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Research article

Biocontrol competence of *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* against tomato leaf miner, *Tuta absoluta* Meyrick 1917 under greenhouse and field conditions

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ABSTRACT

Tomato is one of the most important crops grown under both greenhouse and field conditions throughout the world. Its production is highly challenged by infestation of leaf miner insect, *Tuta absoluta* Meyrick regardless of excessive insecticide application. The chemical insecticides results insect resistance, environmental pollution, and health problems and there is urgent need for management options such as integrated pest management (IPM) to obviate these problems. Thus, the present study aims to evaluate the effectiveness of single and combination treatments of entomopathogens; *Beauveria bassiana, Metarhizium anisopliae, Bacillus thuringeinsis,* and an insecticide against *T. absoluta* under greenhouse and field conditions. Two varieties (*Awash* and *Venes*) of tomato for greenhouse experiment and one (*Gellila*) variety for field experiment were used with Tutan36%SC (insecticide with active ingredient of Chlorphenapyr 36%SC) and untreated plots as positive and negative controls, respectively. The results showed significant leaf and fruit damage reduction in all the treatments. *B. bassiana*-AAUB03, *M. anisopliae*-AAUM78, and *B. thuringiensis*-AAUF6 showed the highest (93.4%, 89.7% and 90.1%) leaf and (93.5%, 94.4% and 95%) fruit protection under greenhouse condition. The combined treatments improved leaf protection efficacy up to 95.3% under field condition. When the entomopathogens were combined with half or quarter reduced concentrations of Tutan36% SC, it showed 94.4% of pest protection. In all the treatments, 72–96% of marketable fruit was obtained as par insecticide treatment scored 85–93%. All the entomopathogens did not cause any adverse effect on the growth of tomato rather improved shoot length, shoot branching, leaf and fruit numbers. Therefore, application of entomopathogens in single, consortium or in combination reduced the recommended concentration of Tutan36%SC to control *T. absoluta*.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the horticultural plants affected by insect pests, microbial diseases, and nematode infections [1, 2]. According to Santana *et al.* [3], tomato leaf miner, *Tuta absoluta* Meyrick is one of the emerging insect pests that severely affect tomato production. The larval stages attack the aerial parts (leaves, stems and fruits) of tomato and destroy 80–100% of production [4]. The mouthpart and antenna of the insect have gustatory and olfactory sense organs (different types of sensilla) to select host plants [5].

Most domesticated and cultivated tomato varieties are vulnerable to the insect infestations. However, wild species of tomato, such as, *S. habrochaites* and *S. cheesmaninae* that are capable of synthesizing biomolecules, methyl-ketones (2-tridecanone), sesquiterpenes (zingiberene) and acyl sugar (acylglucose and acylsucrose) showed high resistance against *T. absoluta* [6, 7, 8]. This gives insight to develop pest resistant cultivars against the insect pests [6].

Although there are various methods to control insects, currently, chemical insecticides are principally used in the management of leaf miner. Several studies, however, showed that continuous use of the chemicals are implicated with destruction of natural enemies, buildup of insecticide residues on tomato fruits, and the environment, rapid development of insecticide resistance, and reduced profits from high insecticide costs [9, 10, 11]. This necessitates a shift from conventional pest

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control practices to integrated pest management (IPM), in order to reduce application of pesticides and produce healthy food [12].

Microbial entomopathogens are a natural component of IPM to control insect pests and naturally regulated by ecological factors with negligible effects on biodiversity [13]. *Beauveria bassiana*, *Metarhizium anisopliae,* and *Bacillus thuringiensis* are the most important microorganisms that are widely used to protect plants from insect pests for horticultural production [14, 15, 16, 17].

These microorganisms establish themselves endophytically in the plant tissue and augment synergistic pest control [18], promote plant growth, and crop productivity [19]. The hitherto studies showed successful endophytic colonization of tomato plants with entomopathogens [20]. The authors reiterated that *B. bassiana* showed significant growth improvement in tomato plants with direct (87.5%) and indirect (72.5%) protection activity against *T. absoluta*. Other studies showed that *B. bassiana* produce large amount of bioactive compounds of flavonoids, phenols and alkaloids that have antagonistic effect against several types of insects and microbial pathogens [21, 22, 23]. Similarly, *Metarhizium* spp. produce plant growth promoting hormones, cell division stimulators, and nutrient bioavailability modifiers to improve host plant growth besides management of insect pests [24].

It is established that success in pest control and plant promotion by entomopathogens depends on strain selection, appropriate formulation, proper application, suitable environmental condition for application, and application timing [25, 26]. To this end, entomopathogens are screened for effective control of insect pests and plant growth promotion function.

In Ethiopia, one study showed that some strains of *B. bassiana* and *M. anisopliae* reduced 84 and 76% of *T. absoluta* infestation under field conditions [27]. However, there is still a dearth of information on the synergistic role of entomopathogenic fungi (EPF) and bacteria (*B. thuringiensis*) against *T. absoluta* under greenhouse and field conditions. Therefore, in this study, effective strains of *B. bassiana*, *M. anisopliae*, and *B. thuringiensis,* those previously screened under laboratory condition [28, 29, 30] were evaluated under greenhouse and field conditions.

2. Materials and methods

2.1. Tomato varieties

Three tomato varieties were selected for this experiment. The newly released “*Awash*” variety with large fruit size and good aroma for consumption and better marketing [31], and the heat tolerant and disease resistant “*Venes*” tomato variety were collected from Merkamba PLc., Addis Ababa, Ethiopia. These two varieties were used for greenhouse experiment which was conducted at Addis Ababa University (AAU), Collage of Natural and Computational Sciences.

For the field trial, the high yielding commercial hybrid “*Gellila*” variety were bought from Florensis PLc., Addis Ababa, Ethiopia. The experiment was conducted between January 12 to May 26, 20/2021 under irrigation cropping system at Walda Qelina Kebele, Dugda Borra (Meki) district, Oromia Regional State, Ethiopia (Figure 1).

