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# Endophytic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, confer control of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in two tomato varieties

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## Abstract

**Background:** The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is an important pest of tomatoes that reduces yield and fruit quality resulting in losses of up to 100%. The pest has developed resistance to chemical insecticides, therefore necessitating alternative control measures. The entomopathogenic fungi (EPF) *Beauveria bassiana* and *Metarhizium anisopliae* have been exploited extensively for biological control of the insect pests of economic importance.

**Results:** The potential of *B. bassiana* (Bals.) Vuillemin (Hypocreales: Cordycipitaceae) (BbC1) and *M. anisopliae* (Metchn.) Sorokin (Hypocreales: Clavicipitaceae) (M150) to persist as endophytic EPF in two tomato varieties, Matina and Harzfeuer, which spread within host tissues and protect plants against larval attack of *S. frugiperda* was evaluated. Tomato seedlings were inoculated by dipping roots in either BbC1 or M150 spore suspensions ( $10^8$  spores/ml). The qPCR analyses revealed the presence of both fungi in > 65% of the tested samples. Second-instar larvae of *S. frugiperda* were introduced onto inoculated plants that revealed the presence of fungal DNA and non-inoculated plants, 14 days after inoculation. Treating tomato plants with the EPF resulted in reducing weights and slowing larval development of *Spodoptera*. Larvae fed on BbC1 and M150-treated Matina significantly reduced weights than those treated with Harzfeuer tomato plants. The tomato variety Matina conferred enhanced level of resistance to the *S. frugiperda* larvae, further aggravated by the presence of both fungi through dietary stress resulting in slow development and reduce larval weight.

**Conclusion:** These results may serve the tomato production systems and the *S. frugiperda* integrated management programs.

**Keywords:** Fall armyworm, *Spodoptera frugiperda*, *Beauveria bassiana*, *Metarhizium anisopliae*, Endophyte, Tomato varieties

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## Background

Tomato is the second largest grown and arguably most consumed vegetable in Kenya (Government of Kenya (GoK) 2018). Production of tomato is threatened by infestation with the invasive fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *S. frugiperda* is an economic pest of > 60 different crops including sorghum, rice, wheat, beans, cotton, potatoes, and tomatoes. It reduces yield and fruit quality of tomatoes grown in greenhouse and open fields and may result in losses of up to 100%. Chemical sprays are minimally effective due to the resistance of *S. frugiperda* to many pesticides and also because of the pest's development that takes place inside the plant or the soil, and therefore, they are out of reach of pesticides. The damages caused by larvae begin after transplanting the seedlings to the field and can extend throughout the plant's development.

The entomopathogenic fungi (EPF) *Beauveria bassiana* (Bals.) Vuillemin (Hypocreales: Cordycipitaceae) (Bb) and *Metarhizium anisopliae* Sorokin (Hypocreales: Clavicipitaceae) (Ma) are ubiquitous and biological control agents of a wide range of insects. They have been exploited extensively in biological control programs. The potential of these fungal pathogens to exist as endophytes in plants and to protect their colonized host plants against the primary herbivore pests has been widely reported. *Bb* has been reported to control many crop pests such as stem borers, beetles, aphids, mites, termites, whiteflies, mealy bugs, and thrips (Shah and Pell 2003). *Bb* has been reported as an endophytic colonizer of several plant species including corn (*Zea mays*), potato (*Solanum tuberosum*), cocoa (*Theobroma cacao*), date palm (*Phoenix dactylifera*), opium poppy (*Papaver somniferum*), coffee (*Coffea arabica*), banana (*Musa spp.*) tissue cultures, sorghum (*Sorghum bicolor*), bean (*Phaseolus vulgaris*), cotton (*Gossypium hirsutum*), pumpkin (*Cucurbita maxima*), tomato (*Solanum lycopersicum*), jute (*Corchorus olitorius*), pine (*Pinus radiata*), squash (*Cucurbita pepo*), artichoke (*Cynara scolymus*), grapevine (*Vitis vinifera*), oilseed rape (*Brassica napus*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), cassava (*Manihot esculenta*), and wheat. The endophytic potential of *Bb* has also been reported to reduce pests' damage to plants indirectly by inhibiting insect development and reproduction or directly by causing insect mycosis resulting in mortality (Rondot and Reineke 2018) while in exchange receiving nutrition and refuge from plants (Saikkonen et al. 2004).

