

## Research Article

# Integrated Potential of Microbial, Botanical, and Chemical Pesticides for the Control of Viral Disease Vector Whiteflies (Hemiptera: Aleyrodidae) on Tomato under Greenhouse and Field Perspectives

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Whiteflies are one of the most devastating horticultural pests attacking tomatoes. Although there are several control methods for the control of whitefly pests, the integrated application of entomopathogenic fungi (IPM) with chemical and botanical insecticides has proven more effective than individual control agents. This study was carried out to evaluate individual and combined treatments of entomopathogens *B. bassiana*, *M. anisopliae*, *B. thuringiensis*, Hunter 40 EC, and neem oil for the control of whitefly species on tomato (*Solanum lycopersicum*) under greenhouse and field condition. The greenhouse study showed that the different treatments resulted in a 58.48 to 100% reduction of nymphs and a 52.06 to 100% reduction of adults on both Galilea and Melkashola tomato varieties under greenhouse conditions. The combined treatments of AAUMB-29 + Neem oil displayed a higher yield (423.3 g fruits/plant) on the Galilea tomato variety, and AAUDM-43 + Hunter 40 EC displayed a yield of (376.66 g/plant) on the Melkashola tomato variety. Under field conditions, the application of AAUMB-29 + Hunter 40 EC + Neem oil significantly decreased the whitefly population by 91.93% ( $P < 0.001$ ) after 10 days of the fourth spray. The result of fruit yield of tomato was significantly higher in all treatments (31.17 t to 70.42 t·ha<sup>-1</sup>) compared to untreated control (25.83 t·ha<sup>-1</sup>). Among the treatments, AAUMB-29 + Hunter 40 EC + Neem oil gave the highest fruit yield of 70.42 t·ha<sup>-1</sup> followed by AAUMB-29 + Hunter 40 EC (64.50 t·ha<sup>-1</sup>) on the Galilea tomato variety under field conditions. The combined treatment of AAUMB-29 + Hunter 40 EC + Neem oil was the most effective with lower whitefly infestation, higher marketable yields, and less percentage of yield losses. Further investigations are required to determine the optimization and practicability of this integrated application of treatments for the control of both sucking and chewing insect pests under field conditions.

## 1. Background

Tomato (*Solanum lycopersicum* Linnaeus) (Solanales: Solanaceae) is one of the most important horticultural vegetables grown all over the world for its high commercial and nutritional value [1]. It is the 3<sup>rd</sup> largest vegetable crop after potato and sweet potato, and as a processing crop, it ranks

first among all vegetables [2]. The tomato fruit is valuable used in the processing of ketchup, juice, paste, and soups in the food industry [3]. Nutritionally, the fruit contains calcium, niacin, flavonoids, lycopene, beta-carotene, vitamins (A, C, and E), and antioxidant compounds such as ascorbic acid, lycopene, and retinol that protect humans against diabetes, cardiovascular diseases, and cancer [4, 5].

In Ethiopia, tomato is the most important and widely cultivated vegetable under rain-fed and irrigated conditions for fresh consumption and as a source of income in the rift valley areas [6]. Regardless of its nutritional, economic, and health importance, the national average production of tomatoes in the country is very low which accounts for 5.3 tons-ha<sup>-1</sup> in comparison with China (59.4 tons-ha<sup>-1</sup>), India (24.6 tons-ha<sup>-1</sup>), the USA (96.8 tons-ha<sup>-1</sup>), Turkey (68.8 tons-ha<sup>-1</sup>), and Egypt (40.9 tons-ha<sup>-1</sup>) [7]. This low level of tomato production is associated with many biotic and environmental constraints. The prominent constraints are pests and diseases which reduce yield [8] and the quality of marketable fruits [9].

Whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), are the most damaging agricultural insect pests for tomato production [10]. These polyphagous pests cause direct damage to tomato by feeding on plant sap, opening up to secondary infection by sooty molds which reduces photosynthesis and decrease plant vigor [11], and transmitting different tomato viral diseases [12] caused by Begomovirus, Crinivirus, Ipomovirus, Carlavirus, and Torradovirus [13]. According to [14], tomato production is threatened worldwide by the occurrence of whitefly-transmitted Begomoviruses, which are associated with tomato leaf curl diseases.

In Ethiopia, whiteflies are the major insect pests that are severely damaging tomato production in north Gondor and the central Rift valley of Ethiopia [15, 16]. Although several whitefly-transmitted tomato viral diseases are detected in the Rift Valley of Ethiopia, tomato yellow leaf curl disease showed a higher prevalence [17]. Therefore, it is important to control tomato Whitefly pests to reduce virus transmission and agronomic losses thereby enhancing the growth, yield, and quality of tomatoes. In this regard, the use of integrated pest management is critical to control pests effectively with the integration of biopesticides and bio-rational insecticides [18].

Integrated pest management (IPM) is a comprehensive approach to crop production that combines a broad array of compatible techniques such as sanitation, use of resistant varieties, cultural manipulation, trap, biological control, and agricultural recommended chemicals to maintain pests below economic damage levels [19]. Thus, the IPM method is applied to insect pest management approaches in diverse agricultural systems with effective, environmentally sound, and sustainable control measures.

Microbial-based biopesticides from entomopathogenic fungi and bacteria, chemical pesticides, and botanical biopesticides from neem (*Azadirachta indica* A. Jussieu) (Sapindales: Meliaceae) extracts are important components of IPM systems for the management of insect pests [20, 21]. Entomopathogenic fungi (EPF), *Beauveria bassiana* (Balsamo-Crivelli), Vuillemin (Hypocreales: Cordycipitaceae), and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) are the most widely studied biocontrol agents against many economically important insect pests in agriculture [22, 23]. They effectively control the different life stages of whiteflies from different host

plants including tomato [24]. A recent report showed that the two entomopathogens can infect over 750 species of host insects [25].

Apart from that the entomopathogenic bacterium, *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) plays an important role in insect pest control around the globe [26]. Insecticidal activity of *B. thuringiensis* (Bt) is usually attributed to the proteinaceous toxins, namely Vip (vegetative insecticidal protein) and Sip (secreted insecticidal protein) families [27], that are produced at various stages of the bacterial life cycle. On the transition to sporulation, Bt shifts to the production of insoluble  $\delta$ -endotoxins which include two families of nonselective pore-forming proteins, namely Cry (crystal) and Cyt (cytotoxic) [28]. *B. thuringiensis* is an effective bio-pesticide agent against whiteflies from different host plants, with more than 92% whitefly nymph mortality [29]. Recently, the Colombian native strains of *B. thuringiensis* induced 18 to 69% mortality against whitefly *B. tabaci* on tomato [30].