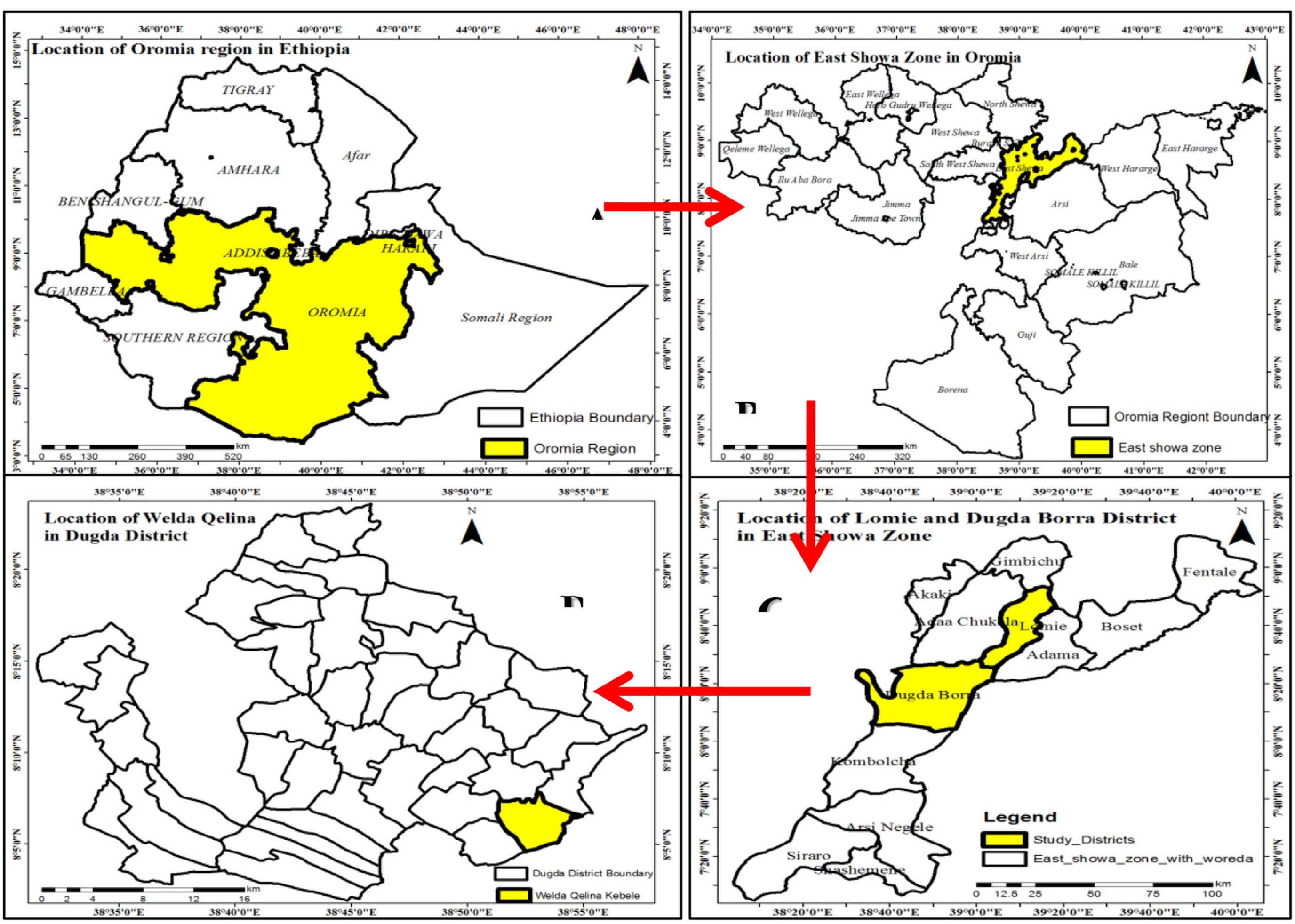

Figure 1. Map of field experiment sites in the East Showa Zone, Oromia Regional State, Ethiopia.

2.2. Microbial bioinsecticides

Four entomopathogenic fungi; *B. bassiana*-AAUB03 (MT588402), *B. bassiana*-AAUB28 (MT588414), *M. anisopliae*-AAUM39 (MT463525), *M. anisopliae*-AAUM78 (MT463530), and two entomopathogenic bacteria; *B. thuringiensis*-AAUF6 (MW250834), and *B. thuringiensis*-AAUMF9 (MW250856) were selected on the basis of their effective larvicidal activity against *T. absoluta* under laboratory conditions [28, 29, 30] for greenhouse tests. From which, *B. bassiana*-AAUB03, *M. anisopliae*-AAUM78, and *B. thuringiensis*-AAUF6 were selected for the trial under field conditions.

2.3. Preparation of entomopathogenic fungi (EPF)

The fungal strains were produced in diphasic (liquid and solid state) fermentations following standard methods [32]. They were grown on potato dextrose agar (PDA) medium at 25 °C until conidiation for 20 days. The conidia were scraped into sterile test tube containing 20 mL of water and 0.01% Tween 80 to produce 1×10^6 conidial suspensions per milliliter. Of which, 15 mL of suspension from each strain was inoculated into 150 mL of potato dextrose broth (PDB) in 250 mL Erlenmeyer flasks and incubated at 28 °C for 7 days with manual agitation every two days to improve aeration. Then, 120 mL of blastoconidia was transferred into 1 kg of autoclaved wheat bran substrate and moistened with sterile water in 1.5 kg capacity of polyethylene bags having pores to allow free flow of air for solid-state fermentation. The culture was incubated at 30 °C for one month, and mixed in a two-day interval at the first week to profuse conidiation.

2.4. Conidial harvesting

The conidia were harvested from the culture by using a manual sifter method [33]. The culture substrates were air-dried, squashed and sieved using a pore size of 500 µm fine wire mesh. The conidia were weighed, collected in sterile 100 mL reagent bottle, and stored at 4 °C for further work.