The insect-pathogenic fungus, *Metarhizium*, is a common inhabitant of soils worldwide and has been studied and used as an insect pathogen for biological control (Clifton et al. 2018). However, this fungus is not only an insect pathogen but it also readily colonizes the plant

rhizosphere (St. Leger et al. 2011). In contrast to the high number of studies on the endophytic *Bb*, only a few studies have been conducted on the endophytic *Metarhizium* isolates. For instance, *Metarhizium* sp. has been reported as an endophyte of corn (Kabaluk and Ericsson 2007), tomato (Garcia et al. 2011), oilseed rape (Batta 2013), and cassava (Greenfield et al. 2016), and its endophytic activity against insect pests has been reported in oil seed rape against larvae of *Plutella xylostella* larvae (Batta 2013).

The present study aimed to evaluate the persistence of two EPF isolates as endophytes in tomato plants, which spread within host tissues and protect the plants against larval infestation of *S. frugiperda*.

## Main text

### Methods

#### Fungal isolates

Two fungal isolates; BbC1 and M150, available in the laboratory, at the Department of Crop Sciences, Agricultural Entomology, Georg-August University, Germany, were used in the study. Spores were harvested from 2-week old PDA cultures, by adding two drops of Tween 20 to the culture, and then gently scraping the conidia and placing into a 200-ml flask containing 100 ml sterile water. The conidia were harvested by vortexing, followed by filtration of the spore suspension, using a Buchner-type funnel through a 2-layered filter paper (90 mm) to remove mycelial fragments and aggregated conidia. The spore densities were adjusted to  $10^8$ /ml.

#### Insect species

Second larval instar of *S. frugiperda* were obtained from Bayer Crop Science. The larvae were reared on tomato seedlings for several generations at 25 °C, RH 70%, and at a photoperiod of 8 h. The adults were fed on 30% honey solution and newly hatched larvae were transferred to 200 ml disposable plastic cups and reared on artificial medium (Gupta et al. 2005) until reaching the second larval instar.

#### Plant species

Two tomato varieties, Harzfeuer and Matina, originated in the Middle East were sown in pots containing a mixture of peat- moss and sand (3:1). Plants used in experiments were at a similar size and were randomly assigned to each treatment. In three separate repeated experiments, 36 seedlings of each tomato variety were left to grow under greenhouse conditions until the 3rd leaf growth stage. After shaking off excess soil, the roots were rinsed, using running tap water. The roots of 12 seedlings of each tomato variety were dipped in 25 ml of either BbC1 or M150 spore suspensions ( $10^8$  /ml) for 2 min. Another 12 plants from each variety were dipped in

25 ml of sterile distilled water and used as controls. These plants were then transplanted into 11-cm plastic pots containing the same soil and sand mixture and left to grow under greenhouse conditions for 7 days and used for bioassays.

#### Determination of endophytic colonization

##### *Re-isolation of fungi on selective media*

In order to assess the potential of BbC1 and M150 to endophytically colonize tomato plants, 4 leaf samples/plant were taken both from the inoculated and control plants every 7 days, cut into 4–6 pieces; their surfaces were sterilized, using 70% alcohol for 3 min, 5% sodium hypochlorite for 2 min, and then 70% alcohol for 2 min; and finally, they were rinsed in sterile distilled water 3 times, and then dried out on sterile filter paper. The fragments were then placed on modified selective medium consisting of 39 g PDA, 100 mg NaCl, 50 mg chloramphenicol, and 0.5 mg crystal violet per 1 l. The plates were incubated at 25 °C for 2 weeks. Three replicates of randomly selected leaf fragments of each treatment were assessed for fungal colonization and repeated with each experimental set-up.

