassiana* Bb115 at 10^9 conidia/ml; Bb11 10^7 conidia/ml = *Beauveria bassiana* Bb11 at 10^7 conidia/ml; Bb11 10^9 conidia/ml = *Beauveria bassiana* Bb11 at 10^9 conidia/ml

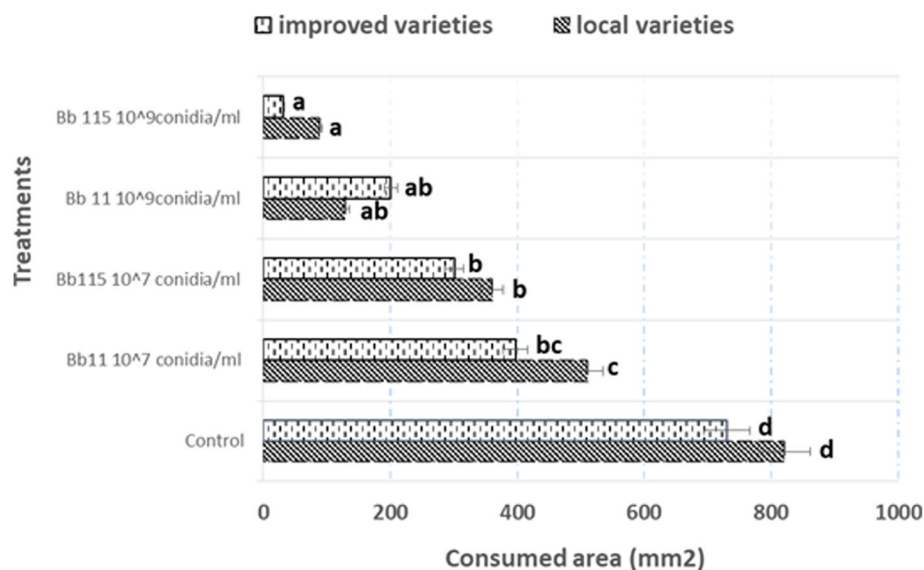


Fig. 5 Leaf areas consumed by *Helicoverpa armigera* larvae when offered inoculated and non-inoculated tomato plants. Histograms with the same letters are not significantly different at the 5% level (SNK). With Bb115 10^9 conidia/ml = *Beauveria bassiana* Bb115 at 10^9 conidia/ml; Bb11 10^9 conidia/ml = *Beauveria bassiana* Bb11 at 10^9 conidia/ml; Bb115 10^7 conidia/ml = *Beauveria bassiana* Bb115 at 10^7 conidia/ml; Bb11 10^7 conidia/ml = *Beauveria bassiana* Bb11 at 10^7 conidia/ml

varieties, respectively). On the other hand, regardless to the fungal concentrations, the mean survival times (MSTs) of larvae fed using leaf from the improved variety seemed to be lower than those obtained with the local variety. The lowest MSTs were recorded at the Bb 115 in 1.5 ± 0.2 days at 1×10^9 conidia/ml, i.e., 7 days less than the surviving larvae in untreated control with MSTs of 8.4 ± 0.9 . However, no difference was observed between the 2 varieties for the MSTs ($F = 0.51$, $P = 0.6350$) (Table 1).

Discussion

The development of alternative methods for plant protection has become an attractive option. Among these alternative methods to control crop pests, pathogenic microorganisms including *B. bassiana* are promising.

The entomopathogen, *B. bassiana* was isolated from the leaves, stems, and roots of local and improved varieties of tomatoes, indicating the potential of both isolates as effective endophytic agents on tomato plants. Posada and Vega (2005) reported that the endophytism of the fungus *B. bassiana* is not harmful to plants growth. Also, this fungus can colonize various plant tissues without affecting their physiological activities. Similar results have been observed by many authors, where *B. bassiana* has been reported as an endophyte in tomato (Ownley et al. 2004).

Regardless of the inoculation concentration and variety of the tomato plants, the colonization rate of the *B. bassiana* Bb 115 isolate was high in all sampled organs (leaves, stems and roots) than in the isolate Bb 11. This difference could be attributed to the compatibility of Bb 115 genome which may be more compatible with the internal environment of the plant. Several factors such as the origin, the nature of the entomopathogen, the penetration site, the inoculation method, and the compatibility with the host plant can also influence the endophytic capacity of a fungal isolate compared to another isolate

(Renuka et al. 2017). However, the lower rate of endophytic colonization of *B. bassiana* observed at the isolate Bb 11 at the concentration of 1×10^7 conidia/ml could be explained by a limited penetration of germinating conidia in tomato plants.