2.5. Conidial viability test

Conidial viability of the entomopathogens was evaluated by germination test on PDA medium through incubating at 28 °C for 24 h [34]. Percent germination of conidia was determined after having treated the medium with 70% ethanol to halt germination. The number of germinated conidia was detected based on the formation of conidia germ tubes under 40× magnification of a compound microscope. The percentage germination was calculated using the following formula;

$$G = \frac{TC - NG}{TC} \times 100$$

where, present of G: germinated conidia; NG: non-germinated conidia and TC: total count of conidia.

Of these, an appropriate amount of conidia were suspended into sterile water containing 0.01% of Tween 80 and the concentration was adjusted to 1×10^8 conidia mL^{-1} to use for greenhouse and field applications.

2.6. Culturing of Bacillus thuringiensis

Pure isolates of *B. thuringiensis*-AAUF6, and *B. thuringiensis*-AAUMF9 were cultured on nutrient agar medium adjusted at pH of 7.1 [35]. A portion of an active colony was inoculated in to 250 mL of nutrient broth medium prepared in 500 mL Erlenmeyer flask and incubated at 28 °C with subsequent hand shaking for 84 h. The bacterial growth was checked through visual observation of cloud formation in culture medium and the cell concentration was adjusted to 1×10^8 cfu (colony forming units) mL^{-1} using serial dilution techniques for greenhouse and field applications.

2.7. Design of greenhouse experiments

For greenhouse experiment, seeds from each of *Awash* and *Venes* tomato varieties were surface sterilized using sodium hypochlorite (3%) and ethanol (70%) for 3 min and rinsed with sterile water five times [36]. Five seeds of each variety were sown in 3 kg capacity pots filled with a 2:1 ratio of sandy soil and compost mixture. They were thinned down into three after a week of germination. All the seedlings were grown and watered as required and fertilized twice monthly with nitrogen (0.05% NPK).

The greenhouse experimental design includes six single entomopathogen applications such as, *B. bassiana*-AAUB03, *B. bassiana*-AAUB28, *M. anisopliae*-AAUM39, *M. anisopliae*-AAUM78, *B. thuringiensis*-AAUF6, and *B. thuringiensis*-AAUMF9, and positive (Tutan 36% SC (insecticide with active ingredient of chlorphenapyr 36% SC)) and negative (untreated) control. The treatments were arranged in a complete randomized design (CRD) with three replications. Four pots with tomato plantation were arranged separately by enclosing with cotton meshed net to prevent insect transfer from one treatment in the greenhouse with photoperiod of 12/12 h of day and night at College of Natural Sciences, Addis Ababa University.

Then, after four days of hatching, three pairs of *T. absoluta* moths (male and female) were released on each treatment replica using wet cheese close wrap method. The formulated fungal conidia and bacterial culture cells were sprayed using a hand sprayer nozzle after five days of insect release after one month of plantation with a frequency treatment twice a week in the first month and once per week thereafter for a period of three months.

2.8. Design of field experiments

2.8.1. Preparation of land, seedling transplant and treatments

The field site was ploughed and prepared according to the cultural practices of the tomato farming system in the area. A total of 392 m^2 (14 m × 28 m) land was divided into six blocks or replications separated by a canal with a width of 0.75 m. Each block was sub-divided into nine plots (each 3 by 45 cm apart separated by 0.5 m canal) corresponding to nine treatments (Table 1S). Treatments were designed in complete randomized block design (CRBD) with six replications. Three rows were made on each plot and one month old seedlings of *Gellila* variety was obtained from greenhouse of FlorensisPLc. located around Awash River, center for seed propagation following the standards of tomato seedling propagation requirements [37]. They were transplanted in 40 cm apart from each other in a row (Fig S1). All tomato plants were fertilized twice monthly with recommended rate (32 kg ha^{-1}) of the fertilizer.

The transplanted tomato plants were left open for natural infestation of *T. absoluta* and after a week, the presence of eggs, larvae, adult insects as well as lesion on plant leaves were checked. Following the detection of insect manifestation, the formulated fungal conidia, and bacterial culture were sprayed on the plants in single and in combination using a knapsack

Table 1. Production of conidia and conidial viability of *B. bassiana* and *M. anisopliae* strains.

EPF strains	Conidia per kg of substrate in g	Conidial density g^{-1}	% of conidial germination
B. bassiana-AAUB03	10.5 ± 0.67^a	5.2×10^{10a}	95.5 ± 0.91^a
B. bassiana-AAUB28	7.8 ± 0.89^b	6.2×10^{9b}	89.2 ± 0.23^c
M. anisopliae-AAUM39	5.9 ± 1.23^c	1.3×10^{8c}	88.5 ± 0.67^d
M. anisopliae-AAUM78	8.7 ± 0.98^b	3.1×10^{10a}	92.7 ± 1.02^b

Same superscript letters within the column indicate no significant differences between strains ($p < 0.05$).

sprayer. The combined treatments were composed of IPM components that included single, two or three entomopathogens together or entomopathogens with 25% or 50% of the full dose of Tutan36% SC (insecticide). Full dose of Tutan36% SC was sprayed on plots as positive control, whereas plots without any spray were served as negative control. The application of treatments on the field experiments follows the same procedures used for greenhouse experiment.

2.8.2. Data collection

In order to determine damages caused by *T. absoluta* in tomato seedling, twelve plants (one plant per each pot per replica) from the greenhouse experiment and eighteen plants (one plant per each line per replica) from the field experiments were randomly selected and total leaves, leaf lesions, total fruits and fruit bores were counted weekly. Leaf lesion data were collected right after a week of the first application of treatments, whereas fruit bores data were gathered just after one week of tomato fruit sets. The damaged leaves and fruits were recorded weekly until the time of harvest.