Botanical bioinsecticides from neem (*Azadirachta indica*) extract are ideal for the control of insect pests with safe, inexpensive, and effective control measures against numerous agriculturally important insects [31]. The neem tree produces more than 300 biologically active secondary metabolites [32], most notably Azadirachtin [33]. It is a better source of pesticide due to its antifeedant and repellent, insecticidal, nematocidal, bactericidal, and fungicidal activities [34]. The neem oil, leaf extracts, and bark extracts have insecticidal properties, and neem oil is widely used for pest control activities worldwide [35]. The study of [36] indicated that neem extracts act as a strong antifeedant and repellent; delay and prevent molting; reduce growth, development, and oviposition; and can cause high mortality of whiteflies.

The cocktail application of entomopathogenic fungi with chemicals [37] and botanical insecticides (neem product) [38] has proven more effective for the control of whitefly pests than individual control agents. This integrated control measure is beneficial because it improves the efficacy of pest control while decreasing the dose of insecticide application and reduction of pest resistance [39]. All taken together, it appears that the combined use of entomopathogenic fungi, neem extracts, and chemical insecticides at sublethal doses is ideal to control tomato pests and improve productivity. Therefore, the objective of this study is to evaluate the dual use of biopesticides and chemical pesticides for managing whitefly (Hemiptera: Aleyrodidae) pests in tomatoes under greenhouse and field conditions.

## 2. Materials and Methods

*2.1. Sources of Microbial Bioinsecticides.* Entomopathogenic microbial isolates of AAUMB-29 (*B. bassiana*), AAUMFB-77 (*B. bassiana*), AAUDM-43 (*M. anisopliae*) [40], and AAUES-69D (*B. thuringiensis*) [41] were isolated from soil and identified using molecular identification methods (Table 1).

TABLE 1: Treatments of biopesticides and their dose of field applications.

No.	Treatments	Concentration/dose
T1	Hunter 40 EC	500 ml/ha (2.5 ml/L)
T2	<i>B. bassiana</i> (AAUMB-29)	10 <sup>8</sup> conidia/ml
T3	<i>M. anisopliae</i> (AAUDM-43)	10 <sup>8</sup> conidia/ml
T4	<i>B. bassiana</i> (AUMB-29) + Hunter 40 EC	10 <sup>8</sup> conidia/ml + 1.25 ml/L Hunter 40 EC
T5	<i>B. bassiana</i> (AUMB-29) + neem oil	10 <sup>8</sup> conidia/ml + 0.5% neem oil
T6	<i>M. anisopliae</i> (AAUDM-43) + Hunter 40 EC	10 <sup>8</sup> conidia/ml + 1.25 ml/L Hunter 40 EC
T7	<i>B. bassiana</i> (AUMB-29) + neem oil + Hunter 40 EC	10 <sup>8</sup> conidia/ml + 0.5% neem oil + 1.25 ml/L Hunter 40 EC
T8	Control (water)	—

**2.2. Source of Neem Extract.** Seeds and fresh leaves of neem (*Azadirachta indica*) were collected from Raya Kobo, North Wollo, Amhara region, Ethiopia. They were washed thoroughly with water and air-dried to a constant weight under shade for 10–15 days. They were ground with an electric grinder (NM-8300, Japan), sieved, and collected in polyethylene bags. The oil of *A. indica* (neem) was extracted using a Soxhlet apparatus at room temperature as described by [42]. Thus, 100 g of seed powder was extracted with 1000 ml of ethanol + n-Hexane (50:50%). The solvents were removed using rotary evaporation at a temperature below 45°C.

**2.3. Source of Chemical Pesticides and Tomato Varieties.** The chemical pesticide of Hunter 40 EC, commonly used for the control of sap-sucking pests in the Rift valley of Ethiopia under field conditions, was purchased from the local market and used with a recommended dose of 400 ml/ha. The Melkashola tomato variety (local variety) was obtained from Melkassa Agricultural Research Center, while the Galilea tomato variety (hybrid variety) was purchased from the local market.

**2.4. Mass Production of *B. thuringiensis*.** The stock culture of *B. thuringiensis* AAUES-69D was transferred to a nutrient agar medium (Difco Labs Ltd) with a sterile loop and incubated for 48 hrs at 28°C. A loop full of the colony on nutrient agar plates was inoculated into a 250-mL Erlenmeyer flask containing 50 mL of sterilized nutrient broth (Difco Labs Ltd). The flasks were incubated at 28°C for 24 hours on a rotary shaker at 120 rpm. The actively growing cells were used as a seed culture for mass production [43]. Then, 5% of the seed culture was added to a 250-mL flask containing 50 mL of sterilized nutrient broth medium and incubated at 28°C for 72 hours on a rotary shaker at 120 rpm. A stock culture suspension was diluted and adjusted to 10<sup>9</sup> CFU/ml concentration.

**2.5. Mass Production of Entomopathogenic Fungi *B. bassiana* and *M. anisopliae***

**2.5.1. Inoculum Preparation and Harvesting Conidia.** The seeding inocula were prepared according to [44]. Isolates, namely AAUMB-29, AAUMFB-77, and AAUDM-43, were cultured on to PDA medium at 25°C for 15 days, and conidia were harvested and suspended in sterile distilled water containing 0.1% Triton X-100 and adjusted to 1 × 10<sup>7</sup>

conidia/ml using a hemocytometer. Then, 1 ml of conidia suspension (1 × 10<sup>7</sup> conidia/ml) from each isolate was inoculated into 250 ml of potato dextrose broth in 500-ml Erlenmeyer flasks. They were kept on an orbital shaker for 14 days to induce the development of blastopores used for mass production in solid-state fermentation.

Mass production of isolates was undertaken on three solid substrates, namely wheat bran, millet, and sorghum, according to [45]. Five hundred grams of each substrate was washed, dried, and boiled in a water bath at 100°C for 1 hour. The excess water was removed by decanting and autoclaved at 15 psi for 1 hr at 121°C. The substrates were then inoculated with 1 ml of liquid culture and incubated at 25°C for 3 weeks by periodically shaking every 4–5 days to avoid clumping and separating the grains and breaking the mycelia mat. Aerial conidia were harvested by manually shaking the culture substrate and allowing them to pass through a 300-µm sieve before being weighed and stored in plastic bags.