##### *Molecular analysis of fungal colonization in tomato plants*

DNA extraction and real time PCR were used to assess plants BbC1 and M150 growth inside plant tissues.

##### *DNA extraction*

The upper 3 newly emerged unfolded leaves leaf samples were picked from inoculated and control plants 10 days after inoculation, and their surfaces were sterilized before freeze-drying and then milled into powder. A 50-mg sample of plant material, in a 2-ml Eppendorf tube, was centrifuged and used for DNA extraction. Total DNA was extracted from the plant materials, using the standard cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1987) after which the DNA samples were stored overnight in the fridge. Thereafter, the quality of the extracted DNA was checked, using the electrophoresis by adding 5 µl of extracted DNA and 2 µl loading dye and loading them into 0.8% agarose gel, which was run for 120 min at 60 V then stained with Ethidium bromide and visualized using UV light.

##### *Fungal DNA quantification using the real-time PCR*

Specific primers designed for both BbC1 and M150 were used for optimizing the qPCR protocol. Thereafter, standard curves for both fungi were established with a minimum quantity of 200 femtogram/µl. Diluted (1/10 and 1/20) and un-diluted DNA samples were run alongside with the standards, following the optimized qPCR

protocol for quantifying the fungal DNA presence in the tested samples.

##### *Introduction of larvae onto inoculated plants*

Second-instar larvae (4 day old) of *S. frugiperda* (one per plant) were introduced onto 12 plants, each of inoculated (that tested positive for fungal DNA) and non-inoculated plants after 14 days of inoculation. One larva was secured per plant, using a clip-cage, and that was moved onto a different leaf on the same plant every 24 h. The plants were arranged in a completely randomized design in a controlled environmental chamber (25 ± 1 °C, RH 80% and L to D 8:16 h). This set-up was repeated 3 times.

##### *Determination of the effect of endophytes on Spodoptera frugiperda larvae*

The initial weights of *Spodoptera* larvae were taken before introduction to the plants. Larvae were weighed every 7 days for 21 days. All larvae were removed from both control plants after 21 days, when the larvae of control plants developed into pupae. Larvae feeding on BbC1- and M150-treated tomato plants never pupated. Dead larvae were removed on a daily basis and placed in dishes with moistened filter paper to evaluate for mycosis.

##### *Data analysis*

A factorial ANOVA was used to assess the impact of root-inoculated BbC1 and M150 tomato plants on larval weights. The dependent variables were the weight of larvae, and factors were tomato variety and fungal treatment. Tukey's test was used for multiple comparisons of means ( $P < 0.05$ ) (Statistica).

## Results

#### *Determination of endophytic colonization*

##### *Re-isolation on selective media*

Both BbC1 and M150 were re-isolated in leaf fragments, using selective medium (Table 1). In addition, the fungal DNA detection in the leaves and significant reduction in

**Table 1** Presence (%) of *Beauveria bassiana* (BbC1) and *Metarhizium anisopliae* (M150) in leaves of two tomato varieties at different times following root inoculation

Tomato variety	Matina		Harzfeuer	
	7 dpi	14 dpi	7 dpi	14 dpi
<i>B. bassiana</i>	81.5 ± 11.7	100	74.5 ± 11.2	100
<i>M. anisopliae</i>	87.5 ± 11.5	100	79.5 ± 11.6	100

Percentages are means of positive scores for three experiments (± SD). Four leaf samples from each plant were taken both from the inoculated and control plants every 7 days and were tested for the presence of the fungi every 7 days. Control plants were negative for *B. bassiana* and *M. anisopliae*, and therefore the results were not shown. dpi days post inoculation

weight of *S. frugiperda* larvae were evidence of fungal presence in the leaves.