The biocontrol effectiveness (E_{biocon}) of the treatments was calculated by using the following formula in order to determine healthy leaves or fruits:

$$\% \ (E_{biocon}) = \frac{TNF - NIF}{TNF} \times 100$$

where TNL, the total mean number of leaves; NIL, the mean number of infected leaves; TNF, the total mean number of fruits; NIF, the mean number of infected fruits per plant.

The percentage of fruit marketability (F_{mark}) per plant was assessed by using formula of;

$$\%(F_{mark}) = [1 - (B_F \text{Treatment} / T_F \text{Counted})] \times 100$$

where B_F, the mean number of bored fruits per plant; T_F, mean number of total fruits counted per plant.

2.8.3. Data analysis

All the data were analyzed by using SPSS software version 25 with one way ANOVA and compared with positive control. Means separation of treatments and control was calculated using Tukey's Least Significant Difference (LSD) test at $p \leq 0.05$.

3. Results and discussion

3.1. Conidial production of entomopathogenic fungi

The entomopathogenic fungi showed significant ($p < 0.05$) variation in the production of conidia, conidial density and germination rate when grown on the wheat bran (Table 1). *B. bassiana*-AAUB03 produced the highest conidial biomass (10.5 g conidia kg^{-1} of substrate) with the largest density (5.2×10^{10} conidia g^{-1}) and germination rate (95.5%), followed by *M. anisopliae*-AAUM78 with production of 8.7 g conidia kg^{-1} of wheat bran, 3.1×10^{10} conidia g^{-1} (density) and conidial germination rate of 92.7% (Table 1). Although the two isolates displayed much lower conidial biomass than produced (40.5 g kg^{-1} of substrate) from optimized rice [38], and lower conidial density (8.7×10^7 conidia g^{-1}) of *B. bassiana* strain grown on brewer's spent grain [39], their germination rate was as good as those obtained from both substrates. This deviation may come from substrate difference since the latter differ in their nitrogen, carbon and amino acids contents that significantly enhance growth and conidial production of entomopathogens [32, 40].

Although *M. anisopliae*-AAUM39 induced the lowest conidial density (1.3×10^8 conidia g^{-1}) on wheat bran, its production was much higher than the conidial density of 4×10^6 conidia g^{-1} produced in the earlier study [41]. This variation in conidial production is due to many factors include; types of substrates, moisture content, incubation temperature, age of initial inoculums, initial concentration of conidia, inoculums size, incubation period, the presence of substrate degrading enzymes and aeration of culture during fermentation process [38, 42].

3.2. Greenhouse study

3.2.1. Leaf protection

The entomopathogens significantly ($P < 0.05$) reduced the leaf damage inflicted by the insect pest, *T. absoluta* ranged from 76% with *B. thuringiensis*-AAUFM9 to 93% with *B. bassiana*-AAUB03 on *Awash* tomato variety and 65–83% on *Venes* variety compared to the untreated control (Table 2). This is best performance compared to previous report of reduction in the insect infestation of 46–75% on tomato plants [43]. The entomopathogen treatments showed significant variation in suppressing the insect infestation to reduce the percent leaf damage of *Awash* variety that was not different from 96% of protection by chemical treatment (Table 2). They also showed the same pattern, but with less percentage reduction of leaf lesion (65–83%) on *Venes* variety compared to the insecticide that scored 87% of protection. Qayyum *et al* [44] indicated that variation in effectiveness is attributed to the difference in the inherent ability of entomopathogens to successfully colonize plant species and attack insect pests.

Among the entomopathogens, *B. bassiana*-AAUB03 showed slightly higher percentage leaf damage reduction (88%) on both tomato varieties, followed by percentage leaf damage decrease of plants treated with *M. anisopliae*-AAUM78 (83%) and *B. thuringensis*-AAUF6 (84%). This was similar to the previous report of 73 and 84% leaf damage reduction of *T. absoluta* on *Coshoro* variety treated with *B. bassiana* and *M. anisopliae,* respectively [45]. However, *B. thuringensis*-AAUF6 induced 78% of leaf damage reduction on the *Venes* variety which was slightly higher reduction in leaf damage of 67% reported by Hosseinzadeh *et al.* [46]. These isolates were also significantly more effective in reducing leaf damage than the commercially recommended dose of *B. thuringiensis* that only reduced 31% of leaf damage on tomato variety [47]. All the

Table 2. Effectiveness of the entomopathogens in reducing leaf damage of tomato by *T. absoluta* under greenhouse conditions.

Treatments	*Awash* variety		*Venes* variety	
	Mean LI (%)	Protection (%)	Mean LI (%)	Protection (%)
B. bassiana-AAUB03	6.6 ± 3.2^{g}	93.4^{ab}	16.5 ± 1.5^{e}	83.5^{b}
B. bassiana-AAUB28	13.9 ± 2.0^{de}	86.1^{b}	19.3 ± 2.0^{ef}	80.2^{bc}
M. anisopliae-AAUM39	20.8 ± 3.2^{c}	79.2^{c}	27.6 ± 1.7^{cd}	72.4^{d}
M. anisopliae-AAUM78	10.3 ± 3.7^{ef}	89.7^{b}	19.8 ± 2.1^{ef}	80.7^{bc}
B. thuringeinsis-AAUF6	9.9 ± 2.4^{f}	90.1^{ab}	22 ± 2.0^{de}	78^{c}
B. thuringeinsis-AAUMF9	23.1 ± 1.7^{bc}	76.9^{c}	34.6 ± 2.2^{bc}	65.4^{e}
Tutan36% SC (insecticide)	4.0 ± 2.12^{hj}	96.0^{a}	13 ± 1.2^{gh}	87^{a}
Untreated control	69.7 ± 4.1^{a}	-	73.5 ± 10.2^{a}	-
Mean		86		77

LI, Leaf infestation; same letters within the column indicate no significant differences $p < 0.05$).

Table 3. Effectiveness of treatments in reducing fruit damage of tomato by *T. absoluta* under greenhouse conditions.