**2.6. Evaluation of Spore Concentration and Conidia Viability.** After spore powders were harvested, the spore concentration was determined as described by [46]. One gram of harvested spore was transferred into 10 ml distilled water containing 0.1% of Triton X-100 solution in flasks, vigorously shaken to mix for 10 min, and filtered through a double-layered muslin cloth. The number of conidia (concentration) per gram of spore powder was quantified, after appropriate dilution, using a Neubauer hemocytometer under a light microscope at 400× magnification. The conidia viability of each treatment was performed according to [47]. To this end, 200 µl spore suspension (10<sup>7</sup> conidia/ml) was dispensed to the PDA plate. All plates were sealed with parafilm and incubated in darkness at 25°C. After 24-h incubation, one drop of lactophenol cotton blue was spotted on the different corners of the plates and covered with coverslips. The percentage of germination was determined by randomly counting 300 spores for each plate under a light microscope at 400× magnification. Conidia were considered to have germinated when the germ tube of any length was visible.

After mass production, isolates were formulated with solid carriers following the protocol developed by [48]. Following solid fermentation, conidial mass was mixed with 10 g of talc powder at a 1:10 ratio (1 g conidia: 10 g carrier). Then, 1% CMC (carboxymethyl cellulose) was added as a sticker, and the mixture was dried at room temperature, powdered, and stored in polyethylene bags.

**2.7. Greenhouse Evaluation.** Adult whiteflies (sex ratio  $\approx 1:1$ ) were mass-reared in whitefly-rearing cages on tomato [49]. Melkassa and Galilea tomato varieties were selected for the greenhouse experiment. Seeds were surface-sterilized and allowed to germinate in a Petri dish for 2 days and transferred into pots (330 cm<sup>3</sup>) filled with a mixture of compost, loam soil, and sandy soil in the ratio of 1:1:2. When the tomato plants reached the 4–5 leaf stage, they were kept inside whitefly-proof cages (2 × 1.5 × 10 m) with fine mesh materials, and each plant was separated with a plastic sheet inside fine mesh to block the jumping of whiteflies from one treatment to other. Then each plant was infested with adult whiteflies (20 adult whiteflies/plant) and kept for 15 days until the nymph reached the 2<sup>nd</sup> to 3<sup>rd</sup> instar. After 15 days of infestation, they were treated with entomopathogens, neem, and chemical pesticides alone and in combinations, in triplicate. The experiment was conducted in fourteen treatments in triplicate with a randomized complete block design (RCBD). All treatments were repeated (sprayed) three times at 12 days of intervals. Before and after the experiment, the number of nymphs and adults were counted.

Treatments:

Treatment 1 (T1) = Control (water)

Treatment 2 (T2) = *B. thuringiensis* AAUES-69D (10<sup>9</sup> CFU/ml)

Treatment 3 (T3) = *B. bassiana* AAUMB-29 (10<sup>8</sup> conidia/ml)

Treatment 4 (T4) = *M. anisopliae* AAUDM-43 (10<sup>8</sup> conidia/ml)

Treatment 5 (T5) = Neem oil (1%)

Treatment 6 (T6) = Chemical treatment with Hunter 40 EC (500 ml/ha)

Treatment 7 (T7) = *B. bassiana* AAUMB-29 (10<sup>8</sup> conidia/ml) + *B. thuringiensis* AAUES-69D (10<sup>9</sup> CFU/ml)

Treatment 8 (T8) = *B. thuringiensis* AAUES-69D (10<sup>9</sup> CFU/ml) + Neem oil (0.5%)

Treatment 9 (T9) = *B. thuringiensis* AAUES-69D + Hunter 40 EC (1.25 ml/L)

Treatment 10 (T10) = *B. bassiana* AAUMB-29 (10<sup>8</sup> conidia/ml) + Hunter 40 EC (1.25 ml/L)

Treatment 11 (T11) = *B. bassiana* AAUMB-29 (10<sup>8</sup> conidia/ml) + neem oil (0.5%)

Treatment 12 (T12) = *M. anisopliae* AAUDM-43 (10<sup>8</sup> conidia/ml) + neem oil (0.5%)

Treatment 13 (T13) = *M. anisopliae* AAUDM-43 (10<sup>8</sup> conidia/ml) + Hunter 40 EC (1.25 ml/L)

Treatment 14 (T14) = *B. bassiana* (AAUMB-29 (10<sup>8</sup> conidia/ml) + neem oil (0.5%) + Hunter 40 EC (1.25 ml/L).

**2.8. Field Trial.** The experiment was laid out on the farmland of Meki, East Shoa Oromia region from

December 2020 to May 2021. The Meki town is located in the central rift valley at 8° 01' to 8° 25' N Latitude and 38° 32' to 39° 04' E Longitude. It has an altitude range from 1600 to 2000 m.a.s.l. The mean annual temperature and rainfall are 22 to 28°C and 700 to 800 mm, respectively (CSA, 2011). The experimental plot was prepared in an area 4 m long and 2 m wide with a total area of 6 m<sup>2</sup>. The spacing between two plots in each replication and between adjacent blocks was 50 and 100 cm, respectively. The commercially important Galilea variety seedlings were raised in the Meki seedling center. Seedlings were transplanted to the plot after 45 days of sowing at a recommended spacing of 100 cm between rows and 40 cm between plants [50]. The experiment was conducted in an RCBD with eight treatments in three replications (Table 1).

All agronomic practices, fertilizer application, and irrigation frequencies were carried out according to the recommendations of the Ethiopian Institute of Agricultural Research (EARO, 2004). All treatments were repeated (sprayed) four times at 12 days of intervals after the infested plants reached at economic threshold level (ETL) of 5 adult or nymph whitefly per leaf. The population density of whitefly was recorded by taking three leaves (upper, middle, and lower) from randomly selected plants during early morning hours one day before and after 5 and 10 days of each spray.

The population reduction of whiteflies was estimated using the following formula [51]:

$$\text{population reduction (\%)} = \left[ \frac{1 - T_a X C_b}{T_b X C_a} \right] \times 100, \quad (1)$$

where  $T_a$  is the number of insects in treatment after spray,  $T_b$  is the number of insects in treatment before spray,  $C_a$  is the number of insects in untreated check after spray, and  $C_b$  is the number of insects in untreated check before spray.

Data on yield and yield-related parameters including the number of fruits per plant, marketable yield (t ha<sup>-1</sup>), unmarketable yield (t ha<sup>-1</sup>), and total yield (t ha<sup>-1</sup>) were collected and analyzed.

The control index and potency level of biopesticides on whiteflies were calculated using the following formula [52]:

$$\text{control index} = \left\{ \frac{\% \text{ overall reduction in treatment}}{\% \text{ overall reduction in the most effective treatment}} \right\} \times 100,$$

$$\text{potency level} = \frac{\% \text{ overall reduction in treatment}}{\% \text{ overall reduction in the least effective treatment}}. \quad (2)$$

**2.9. Data Analysis.** The greenhouse and field data were subjected to analysis of variance (ANOVA). The mean values were compared by Duncan's multiple range test (DMRT) at  $p = 0.05$ , using SAS program, version 9.4 [53].