#### Fungal growth quantification using the real-time PCR

The qPCR analyses proved the presence of both fungi, BbC1 and M150, and they were found in > 65% of the tested samples (Fig. 1). Although the detected quantities were so low (even in 1:10 as well as 1:20 dilutions of DNA samples), it was important to note that the sampled leaves for this test were always the upper 3 newly emerged unfolded leaves.

#### Effect of *B. bassiana* and *M. anisopliae* on the weight of *S. frugiperda* larvae

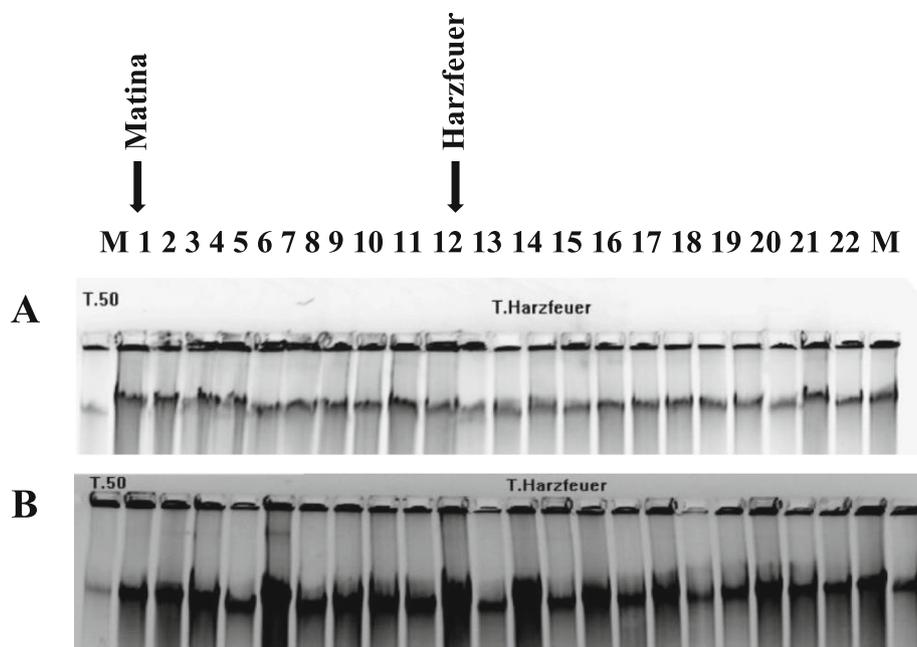
Treating tomato plants with either BbC1 or M150 resulted in reducing weights of *Spodoptera* larvae (Table 2, Fig. 2). Weights of the larvae fed on tomato plants, treated with either BbC1 or M150, significantly differed between tomato varieties and treatments ( $P < 0.001$ : df 1) than the control plants. Significant interactive effects were also observed between M150-treated tomato plants of either tomato varieties Matina ( $P = 0.006$ : df 1) and Harzfeuer ( $P = 0.014$ : df 1) varieties compared to the BbC1-treated tomato plants. These differences were observed during weeks 1 and 2, but they were not as apparent as the differences observed in the larval development after 3 weeks of feeding on M150-treated tomato plants (Table 2, Fig. 2).

The obtained results also showed that the weights of larvae, fed on BbC1- or M150-treated Matina tomato variety, were reduced compared to those feeding on either BbC1- or M150-treated Harzfeuer variety (Fig. 2). This was also true at the controls of both plants. For instance, in controls, weights of larvae fed on Matina were reduced by (33%) more than those fed on the Harzfeuer, while weights of larvae fed on BbC1-treated Matina plants were reduced 45% more than those feeding on BbC1-treated Harzfeuer tomato plants. When *S. frugiperda* larvae were fed on M150-treated Matina plants, their weights were reduced by 59% higher than M150-treated Harzfeuer plants.