Treatments	*Awash* variety			*Venes* variety		
	TNF	NMF	Protection (%)	TNF	NMF	Protection (%)
B. bassiana-AAUB03	39.3 ± 2.4^{a}	36.7 ± 1.2^{a}	93.5^{b}	28.5 ± 1.4^{a}	23.9 ± 1.0^{a}	83.7^{a}
B. bassiana-AAUB28	35.7 ± 2.7^{b}	25.2 ± 0.8^{d}	70.6^{ef}	22.4 ± 1.7^{c}	15.1 ± 0.8^{d}	67.4^{c}
M. anisopliae-AAUM39	29.7 ± 1.9^{d}	22.8 ± 1.0^{ef}	76.9^{cd}	26.7 ± 1.4^{b}	16.2 ± 1.2^{cd}	60.5^{d}
M. anisopliae-AAUM78	33.7 ± 2.7^{c}	31.8 ± 0.8^{b}	94.4^{b}	28.8 ± 1.8^{a}	24.2 ± 1.1^{a}	84.2^{a}
B. thuringeinsis-AAUF6	30 ± 2.5^{d}	28.5 ± 0.8^{c}	95.7^{a}	29.3 ± 2.1^{a}	24.5 ± 0.8^{a}	83.7^{a}
B. thuringeinsis-AAUMF9	29.3 ± 2.2^{de}	21.9 ± 1.1^{f}	75.4^{d}	27.6 ± 2.3^{a}	19.9 ± 1.0^{b}	72.1^{bc}
Tutan36% SC (insecticide)	29.3 ± 2.2^{de}	27.5 ± 0.8^{c}	94.2^{b}	23.9 ± 1.6^{c}	21.5 ± 1.0^{b}	90.0^{a}
Untreated control	16.7 ± 2.1	3.4 ± 1.1	$20.4 \pm 5.2^{g\text{-}j}$	12.2 ± 1.4	2.74 ± 0.8	$22.5 \pm 6.3^{f\text{-}i}$

TNF, mean total number of fruits; NMF, mean number of marketable fruits; same letters within the column indicate no significant differences between treatments ($p < 0.05$).

treatments showed significant (F 19, 32 = 10. 23, $p < 0.001$) variation (4–34%) of leaf infestation (LI) by *T. absoluta* on both varieties of tomato compared to high damage (>70%) observed from untreated control plants (Table 2). In the other study infestation rate of *T. absoluta* was vary from cultivar to cultivar in tomato production [48].

Based on the mean evaluation of all the treatments, entomopathogens induced more protection (86%) on *Awash* variety than they did (77%) on *Venes* variety (Table 2). In general, *B. thuringeinsis*-AAUF6 performed as good as the other entomopathogenic fungi (*B. bassiana*-AAUB28 and *M. anisopliae*-AAUM78) in protecting the two tomato varieties from leaf miner pest under greenhouse conditions. *B. thuringeinsis*-AAUF6 was better induced leaf protection than the commercial *B. thuringeinsis* showed that less performance on leaf protection compared to chemical insecticides [53].

3.2.2. Fruit protection

In this study, the findings showed significant (F 8, 57 = 12.23, $p < 0.002$) variation among treatments to protect the fruits of both *Awash* and *Venes* varieties from insect damage (Table 3). Thus, *B. bassiana*-AAUB03 treated plants produced the largest (93%) number of marketable fruits (36 fruits/plant) on *Awash* variety with 13–40% increase in the number of marketable fruits over other entomopathogens and the standard insecticide treatments. In another study, *B. bassiana* was less effective (29%) on fruit protection from the *T. absoluta* as reported by Shahini *et al.* [47].

The other entomopathogens induced the production of 70–95% of marketable fruits with less number of fruits (21–32 fruits/plant) compared to *B. bassiana*-AAUB03 treated plants (36 fruits/plant) on the *Awash* variety (Table 3). On the other hand, *Venes* variety showed significant variation (F 27, 43 = 6.23, $p < 0.001$) in different treatments compared to *Awash* variety (Table 3). Thus, *B. thuringeinsis*-AAUF6, *M. anisopliae*-AAUM78 and *B. bassiana*-AAUB03 treated plants produced 24 marketable fruits/plant followed by insecticide and *B. thuringeinsis*-AAUMF9 that produced 22 and 20 marketable, respectively showing 38% increase compared to the least effective treatments, *B. bassiana*-AAUB28 and *M. anisopliae*-AAUM39. In general, the various treatments produced 60–84% of marketable fruits from the total count and displayed 5-9-fold higher number of fruits than the untreated control plants that were almost totally infected with the insect pest.

3.2.3. Fruit yield

The marketable fruit yield obtained from different treatments was significantly ($P < 0.05$) higher than the yield of untreated *Awash* tomato variety (Table 4). The data showed that *B. bassiana*-AAUB03 treatment induced the highest fruit yield (3.6 kg/plant) which was twice more than the ones treated with *B. thuringeinsis*-AAUMF9. However, the marketable fruit yield (3–3.2 kg/plant) from insecticide, Tutan 36%SC treated plant was not significantly different from plants treated with *M. anisopliae*-AAUM78, *B. thuringeinsis*-AAUF6.

The fruit yield pattern was similar to the same treatments on *Venes* variety in that *B. thuringeinsis*-AAUF6 produced 3.5 kg/plant of marketable fruits, followed by *M. anisopliae*-AAUM78 (3.3 kg/plant), *B. bassiana*-AAUB03 (3.1 kg/plant) and Tutan36%SC (2.8 kg/plant) that were twice more protective than the least effective strain (*B. thuringeinsis*-AAUMF9). In all cases, marketable fruit yield obtained from the above mentioned isolates was more than 90% of the total yield. The rest of the isolates also exhibited 72–89% marketable fruit yield with both tomato varieties. This implies that most of the isolates performed as good as those treated with Coragen 200 SC (insecticides) that protected the tomato and produced up to 94% marketable fruit yield with local *Coshoro* variety in Ethiopia [49]. Buragohain *et al.* [50] in India also reported that commercially formulated *B. bassiana* (Green *Beauveria*® and BB Power®) and *B. thuringeinsis* (Green Larvicide® and Delfin® WG) showed more than 90% of marketable fruit yield of tomato by protecting them from *T. absoluta.*

Table 4. The fruit weight and marketability of tomato per plant treated with entomopathogens under greenhouse conditions.