### 3. Results

**3.1. Mass Production.** The mass production effects of *B. bassiana* AAUMB-29, *B. bassiana* AAUMFB-77, and

TABLE 2: Germination, spore harvesting, and spore concentration (Mean  $\pm$  SE) of entomopathogenic fungal isolates on the sorghum, wheat bran, and millet substrates.

Treatments	Sorghum substrate			Wheat bran substrate			Millet substrate		
	Spore harvesting (g/ 500 g)	Spore concentration (x10 <sup>9</sup> conidia/g of spore powders)	Germination (%)	Spore harvesting (g/ 500 g)	Spore concentration (x10 <sup>9</sup> conidia/g of spore powders)	Germination (%)	Spore harvesting (g/ 500 g)	Spore concentration (x10 <sup>9</sup> conidia/g of spore powders)	Germination (%)
AAUMB-29	8.33 <sup>a</sup>	10.60 <sup>a</sup>	95 <sup>a</sup>	5.33 <sup>a</sup>	6.40 <sup>a</sup>	92 <sup>a</sup>	3.34 <sup>a</sup>	3.73 <sup>a</sup>	88 <sup>a</sup>
AAUMFB-77	4.57 <sup>b</sup>	8.00 <sup>b</sup>	88.3 <sup>b</sup>	4.37 <sup>ab</sup>	2.77 <sup>b</sup>	87 <sup>a</sup>	3.4 <sup>a</sup>	3.60 <sup>a</sup>	79.67 <sup>a</sup>
AAUDM-43	6.77 <sup>a</sup>	5.47 <sup>c</sup>	91.67 <sup>ab</sup>	3.83 <sup>c</sup>	5.13 <sup>ab</sup>	86.67 <sup>a</sup>	4.67 <sup>a</sup>	4.20 <sup>a</sup>	85.33 <sup>a</sup>

Means with different letters in a column indicate the significant difference in Duncan's multiple range test (DMRT) ( $P = 0.05$ ). SE, standard error.

TABLE 3: The effects of single and combined application of biopesticides in the reduction of nymph and adults (%) of the whitefly *T. vaporariorum* on the Galilea tomato variety.

Treatment	Nymph					Adult				
	1 <sup>st</sup> spray*	2 <sup>nd</sup> spray**	3 <sup>rd</sup> spray**	CI	PV	1 <sup>st</sup> spray*	2 <sup>nd</sup> spray**	3 <sup>rd</sup> spray**	CI	PV
AAUMB-29	—	—	84%	71.34	1.56	52%	—	82%	80.82	1.54
AAUES-69D	—	—	—	45.66	1.00	—	—	—	52.59	1.00
AAUDM-43	—	—	—	64.78	1.42	—	—	87	80.59	1.52
Neem oil	—	—	—	63.12	1.38	—	—	87%	70.01	1.33
Hunter 40 EC	—	90%	97%	90.08	1.97	59%	90%	100%	100	1.90
AAUMB-29 + AAUES-69D	—	—	89%	76.46	1.67	55%	—	93%	88.82	1.67
AAUMB-29 +Neem oil	—	—	98%	80.81	1.77	—	—	100%	87.56	1.67
AAUMB-29 + Hunter 40 EC	—	82%	100%	87.96	1.93	56%	89%	96%	89.72	1.71
AAUES-69D + Neem oil	—	—	87%	65.50	1.52	—	—	—	64.51	1.23
AAUES-69D + Hunter 40 EC	—	—	82%	68.71	1.50	—	—	—	70.84	1.35
AAUDM-43 + Hunter 40 EC	—	92%	93%	89.67	1.96	53%	—	94%	87.01	1.65
AAUDM-43 +Neem oil	—	93%	96%	86.53	1.89	54%	—	95%	89.38	1.70
AAUMB-29 + Hunter 40 EC + Neem oil	64%	97%	100%	100	2.19	60%	89%	100%	99.82	1.90

\*Values with more than 50% white fly reduction. \*\*Values more than 80% white fly reduction. CI, control index; PV, potency value.

TABLE 4: The effects of single and combined application of biopesticides in the reduction of nymph and adults (%) of whitefly *T. vaporariorum* on the Melkashola tomato variety.

Treatment	Nymph					Adult				
	1 <sup>st</sup> spray*	2 <sup>nd</sup> spray**	3 <sup>rd</sup> spray**	CI	PV	1 <sup>st</sup> spray*	2 <sup>nd</sup> spray**	3 <sup>rd</sup> spray**	CI	PV
AAUMB-29	—	—	87%	66.99	1.61	52%	—	80%	70.43	1.68
AAUES-69D	—	—	—	41.72	1.00	—	—	—	41.83	1.00
AAUDM-43	—	—	86%	61.88	1.48	72%	—	80%	62.84	1.51
Neem oil	—	—	—	60.08	1.54	—	—	80%	81.16	1.94
Hunter 40 EC	53%	88%	100%	87.23	2.09	82%	84%	100%	91.10	2.32
AAUMB-29 + AAUES-69D	—	—	89%	71.11	1.70	60%	—	86%	81.15	1.94
AAUMB-29 + Neem oil	—	87%	97%	78.31	1.88	—	—	100%	77.96	1.86
AAUMB-29 + Hunter 40 EC	—	95%	100%	86.57	2.08	71%	80%	100%	91.31	2.18
AAUES-69D + Neem oil	—	—	87%	64.80	1.55	—	—	80%	64.72	1.55
AAUES-69D + Hunter 40 EC	—	—	86%	63.83	1.53	57%	—	—	72.65	1.74
AAUDM-43 + Hunter 40 EC	51%	93%	96%	86.67	2.07	74%	80%	97%	90.31	2.16
AAUDM-43 +Neem oil	68%	91%	98%	93.06	2.26	—	81%	94	80.26	1.92
AAUMB-29 + Hunter 40 EC + Neem oil	78%	98%	100%	100	2.40	84%	89%	100%	100	2.39

\*Values with more than 50% white fly reduction. \*\*Values more than 80% white fly reduction. CI, control index; PV, potency value.

*M. anisopliae* AAUDM-43 on sorghum, wheat bran, and millet substrates were determined. The highest conidia yield was recorded by *B. bassiana* AAUMB-29 on sorghum (8.33), *B. bassiana* AAUMB-29 on wheat bran (5.33 g), and *M. anisopliae* AAUDM-43 on millet (4.67 g) (Table 2). The highest spore germination obtained from sorghum, wheat bran, and millet substrates were 95%, 92%, and 88%, respectively (Table 2). Concerning spore concentration, *B. bassiana* AAUMB-29 showed better concentration on both sorghum ( $10.6 \times 10^9$  conidia/g) and wheat bran ( $6.4 \times 10^9$  conidia/g), whereas isolate *M. anisopliae* AAUDM-43 displayed the maximum numbers of spore on millet substrate ( $4.2 \times 10^9$  conidia/g) (Table 2). All taken together, among all substrates tested, sorghum substrate supported better biomass production of isolates followed by wheat bran and millet substrates. In general, *B. bassiana* AAUMB-29 isolate achieved the highest harvested conidia (8.33 g/500 g substrate), spore concentration per gram of

spore powder ( $10.60 \times 10^9$  conidia/g), and conidia germination (95%) on sorghum substrate.