#### Discussion

Fungal presence in the leaves was evidenced by reisolation of fungal DNA in the treated leaves and reduced weights of *S. frugiperda* larvae feeding on the treated leaves. Ramirez-Rodriguez and Sánchez-Peña (2016) reported similar results, using endophytic *B. bassiana* in *Zea mays* acting as a systemic entomopathogen to larvae of *S. frugiperda*.

The results of the present study revealed that the weights of *S. frugiperda* larvae were affected when the larvae were fed on BbC1 and M150 root-inoculated tomato plants. In addition, none of the larvae fed on either of the tomato varieties could develop beyond the larval stage. Clearly, the development of the larvae was delayed as a result of BbC1 or M150 fungal treatments of the



**Fig. 1** Total DNA of A. endophytic *Beauveria bassiana* (BbC1) and B. *Metarhizium anisopliae* (M150) in on agarose gel in two tomato varieties. 1<sup>st</sup> and last lanes Marker: Lane 1- 11: Total fungal DNA isolated in Matina tomato variety: Lanes 12 to 22: Total DNA isolated from Harzfeuer tomato variety

**Table 2** Weight gain of *Spodoptera frugiperda* larvae feeding on root-inoculated tomato varieties (Harzfeuer and Matina) either with *Beauveria bassiana* (BbC1) or with *Metarhizium anisopliae* (M150) for 21 days

Treatment		Mean weight gain of <i>S. frugiperda</i> larvae (mg) $\pm$ se*		
Tomato variety	Fungal treatment	Days		
		7	14	21
Matina	None (control)	23.40 $\pm$ 2.93a	110.77 $\pm$ 8.69ab	202.78 $\pm$ 19.64bc
	BbC1	20.99 $\pm$ 1.70b	81.29 $\pm$ 11.58bc	147.18 $\pm$ 28.74cd
	M150	21.35 $\pm$ 1.47a	70.16 $\pm$ 9.56c	95.56 $\pm$ 13.56d
Harzfeuer	None (control)	22.17 $\pm$ 1.92ab	127.91 $\pm$ 8.47a	332.38 $\pm$ 30.14a
	BbC1	16.98 $\pm$ 1.81bc	104.40 $\pm$ 17.03ab	300.78 $\pm$ 29.18a
	M150	11.34 $\pm$ 1.60c	72.62 $\pm$ 11.48c	235.96 $\pm$ 36.08b

Means with the same letter within the same column are not significantly different ( $P \leq 0.05$ )

\*Values are means ( $\pm$  se) of three replicates of assays each containing 12 larvae  $\pm$  se

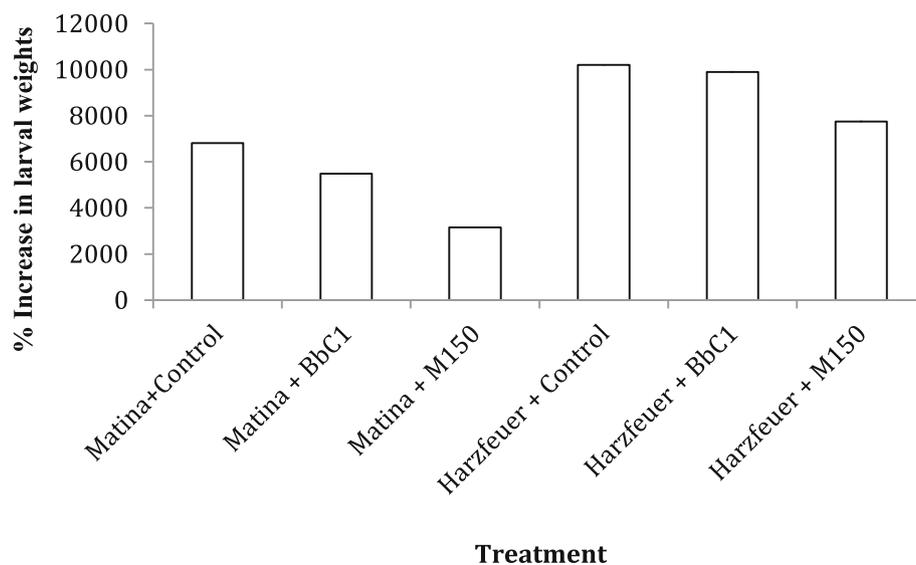
Calculated from a mean original weight ( $3.25 \pm 0.15$  mg) of 12 larvae

tomato plants, and unlike the larvae feeding on untreated tomato that pupated after 21 days, it did not pupate.