Treatments	*Awash* variety			*Venes* variety		
	TFW/P (Kg)	MFW/P (Kg)	MF (%)	TFW/P (Kg)	MFW/P (Kg)	MF (%)
B. bassiana-AAUB03	3.8 ± 1.2^{a}	3.6 ± 0.9^{a}	94.7^{a}	3.4 ± 0.6^{a}	3.1 ± 0.9^{a}	91.2^{a}
B. bassiana-AAUB28	3.3 ± 0.9^{a}	2.7 ± 1.2^{ab}	81.8^{b}	3 ± 0.3^{a}	2.4 ± 1.2^{ab}	80^{b}
M. anisopliae-AAUM39	2.8 ± 1.0^{ab}	2.1 ± 0.1^{b}	75.0^{c}	2.9 ± 0.2^{ab}	2.1 ± 1.2^{b}	72.4^{c}
M. anisopliae-AAUM78	3.6 ± 0.8^{a}	3.2 ± 2.3^{a}	88.9^{b}	3.7 ± 1.3^{a}	3.7 ± 1.2^{a}	89.2^{ab}
B. thuringeinsis-AAUF6	3.5 ± 2.1^{a}	3.2 ± 0.9^{a}	91.4^{a}	3.8 ± 1.6^{a}	3.5 ± 1.0^{a}	92.1^{a}
B. thuringeinsis AAUMF9	2.6 ± 0.7^{b}	1.9 ± 1.5^{c}	73.1^{c}	2.2 ± 0.8^{bc}	1.6 ± 1.3^{c}	72.7^{c}
Tutan36%SC (insecticide)	3.2 ± 0.8^{a}	3 ± 1.3^{a}	93.8^{a}	3 ± 1.2^{a}	2.8 ± 1.2^{a}	93.3^{a}
Untreated control	1.1 ± 2.7^{c}	0.28 ± 1.3^{e}	25.5^{efg}	1.3 ± 1.0^{d}	$0.5 \pm 0.8^{d\text{-}f}$	$38.5^{d\text{-}g}$

TFW, total fruit weight per plant; MFW, marketable fruit weight per plant; MF, marketable fruits per plant; same letters within the column indicate no significant differences between treatments ($p < 0.05$).

Table 5. The effect of entomopathogen treatments to reduce tomato (*Gellila* variety) leaf infestation from *T. absoluta* under field conditions.

No	Treatment	Mean LI (%)	Protection (%)
T1	*B. bassiana*-AAUB03	8.27 ± 0.9[c]	91.73[a]
T2	*M. anisopliae*-AAUM78	12.71 ± 0.8[b]	87.29[b]
T3	*B. thuringeinsis*-AAUF6	17.84 ± 1.0[a]	82.16[b]
T4	AAUF6 + AAUM78	8.59 ± 1.0[c]	91.41[a]
T5	AAUB03 + AAUM78 + AAUF6	4.68 ± 1.3[e]	95.32[a]
T6	AAUB03 + AAUM78 + 25% Tutan36%SC	5.62 ± 1.5[d]	94.38[a]
T7	AAUF6 + 50% Tutan36%SC	7.77 ± 0.7[c]	92.23[a]
T8	Tutan36%SC (positive control)	6.12 ± 0.9[d]	93.88[a]
T9	Untreated control	93.88 ± 1.0	-

LI, mean leaf infestation; same letters within the column indicate no significant differences between treatments ($p < 0.05$).

3.3. Field study

3.3.1. Leaf protection

The three effective strains (*B. bassiana*-AAUB03, *M. anisopliae*-AAUM78, and *B. thuringeinsis*-AAUF6) significantly reduced leaf infestation (LI) of *Gellila* tomato variety when applied in single or in combination with each other and Tutan36% SC (insecticide) under field conditions (Table 5). All treatments except *M. anisopliae*-AAUM78 (T2) and *B. thuringeinsis*-AAUF6 (T3) showed 91–95% leaf protection with significant ($F_{18, 67} = 13.12$, $p < 0.001$) variation on leaf infestation (LI) (Table 5). In fact, at the early stage of tomato plantation, the insect infestation rate was between, 12.1 and 39.8% (data not shown), that was reduced to 4.7–9.7%, due to repeated application of entomopathogens and increase in age of the plants (Table 5). Klieber and Reineke [51] also reported the same pattern as a function of time and duration of biopesticide application. Similarly, commercial *B. bassiana* (Beaucitech® WP) and *Metarhizium* strain (Metatech® WP) showed 90–98% and 88–95% leaf protection on *Roma* tomato variety, respectively [52].

The findings showed that the single treatment of *B. bassiana*-AAUB03 (T1) maintained its leaf protection (91%) as good as the combined treatments (T4, T5, T6 and T7) (Table 5). Allegrucci *et al* [20] corroborated that *B. bassiana* is efficient in its endophytic colonization to induced direct (86%) and indirect (73%) leaf damage protection from leaf miner. The IPM treatments with consortia of entomopathogens and reduced dose of the chemical insecticide was equally effective as full dose insecticide (positive control) treated plants (65%) in the control of *T. absoluta* (Table5). This may be associated with the ability of the entomopathogens to produce different kinds of secondary metabolites that synergistically induce systemic and repellant responses against pests [19, 53].