### 3.2. Bioassay Evaluation under Greenhouse Condition.

The single and combined treatments highly reduced the infestation of nymphs and adults of the whitefly *Trialeurodes vaporariorum* on two tomato varieties, namely Galilea (Table 3) and Melkashola (Table 4). The single treatment application showed that the entomopathogenic fungal isolates *B. bassiana* AAUMB-29 and *M. anisopliae* AAUDM-43 significantly reduced the infestation of *T. vaporariorum* nymphs on the Galilea variety after 2<sup>nd</sup> (65%, 84%) and 3<sup>rd</sup> sprays (65%, 80%) compared to insecticide Hunter 40 EC (90%, 97%), respectively (Table 3). The same pattern of whitefly nymphs' reduction was obtained from the Melkashola tomato variety after 2<sup>nd</sup> (65%, 84%) and 3<sup>rd</sup> sprays of *B. bassiana* AAUMB-29 and *M. anisopliae* AAUDM-43,

TABLE 5: The effects of treatments on fruit yield of the Galilea tomato variety under greenhouse.

Treatments	Galilea variety					Increased yield (%) over control
	Total fruit/plant	% Marketable fruit/plant	Total yield (g plant <sup>-1</sup> )	Marketable yield (g plant <sup>-1</sup> )	% marketable yield	
Control	8.66 <sup>c</sup>	73.09	135.00 <sup>f</sup>	71.67 <sup>e</sup>	53.09	—
AAUMB-29	8.66 <sup>c</sup>	92.38	326.66 <sup>bcd</sup>	296.67 <sup>bc</sup>	90.82	75.84
AAUDM-43	9.33 <sup>bc</sup>	89.28	280.00 <sup>cde</sup>	245.00 <sup>cd</sup>	87.50	70.75
AAUES-69	8.67 <sup>c</sup>	80.73	248.33 <sup>de</sup>	188.33 <sup>d</sup>	75.84	61.94
Neem oil	9.33 <sup>bc</sup>	89.28	218.33 <sup>e</sup>	190.00 <sup>d</sup>	87.02	62.28
Hunter 40 EC	13.33 <sup>a</sup>	100	366.67 <sup>ab</sup>	366.67 <sup>ab</sup>	100	80.45
AAUMB-29 + AAUES-69	9.34 <sup>bc</sup>	92.83	355.00 <sup>abc</sup>	330.00 <sup>ab</sup>	92.96	78.28
AAUMB-29 + Neem oil	11.66 <sup>abc</sup>	97.17	423.30 <sup>a</sup>	413.33 <sup>a</sup>	97.57	82.66
AAUMB-29 + Hunter 40 EC	12.00 <sup>ab</sup>	92.25	403.33 <sup>ab</sup>	373.33 <sup>ab</sup>	92.56	80.80
AAUES-69 + Neem oil	10.65 <sup>abc</sup>	87.89	278.33 <sup>cde</sup>	233.33 <sup>cd</sup>	83.83	69.28
AAUES-69 + Hunter 40 EC	10.66 <sup>abc</sup>	87.52	335.00 <sup>bc</sup>	296.67 <sup>bc</sup>	88.56	75.84
AAUDM-43 + Hunter 40 EC	12.33 <sup>ab</sup>	91.89	393.33 <sup>ab</sup>	370.00 <sup>ab</sup>	94.07	80.63
AAUDM-43 + Neem oil	12.00 <sup>ab</sup>	88.92	363.34 <sup>ab</sup>	351.67 <sup>ab</sup>	96.79	79.62
AAUMB-29 + Hunter 40 EC + Neem oil	12.67 <sup>a</sup>	100	386.66 <sup>ab</sup>	386.67 <sup>a</sup>	100	81.46
Average	10.66	—	317.43	—	—	75.37%

Treatment columns bearing different letters are significantly different from other treatments according to Duncan's multiple range test (DMRT) ( $P = 0.05$ ).

respectively (Table 4). The data showed that the highest adult whitefly population reduction ranged from 62% to 100% on the Galilea tomato variety and from 52% to 100% on the Melkashola tomato variety after 3<sup>rd</sup> spray of single treatments.

Interestingly, all combined botanical and chemical treatments with *B. bassiana* AAUMB-29 reduced the whitefly nymph and adult population by 64% and 60% during the 1<sup>st</sup> spray, respectively, which reached almost 100% after the 2<sup>nd</sup> and 3<sup>rd</sup> sprays. After the 3<sup>rd</sup> spray, all combined treatments reduced the nymph population by 84–100% and the adult population by 82–100% except for the co-application of *B. thuringiensis* AAUES-69 with a half dose of neem oil and Hunter 40 EC on the Galilea variety (Table 3). On the Melkashola tomato variety, the combined treatments of *M. anisopliae* AAUDM-43 + Neem oil (68%) and *B. bassiana* AAUMB-29 + Hunter 40 EC + Neem oil (78%) significantly reduced the whitefly nymph population after the 1<sup>st</sup> spray compared to 53% reduction with the application of the standard insecticide (Table 4). It is also important to note that almost all combined treatments reduced the adult whitefly population (41–84%) after the 1<sup>st</sup> spray application (Table 4), and almost all combined treatments significantly reduced both nymph and adult stages after 3<sup>rd</sup> spray (80–100%) on the Melkashola variety.

The control index (CI) values of the different treatments on whitefly nymph and adult populations were in the range of 42–100, and potency values (PV) were between 1.0 and 2.4. In most cases, the combined treatments of entomopathogens with any other components displayed higher values of CI (>70%) and PV (>1.7). All taken together, a single treatment of *B. thuringiensis* AAUES-69 and

combined application of *B. bassiana* AAUMB-29 with all IPM components displayed the lowest and highest CI and PV values, respectively.