Although non-significant differences were observed between BbC1-treated and BbC1-untreated plants, the results indicated that BbC1 reduced larval weights by 27 and 9.5% in Matina and Harzfeuer varieties, respectively. These results are comparable to those reported by Cherry et al. (2004) on the effect of endophytic *Bb* on maize stem borers. The authors demonstrated that even though *Bb* exhibited a non-significant reduction in mean larval weights, there was an evidence of reduction in stem borer effect in the maize plants. Reduced growth effects, as a result of *Bb*, were also reported for *Chortocetes terminifera* by Gurulingappa et al. (2010). Similarly, it was demonstrated that BbC1-inoculated Matina and

Harzfeuer tomato plants displayed reduced growth effects as a result of infestation with the fall armyworm (unpublished report). Most probably, *Bb* acted as an antagonist against *S. frugiperda* larvae by producing metabolites that reduced insect infestations on their host plants (Jaber and Ownley 2018) or changed the plant defense responses and recruited *Bb* as a defensive strategy against insect herbivore (McKinnon et al. 2018).

Interestingly, *S. frugiperda* larvae in the control of variety Matina had significantly lower weights than those fed on Harzfeuer. This was further compounded by the presence of BbC1 and M150 in the variety Matina plant tissues. This indicates that varietal differences of the tomato plants influenced the susceptibility of *S. frugiperda* larvae to the entomopathogens (Tanada and Kaya 1993). The larvae that fed on Matina-inoculated plants most



**Fig. 2** Percent increase in weight of *Spodoptera frugiperda* larvae after 3wks of feeding on *Beauveria bassiana* (BbC1) and *Metarhizium* (M150) root-inoculated tomato (Matina and Harzfeuer)

probably suffered from the reduced consumption as a result of production of secondary metabolites with anti-herbivore properties (Shrivastava et al. 2015), allelochemical (Santiago-Álvarez et al. 2006) changes in the phytosterol profile of plants, or induction of an indirect systemic response (Castillo-Lopez and Sword 2015) that may have affected the feeding behavior in the larvae and growth rate and development of the pest. Furthermore, the presence of endophytic EPF may also influence terpenoid levels in tomatoes, resulting in a strong defense response against insect herbivores (Shrivastava et al. 2015; McCormick et al. 2016). It is therefore possible that the tomato variety Matina increased the susceptibility of *S. frugiperda* larvae to BbC1 and M150 through dietary stress (Tanada and Kaya 1993), therefore influencing their susceptibility to EPF infection and resulting in reduced development and weight of larvae.

## Conclusion

The present study assessed the influence of 2 tomato varieties on the susceptibility of *S. frugiperda* larvae to *B. bassiana* and *M. anisopliae*. The tomato variety Matina conferred some level of resistance to the *S. frugiperda* larvae. This effect was further aggravated by the presence of M150 and BbC1. It is important to use this information in *S. frugiperda* integrated management programs.

## Abbreviations

Bb: *Beauveria bassiana*; BbC1: *Beauveria bassiana* variety C1; Ma: *Metarhizium anisopliae*; M150: *Metarhizium anisopliae* variety 150; CTAB: Cetyltrimethylammonium bromide

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## Author's contributions

The author read and approved the final manuscript.

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## Availability of data and materials

The data used and analyzed during this study is available from the author on request.

## Ethics approval and consent to participate

N/A

## Consent for publication

N/A

## Competing interests

The author declares no conflict of interest.

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