Furthermore, combined treatments of the entomopathogenic fungi and quarter reduced concentration of Tutan36% SC was the second most effective treatment in reducing leaf infestation. This suggests that reduction in pest infestation can be enhanced due to chemical interference on the physiology of the insect and increase their susceptibility to entomopathogens [47, 54].

3.3.2. Fruit protection

The total and marketable fruit production was significantly ($F_{23, 82} = 6.15$, $p < 0.001$) different among the treatments in the field conditions (Table 6). Tomato plants treated with *B. bassiana*-AAUB03 (T1) produced a large number of total (80.3 fruits/plant) fruits per plant and 69.3 fruits/plant with 86.3% marketable fruits. Whereas, combined treatments of entomopathogens with quarter-reduced concentration of Tutan 36%SC (insecticide) (T6) and full dose (T7) of insecticide was scored equal number of total fruits (79 fruits/plant) and 72 fruits/plant with 92.1% of marketability. These treatments showed 15–18 fruits/plant marketable fruit production increment compared to the least effective entomopathogen (*M. anisopliae*-AAUM78) (T2) that scored 45 fruits/plant with 74% of marketability.

Table 6. Efficacy of entomopathogens in reducing fruit damage of tomato (*Gellila* variety) from *T. absoluta* under field conditions.

No	Treatment	Total fruits/ plant	Marketable fruit/plant	Protection (%)
T1	*B. bassiana*-AAUB03	80.3 ± 0.9[a]	69.3 ± 0.9[a]	86.3[b]
T2	*M. anisopliae*-AAUM78	64.3 ± 1.2[d]	54.0 ± 1.2[c]	74.0[c]
T3	*B. thuringeinsis*-AAUF6	76.3 ± 1.0[b]	65.3 ± 1.0[b]	75.6[c]
T4	AAUF6 + AAUM78	71.3 ± 1.2[b]	61.7 ± 2.1[b]	86.5[b]
T5	AAUB03 + AAUM78 + AAUF6	67.0 ± 1.1[d]	61.0 ± 1.9[bc]	91.0[a]
T6	AAUB03 + AAUM78 + 25% Tutan36%SC	78.7 ± 0.6[a]	72.5 ± 0.2[a]	92.1[a]
T7	AAUF6 + 50% Tutan36% SC	65.0 ± 1.4[d]	56.7 ± 1.3[c]	87.2[b]
T8	Tutan36%SC (positive control)	79.3 ± 0.9[a]	72.3 ± 1.0[a]	91.2[a]
T9	Untreated control	40.0 ± 1.5[efg]	12.0 ± 1.4[d-f]	30[b-g]

Same letters within the column indicate no significant differences between treatments ($p < 0.05$).

The tomato plants treated with *B. thuringeinsis*-AAUF6 (T3) showed considerable (75.3 fruits/plant) number of total fruits (65.3 fruits/plant) with 75.6% marketability followed by combined treatment of *B. thuringeinsis*-AAUF6 + *M. anisopliae*-AAUM78 (T4) that showed 71.3 fruits/plant with 86.5% marketable fruits (Table 7). However, the consortium of all entomopathogens (T5) was effective in fruit protection (91%) but relatively less effective on fruit number production. In general, marketable fruit production in all the treatments was highly significant ($p < 0.05$) compared to untreated (T9) control showing 5-folds of improvement by protecting plants from the insect pest.

3.3.3. Fruit yields

The marketable fruit yield (kg/plant) of the single and combined entomopathogens treated *Gellila* variety showed considerable variations (Table 7). Thus, almost all of the strains applied in consortia and combined with insecticide showed the highest percentage of marketable fruit (87.2–93.8%) yields compared to positive control (86.6%). All entomopathogens combined treatments (T5) and the two entomopathogenic fungi (*B. bassiana*-AAUB03 + *M. anisopliae*-AAUM78) combined with quarter-reduced concentration of insecticide (T6) showed marketable fruit yield of 10.9 and 9.7 kg/plant, respectively. Fruits obtained from the plants treated with consortia of entomopathogens showed a 25% significant ($P < 0.05$) difference between the most and the least effective

Table 7. Marketable fruits from tomato (*Gellila* variety) plants treaded with entomopathogens under field conditions.

No	Treatments	Total and Marketable fruit weights		
		TFW/P (kg)	MFW/P (kg)	MF (%)
T1	*B. bassiana*-AAUB03	11.21 ± 1.0[a]	9.67 ± 1.1[a]	86.3[b]
T2	*M. anisopliae*-AAUM78	8.73 ± 0.9[b]	7.33 ± 1.0[c]	78.9[c]
T3	*B. thuringeinsis*-AAUF6	10.75 ± 0.7[a]	8.4 ± 0.7[b]	78.1[c]
T4	AAUF6 + AAUM78	10 ± 0.8[ab]	8.75 ± 0.9[b]	87.5[b]
T5	AAUB03 + AAUM78 + AAUF6	10.42 ± 1.0[a]	9.72 ± 1.0[a]	93.3[a]
T6	AAUB03 + AAUM78 + 25% Tutan36%SC	11.64 ± 0.9[a]	10.92 ± 1.0[a]	93.8[a]
T7	AAUF6 + 50% Tutan36%SC	10.53 ± 0.8[ab]	9.18 ± 0.9[a]	87.2[b]
T8	Tutan36%SC (insecticide)	11.33 ± 0.3[a]	9.81 ± 1.0[a]	86.6[b]
T9	Untreated control	5.25 ± 0.6[c-e]	1.58 ± 0.7[d-f]	30.1[c-f]

TFW, total fruit weight per plants; MFW, marketable fruit weight per plant; MF, marketable fruits per plant; same letters within the column indicate no significant differences between treatments ($p < 0.05$).

Table 8. The effect of entomopathogens on the growth of tomato varieties under greenhouses and field conditions.