### 3.3. Yield Evaluation of Tomato under Greenhouse Conditions.

The single and combined treatments showed variation in the number of fruits and yields of Galilea (Table 5) and Melkashola (Table 6) tomato varieties under greenhouse conditions. The number of total fruits per plant from the Galilea variety was in the range of 8.66 up to 12.67 and 218 g/plant–423 g/plant under various treatments from which 80.73–100% were marketable fruit numbers compared to the control plant (73.09%) (Table 5). The AAUMB-29 + Hunter 40 EC + Neem oil-treated plants (IPM-treated plants) displayed the highest number of fruits per plant with 100% marketable fruit number compared to the full dose pesticide-treated plants with 13.33 fruits/plant (100%). Concerning total yields of the Galilea variety, the AAUMB-29 + neem-treated plants produced the highest total yield of 423 g/plant followed by AAUMB-29 + Hunter 40 EC (403 g/plant). They also produced more than 76% marketable fruits and increased yield by 62%–82% over control (Table 5).

In relation to the Melkashola variety, the different treatments also showed variations in the number of fruit/plant (7–13) and total yield (132–375 g/plant with 76–97% marketable yield (Table 6). The AAUDM-43 + Hunter 40 EC treatment gave the highest yield (375 g/plant) followed by AAUDM-43+Neem (373 g/plant) and IPM-treated plants (352 g/plant) compared to the chemically treated ones (372 g/plant). All single and combined treatments yielded 76–97% marketable fruit yield with a 47–86% yield increase over the control plants (Table 6).

TABLE 6: The effects of treatments on the fruit yield of the Melkashola tomato variety under greenhouse condition.

Treatments	Total fruit/ plant	% Marketable fruit/plant	Melkashola variety			Increased yield (%) over control
			Total yield (g plant <sup>-1</sup> )	Marketable yield (g plant <sup>-1</sup> )	% marketable yield	
Control	8.33 <sup>de</sup>	60.02	126.67 <sup>e</sup>	53.61 <sup>e</sup>	42.32	—
AAUMB-29	8.00 <sup>de</sup>	95.88	330.00 <sup>abc</sup>	305.00 <sup>ab</sup>	92.42	82.51
AAUDM-43	9.00 <sup>cde</sup>	85.22	245.00 <sup>bcd</sup>	208.33 <sup>cd</sup>	85.03	74.40
AAUES-69	7.00 <sup>e</sup>	85.71	131.66 <sup>e</sup>	100.00 <sup>e</sup>	75.95	46.67
Neem oil	7.99 <sup>de</sup>	91.74	153.33 <sup>de</sup>	135.00 <sup>de</sup>	88.05	60.50
Hunter 40 EC	12.67 <sup>a</sup>	100	371.66 <sup>a</sup>	371.67 <sup>a</sup>	100	85.65
AAUMB-29 + AAUES-69	7.67 <sup>de</sup>	91.26	350.00 <sup>ab</sup>	321.66 <sup>a</sup>	91.90	83.42
AAUMB-29 + Neem oil	10.34 <sup>abcd</sup>	93.52	315.00 <sup>abc</sup>	293.33 <sup>ab</sup>	93.12	81.82
AAUMB-29 + Hunter 40 EC	12.33 <sup>ab</sup>	91.89	348.33 <sup>ab</sup>	335.00 <sup>a</sup>	96.17	84.08
AAUES-69 + Neem oil	11.34 <sup>abc</sup>	85.27	225.00 <sup>cde</sup>	171.66 <sup>cde</sup>	76	68.93
AAUES-69 + Hunter 40 EC	9.34 <sup>bcde</sup>	92.83	260.00 <sup>abcd</sup>	230.00 <sup>bc</sup>	88.46	76.81
AAUDM-43 + Hunter 40 EC	13.00 <sup>a</sup>	92.31	375.00 <sup>a</sup>	332.00 <sup>a</sup>	88.14	83.93
AAUDM-43 +Neem oil	11.67 <sup>abc</sup>	91.43	373.33 <sup>a</sup>	326.67 <sup>a</sup>	87.11	83.67
AAUMB-29 + Hunter 40 EC + Neem oil	13.33 <sup>a</sup>	97.52	351.66 <sup>ab</sup>	341.67 <sup>a</sup>	97.16	84.39
Average	10.14	—	282.62	—	—	76.68%

Treatment columns bearing different letters are significantly different from other treatments according to Duncan's multiple range test (DMRT) ( $P = 0.05$ ).

TABLE 7: Field performance of bioinsecticides for the reduction of whiteflies on the Galilea tomato variety after 3<sup>rd</sup> and 4<sup>th</sup> spray application.

Treatment	% reduction of whiteflies				Control index	Potency level
	3 <sup>rd</sup> spray application		4 <sup>th</sup> spray application			
	5DAS	10DAS	5DAS	10DAS		
AAUMB-29	47.87 <sup>d</sup>	60.09 <sup>de</sup>	66.74 <sup>c</sup>	79.31 <sup>ab</sup>	78.91	1.06
AAUDM-43	48.75 <sup>d</sup>	54.44 <sup>e</sup>	67.93 <sup>bc</sup>	74.37 <sup>b</sup>	74.73	1.00
Hunter 40 EC	64.56 <sup>ab</sup>	69.63 <sup>bc</sup>	78.08 <sup>abc</sup>	88.10 <sup>ab</sup>	88.57	1.19
AAUMB-29 + Hunter 40 EC	58.85 <sup>bc</sup>	70.96 <sup>bc</sup>	82.88 <sup>ab</sup>	90.19 <sup>a</sup>	90.38	1.21
AAUMB-29 + Neem oil	63.07 <sup>ab</sup>	75.84 <sup>ab</sup>	78.07 <sup>abc</sup>	87.30 <sup>ab</sup>	86.12	1.15
AAUDM-43 + Hunter 40 EC	51.74 <sup>cd</sup>	64.49 <sup>cd</sup>	81.17 <sup>abc</sup>	78.03 <sup>ab</sup>	85.60	1.14
AAUMB-29 + Hunter 40 EC + Neem oil	68.89 <sup>a</sup>	80.11 <sup>a</sup>	85.07 <sup>a</sup>	91.93 <sup>a</sup>	100	1.30
SE±	1.54	1.84	2.06	1.89	—	—
F-value	5.10	8.61	1.99	1.54	—	—
P value	0.0002	<0.0001	0.065	0.17	—	—
CV%	17.34	17.63	17.37	14.59	—	—

DAS, days after spray. Treatment columns bearing different letters are significantly different from other treatments according to Duncan's multiple range test (DMRT) ( $P = 0.05$ ).