The colonization rate of tomato by *B. bassiana* increased with the fungal concentration, suggesting that high conidia number resulted in fungal growth within tomato plants. This may be related to high secondary fungal metabolites production in the different plant organs. The endophytic colonization of tomato plants by *B. bassiana* was effective with seed coating. However, non-significant difference was observed between the tomato varieties demonstrating the capacity of *B. bassiana* to actively penetrate plant tissues, regardless of tomato variety. The highest colonization rate observed in roots than in leaves and stems may be related to their physiological differences and the fungal characteristics. Indeed, colonization started from roots and progressed vertically within stems and leaves with seed coating.

According to several studies, endophytic fungi present specificity for some plant tissues because they adapted to particular conditions within plant organs. Akello et al. (2007) showed that the seed coating method allowed the roots to provide a route for the fungus development within plant tissue. On the other hand, Agrios (2005) reported that many pathogenic bacteria and fungi such as *B. bassiana* might enter in plant tissues through natural openings including stomata. Progression of *B. bassiana* within tomato plant could be confirmed with the detection of mycotoxins produced by *B. bassiana* in tomato fruit or seeds.

Moreover, the isolate Bb 115 induced significant decrease in leaf consumption by *H. armigera* larvae than the control. The differences between the improved and local tomato varieties could be explained as the fact that the improved variety was more susceptible to conidia penetration of spores than the local one. Induced

Table 1 Mean survival times (MSTs) in days of third-instar *Helicoverpa armigera* larvae fed using tomato leaves sampled from tomato inoculated and non-inoculated plants with seeds coating at different *Beauveria bassiana* concentrations

Treatments	Mean survival time in days (means \pm SE)	
	Local variety	Improved variety
Control	10.7 \pm 1.4 aA	8.4 \pm 0.9 aA
Bb 115 10^9 conidia/ml	2.2 \pm 0.8 bB	1.5 \pm 0.2 bB
Bb 115 10^7 conidia/ml	2.9 \pm 0.3 bB	2.8 \pm 0.6 bcB
Bb 11 10^9 conidia/ml	4.8 \pm 0.5 cC	3.2 \pm 0.7 cC
Bb 11 10^7 conidia/ml	5.1 \pm 1.1 cC	3.6 \pm 0.4 cC
F	11.22	47.13
P	< 0.0001	< 0.0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)

In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)
ES standard error

systemic resistance (ISR) is an important mechanism by which the whole plant is prepared for an enhanced defense against a wide range of insect pests (Pieterse et al. 2014). However, the effective mechanism by which the endophyte *B. bassiana* can induce defense responses in tomatoes to repel pests is still unknown.

It was also shown that the *B. bassiana* Bb 115 isolate at a concentration of 1×10^9 conidia/ml reduced the mean survival times (MST) of third-instar *H. armigera* larvae more than the Bb 11 isolate. The MST of the larvae decreased with the increase in fungal concentration. These results could be considered as indirect effects occurring during the consumption of the *B. bassiana*-colonized leaves. These indirect effects, such as the production of secondary metabolites or the induction of a systemic response in tomatoes, could inhibit larval feeding behavior. Similar results were obtained with Jaber Lara and Ownley (2017) on *H. armigera* fed using leaves of *Vicia faba* plants treated with the endophyte *B. bassiana*. Obtained findings are in agreement with Castillo Lopez and Sword (2015) who observed lower survival rates of *Helicoverpa zea* larvae when fed using tomato plants colonized by *B. bassiana*.

Thus, *B. bassiana* can reduce insect pest's damage through its endophytic colonization by inhibiting insect development. The endophytic relationship between an EPF and a plant suggests possibilities for biological control, in particular the use of fungal inocula as insecticides. In the present study, the endophytic characteristic of *B. bassiana* was proved and its effect on *H. armigera* larvae well established, confirming the usefulness of this fungal species in insect pest control.

Conclusions

The present study demonstrated the endophytic colonization of tomato plants by *B. bassiana* and its effect on the survival of *H. armigera* larvae feeding on leaves of local and improved tomato inoculated varieties. The colonization rate increased fungal concentration with seed coating method, regardless to the fungal isolate. However, *B. bassiana* isolate Bb 115 at a concentration of 1×10^9 conidia/ml was found to be more effective with seed coating method. Although field studies are necessary to support the obtained results, the endophytic characteristic of *B. bassiana* could be included in an integrated strategy for the management of *H. armigera* in tomato.

Abbreviations

Bb: *Beauveria bassiana*; p: p value; SE: Standard Error

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Authors' contributions

JT: conceptualization, methodology, writing—original draft. YLEL, EYD, and OKDK: writing—review and editing. KZ, JA, YG, and AK: collection of the data. AD: supervision, writing—review and editing. All authors have read and approved the manuscript.

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Availability of data and materials

All data and materials are stated in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Laboratoire d'Entomologie Appliquée, Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), B.P. 14, Dassa, Republic of Benin. ²International Institute of Tropical Agriculture (IITA), 08 B.P. 0932, Tri Postal, Cotonou, Republic of Benin. ³Université Nationale d'Agriculture (UNA), 08 BP1055, Cotonou, Republic of Benin.

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