Treatments	*Results from greenhouse condition*			
	Awash variety		*Venes* variety	
	SL (cm)	PB (n)	SL (cm)	PB (n)
AAUB03	48 ± 1.24^{b}	15 ± 1.44^{a}	43 ± 3.07^{c}	16 ± 1.58^{b}
AAUB28	52 ± 1.89^{a}	12 ± 1.44^{c}	47 ± 1.94^{b}	16 ± 1.44^{b}
AAUM39	53 ± 2.36^{a}	16 ± 1.00^{a}	40 ± 2.42^{d}	15 ± 1.23^{b}
AAUM78	43 ± 1.44^{c}	14 ± 1.00^{b}	44 ± 2.30^{c}	16 ± 1.00^{b}
AAUF6	50 ± 2.04^{ab}	14 ± 1.23^{b}	48 ± 1.44^{ab}	18 ± 1.00^{a}
AAUMF9	51 ± 1.62^{a}	15 ± 1.23^{a}	51 ± 2.24^{a}	12 ± 1.41^{d}
Tutan36%SC (Positive control)	49 ± 2.47^{ab}	15 ± 1.44^{a}	49 ± 1.00^{ab}	14 ± 1.23^{c}
Without any (Negative control)	43 ± 2.12^{c}	8 ± 1.23^{d}	48 ± 3.56^{ab}	10 ± 1.44^{e}
Results from field condition		*Gellila* variety		
AAUB03		79.45 ± 2.08^{ab}		35.8 ± 1.45^{c}
AAUM78		79.07 ± 1.54^{ab}		37 ± 2.72^{c}
AAUF6		79.82 ± 2.45^{ab}		39.7 ± 1.34^{b}
AAUF6 + AAUM78		81.64 ± 1.98^{a}		44.93 ± 0.94^{a}
AAUB03 + AAUM78 + AAUF6		83.84 ± 3.12^{a}		45.11 ± 2.54^{a}
AAUB03 + AAUM78 + 25% Tutan36%SC		79.25 ± 2.09^{ab}		38.73 ± 3.21^{b}
AAUF6 + 50% Tutan36%SC		77.32 ± 2.06^{b}		36.52 ± 4.67^{c}
Tutan36%SC (Positive control)		71.05 ± 2.45^{c}		33.18 ± 4.23^{c}
Without any (Negative control)		67.3 ± 4.12^{d}		26.41 ± 1.43^{e}

SL, mean shoot length; PB, mean number of branches per plant; same letters within the column indicate no significant differences between treatments ($p < 0.05$).

treatments with 40–55% yield increase compared to the untreated control.

It is interesting to note that there was no significant ($P < 0.05$) variation in marketable fruits yield between the plants treated with *B. bassiana*-AAUB03, combination of *B. thuringeinsis*-AAUF6 and half concentration of Tutan36% SC and the full dose of Tutan36%SC treated plants. The finding showed that 9.7, 9.2 and 9.8 kg/plant with 86.3, 87.2 and 86.6% of fruit marketability, respectively (Table 7). Similarly, the *Coshoro* variety tomato treated with *B. bassiana* was produced 98.2% of marketable fruit yield in Ethiopia [49]. However, the second least effective entomopathogen, *B. thuringeinsis*-AAUF6 showed more than 2-folds of fruit yields (8.4 kg/plant) compared to 3.2 kg/plant of marketable fruit yields scored by tomato plants treated with commercial *B. thuringeinsis* (Costar®) under field conditions [55].

All these effective treatments showed 20–33% marketable yield increment compared to plants treated with the least effective strain (*M. anisopliae*-AAUM78). Shibru and Getu [49] have reported 78.5% of marketable fruit yield from the tomato treated with *M. anisopliae,* which is comparable with the result (78.9%) obtained from our *M. anisopliae*-AAUM78. Thus, treatments with their own effectiveness in control of *T. absoluta* enhanced the production of marketable yield compared to untreated plants. This suggests that all of the treatment options are effective to control *T. absoluta* and produce tomato on sustainable basis.

3.4. Effect of entomopathogens on the growth of tomato plants

Interestingly, none of the applied entomopathogens caused any negative effect on the growth of tomato, rather they showed better growth performance and productivity in both greenhouse and field conditions (Table 8). All the tomato varieties (*Awash*, *Venes* and *Gellila*) treated with entomopathogens showed 40–83 cm of shoot length and 12–45 number of shoot branches (Table 8). It is almost equal or better compared to tomato treated with Tutan36%SC. In fact, *B. bassiana* showed plant growth promotion and increased height, number of leaves, grain weight, crop yield, and germination on the corn crop besides reducing larvae of *Rachiplusia un* [56].

The entomopathogens might be fully colonize plants and induce growth besides insect damage reduction or entomopathogens could also antagonize certain pathogens and favor the growth of plants [56, 57]. In addition, *Metarhizium* spp. produced plant growth hormones (auxin and cytokinins), the cellular division stimulator [24] and nutrient bioavailability modifier, siderophore [58, 59] that augment growth of plants. Furthermore, endophytic microbes induce adaptation of plants to environmental stresses and improve metabolic pathways through interlinking each other to exchange compounds that improve efficient functioning [60], produce antibiotics and plant growth enhancing substances [61].

4. Conclusion

In conclusion, single and consortium application of *B. bassiana, M. anisopliae* and *B. thuringeinsis* against *T. absoluta* showed considerable leaf and fruit damage reductions. *B. bassiana*-AAUB03, *M. anisopliae*-AAUM78 and *B. thuringiensis*-AAUF6 are the most effective strains in *T. absoluta* management. Their consortia and combination with quarter or half dose of Tutan36% SC showed stronger activity in reduction of *T. absoluta* infestation. Besides to pest control activity, entomopathogens improved crop productivity without any effect on the growth of the plant in both greenhouse and field conditions. Therefore, application of entomopathogens in single, consortium and combining with reduced concentration of recommended rate of insecticide could be utilized in IPM strategy.

Declarations

Author contribution statement

Birhan Aynalem: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Diriba Muleta; Mulissa Jida; Fekadu Shemekite; Fassil Aseffa: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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