**3.4. Field Evaluation.** The bioinsecticidal treatments displayed higher reduction percentages of whiteflies than untreated control on tomato plants after 5 and 10 days of 1<sup>st</sup> and 2<sup>nd</sup> spray applications (data not shown). These treatments effectively reduced the adult whiteflies on the Galilea tomato variety with control index (CI) (75–100) and PV (1.0–1.30). Thus, the IPM components combined with AAUMB-29 showed a CI of 100% followed by *B. bassiana* AAUMB-29 + Hunter 40 EC and Hunter 40 EC with almost a CI of 90. Under the circumstances, the single treatments with entomopathogenic fungal species showed a lower CI of <80. Based on PV, the IPM treatment displayed a PV of 1.30, showing that its effectiveness was almost 30% higher than

the individual treatments, indicating that the IMP packages performed better than the single treatments with the entomopathogens (Table 7).

**3.5. The Effects of Biopesticides on the Yield of Tomato under Field Conditions.** Bioinsecticide treatments indicated significant variation on marketable ( $F = 49.07$ ;  $DF = 12, 35$ ;  $P < 0.0001$ ), unmarketable ( $F = 19.98$ ;  $DF = 12, 35$ ;  $P < 0.0001$ ), and total ( $F = 19.98$ ;  $DF = 12, 35$ ;  $P < 0.0001$ ) number of tomato per plant in field trial (Table 8).

The tomato plants treated with single and combined treatments revealed a total number of fruits range from



TABLE 8: The effects of bioinsecticides on marketable, unmarketable, and total fruit yield (kg/plant) of tomato.

Treatment	Fruit number per plant			Fruit yield (kg plant <sup>-1</sup> )			Increased yield (%) over control
	Total	Marketable	% marketable	Total	Marketable	% marketable	
Control	39.16 <sup>c</sup>	19.33 <sup>f</sup>	49.36	2.73 <sup>b</sup>	1.58 <sup>c</sup>	57.88	—
AAUMB-29	60.00 <sup>cd</sup>	54.17 <sup>d</sup>	90.28	3.37 <sup>b</sup>	3.00 <sup>bc</sup>	89.02	47.33
AAUDM-43	54.33 <sup>d</sup>	47.50 <sup>e</sup>	87.43	3.08 <sup>b</sup>	2.60 <sup>bc</sup>	84.42	39.23
Hunter 40 EC	80.50 <sup>a</sup>	78.67 <sup>a</sup>	97.73	6.45 <sup>a</sup>	6.40 <sup>a</sup>	99.22	75.31
AAUMB-29 + Hunter 40 EC	64.00 <sup>bc</sup>	60.50 <sup>c</sup>	94.53	6.01 <sup>a</sup>	5.93 <sup>a</sup>	98.67	73.35
AAUMB-29 + Neem oil	68.17 <sup>b</sup>	63.33 <sup>bc</sup>	92.90	3.54 <sup>b</sup>	3.31 <sup>b</sup>	93.50	52.26
AAUDM-43 + Hunter 40 EC	67.50 <sup>b</sup>	65.17 <sup>bc</sup>	96.55	4.19 <sup>b</sup>	4.08 <sup>b</sup>	97.37	61.27
AAUMB-29 + Hunter 40 EC + Neem oil	69.17 <sup>b</sup>	68.50 <sup>b</sup>	99.03	7.04 <sup>a</sup>	7.00 <sup>a</sup>	99.43	77.43
SE±	1.86	2.54	—	0.30	0.33	—	—
CV (%)	20.45	30.87	—	45.93	53.30	—	—

SE, standard error; CV, coefficient variance. Treatment columns bearing different letters are significantly different from other treatments according to Duncan's multiple range test (DMRT) ( $P = 0.05$ ).

TABLE 9: The effects of bioinsecticides on marketable, unmarketable, and total fruit yield (ton/ha) of tomato.

Treatment	Fruit yield (t ha <sup>-1</sup> )			Increased yield (%) over control
	Total	Marketable	% marketable	
Control (water)	25.83 <sup>c</sup>	15.83 <sup>c</sup>	61.29	—
AAUMB-29	33.67 <sup>bc</sup>	30.00 <sup>bc</sup>	89.10	47.23
AAUDM-43	31.17 <sup>bc</sup>	26.33 <sup>bc</sup>	84.47	39.88
Hunter 40 EC	60.13 <sup>a</sup>	59.33 <sup>a</sup>	98.67	73.32
AAUMB-29 + Hunter 40 EC	64.50 <sup>a</sup>	64.00 <sup>a</sup>	99.22	75.27
AAUMB-29 + Neem oil	35.42 <sup>bc</sup>	33.17 <sup>b</sup>	93.65	52.28
AAUDM-43 + Hunter 40 EC	41.88 <sup>b</sup>	40.83 <sup>b</sup>	97.49	61.23
AAUMB-29 + Hunter 40 EC + Neem oil	70.42 <sup>a</sup>	70.00 <sup>a</sup>	99.40	77.39
SE±	3.05	3.26	—	—
CV (%)	46.65	53.26	—	—

SE, standard error; CV, coefficient variance. Treatment columns bearing different letters are significantly different from other treatments according to Duncan's multiple range test (DMRT) ( $P = 0.05$ ).

54–81 fruits/plant, fruit yield from 2.6–7.0 kg/plant and percentage of marketable fruit yield from 84–99%. The plants treated with the full dose of chemical insecticide (Hunter 40 EC) showed a number of 78 fruits/plant and 6.4kg/plant fruit yield. The IPM treatment (AAUMB-29 + Hunter 40 EC + Neem oil) produced the highest marketable fruit number (68/plant) and fruit yield (7.0 kg/plant) compared to other IPM packages (Table 8).

When the total fruit yield was computed as t/ha, different treatments showed significant variations in marketable ( $F = 11.70$ ;  $DF = 12, 35$ ;  $P < 0.0001$ ) and total fruit yield ( $F = 8.59$ ;  $DF = 12, 35$ ;  $P < 0.0001$ ) (Table 9). The result showed that the treated plants gave marketable yield within the range of 26 t/ha (AAUDM-43) to 70 t/ha (AAUMB-29 + Hunter 40 EC + Neem oil), which was the highest followed by treatment with AAUMB-29 + Hunter 40 EC with 64 t/ha. The inter-treatment trend showed that the combined treatments with the chemical increased yield by 1.5–2.5 more than the individual treatments. It is interesting to note that the most effective IPM treatment also showed a slightly better yield (1.2 times more fruit yield) than the chemically treated plants indicating that the entomopathogens may incur additional advantages in their PGP properties to enhance productivity other than suppressing the pest.

#### 4. Discussion

Mass multiplication study revealed that entomopathogenic fungus *B. bassiana* AAUMB-29 achieved the highest harvested conidia (8.33 g/500 g substrate), spore concentration per gram spore powder ( $10.60 \times 10^9$  conidia/g), and conidia germination (95%) on sorghum substrate. This study emphasized that sorghum substrate achieved the maximum spore harvesting, spore production, and germination compared to wheat bran and millet substrates. Similar studies showed that sorghum substrate was supported by the considerable spore production (8.48 g/kg), spore concentration ( $4.80 \times 10^{10}$  spore/kg), and germination rate (89%) of *B. bassiana* [54]. Moreover, [55] also attained the 0.42 g/100 g substrate biomass production and  $10.24 \times 10^8$  spore/g spore count of *B. bassiana* from sorghum substrate. The large multiplication of *B. bassiana* and *M. anisopliae* on sorghum substrate may be associated with a rich nutritional composition that enhanced the growth and sporulation of fungal isolates. This implicated that sorghum could be chosen for the mass production of entomopathogenic fungal isolates.

This study demonstrated that the foliar spray applications of single and combined treatments with entomopathogenic fungi with chemical insecticide and neem extracts

highly reduced the infestation of whiteflies as compared to control treatments under greenhouse and field trials. It is also established that the combination of entomopathogenic fungi with sublethal concentrations of insecticides as integrated pest management (IPM) options is a new strategy for effective pest control in crop protection and sustainable agriculture [56].

Under the greenhouse study, most of the biopesticide treatments resulted in 84 to 100% reduction of nymphs and 80 to 100% reduction of adults of *T. vaporariorum* on both Galilea and Melkashola tomato varieties after 3<sup>rd</sup> spray application. The result was similar to the application of different strains of *B. bassiana*, *M. anisopliae*, and neem extract that reduced 85% to 92% of nymphs from the Srijana tomato variety in Nepal [57] and 50 to 90% reduction in adult whitefly populations on two tomato varieties (Shifa and Savera) in Egypt under greenhouse conditions [58]. Furthermore, Ghongade and Sangha (2021) reported that the application of entomopathogenic fungal isolates of *B. bassiana*, *M. anisopliae*, and *L. lecanii* ( $1 \times 10^9$  CFU/ml), neem product (Neem Baan 1% W/W), and chemical pesticide (Malathion 50EC with 4 ml/l) significantly reduced nymphs (82 to 99%) and adults (57 to 98%) of whiteflies on cucumber under greenhouse condition after 3<sup>rd</sup> spray application in India [59].

The combination of entomopathogenic fungi *B. bassiana* and *M. anisopliae* ( $1 \times 10^8$  conidia/ml) with a sublethal dose of neem oil (0.5%) and Hunter 40 EC insecticide (1.25 ml/L) caused a higher percentage reduction of whitefly *T. vaporariorum* than any one of the individuals used alone. In addition, another study showed that the combined application of entomopathogenic fungus *B. bassiana* ( $1 \times 10^7$  conidia/ml) with a sublethal dose of neem product (Azadirachtin) (0.5%) yielded higher mortality of 97% against whitefly *B. tabaci* compared to individual treatment mortality of 77% and 70%, respectively, under greenhouse condition [60]. This high virulence outcome may be due to the combined effects of biopesticides, which increase the susceptibility of target insect pests [61].

The foliar application of entomopathogenic fungal strains *B. bassiana* and *M. anisopliae* alone and integrated with neem extract and chemical pesticide significantly increased the yield of tomato by reducing whitefly infestation in the greenhouse trial. Thus, the treated plants showed variation in the yield of Galilea and Melkashola tomato varieties ranging from 132 to 423 g/plant (Tables 4 and 5). Table 4 shows that the AAUMB-29 + Neem oil treatment induced the highest yield ( $423.3 \text{ g}\cdot\text{plant}^{-1}$ ) followed by AAUMB-29 + Hunter 40 EC ( $403 \text{ g}\cdot\text{plant}^{-1}$ ) on the Galilea tomato variety. Table 5 shows that the AAUDM-43 + Hunter 40 EC induced the highest yield ( $375 \text{ g}\cdot\text{plant}^{-1}$ ) followed by AAUDM-43 + Neem oil ( $373 \text{ g}\cdot\text{plant}^{-1}$ ) on the Melkashola tomato variety. These treatments also produced 87–97% marketable fruits and increased yield by 80%–84% over control. The yield gain in this study was slightly different from the yield variation of different tomato cultivars ( $307\text{--}564 \text{ g}\cdot\text{plant}^{-1}$ ) [62] and Roma variety ( $197\text{--}739 \text{ g}\cdot\text{plant}^{-1}$ ) [63] under different integrated pest management options (combination of *B. bassiana*, *M. anisopliae*, plant extracts,

and chemical insecticides) in the greenhouse conditions. This yield variation in tomato varieties could be due to differences in their genetic makeup, resistance to biotic and abiotic constraints, and pest control efficiencies.

In the field study, the percent reduction of the whitefly population varied from 74 to 92% on the Galilea tomato variety after 10 days of the 4<sup>th</sup> spray. The result showed that *B. bassiana* AAUMB-29 + Hunter 40 EC + Neem oil significantly displayed a higher percentage reduction of whiteflies (91.93%) followed by a 90% reduction obtained from treatment by *B. bassiana* AAUMB-29 + Hunter 40 EC compared to other treatments. Interestingly, the integration of IPM components with *B. bassiana* AAUMB-29 showed a control index value of 100% followed by *B. bassiana* AAUMB-29 + Hunter 40 EC (90%). This result was similar to the finding that showed the integrated application of *L. lecanii* ( $2 \times 10^9$  spores/ml) with neem oil (0.5%) recorded the higher whitefly population reduction (71.57%) on Okra [60, 64] and the combined application of *B. bassiana* ( $1 \times 10^9$  spores/ml) with half-field recommended doses of spinosad (0.25 ml/l) caused up to 89.35% reduction of whitefly populations over control treatments from cucumber [65] under field conditions.

The field study also revealed that combined spray application of entomopathogenic fungi *B. bassiana* and *M. anisopliae* with a sublethal dose of neem oil (0.5%) and Hunter 40 EC (1.25 ml/L) achieved 0.98 to 1.24-fold higher whitefly population reduction compared to single treatment applications. In addition, the study by [66] stated that the combined evaluation of *B. bassiana* and *M. anisopliae* with various insecticides showed a 1.05- to 1.42-fold increase in virulence over the sole treatment application. This indicated that the consortium of microbial entomopathogens with sublethal doses of insecticides achieved the highest percentage mortality of insect pests compared to individual treatment applications. This may be due to the attacking effects of combined treatments independently at the different points of susceptibility in the target insect pests.

The tomato yield evaluation under field conditions indicated that the fruit yield of tomato was significantly higher in all single and combined treatments ( $31.17$  to  $70.42 \text{ t}\cdot\text{ha}^{-1}$ ) compared to the control treatment ( $25.83 \text{ t}\cdot\text{ha}^{-1}$ ). Thus, the integrated treatment of *B. bassiana* AAUMB-29 + Hunter 40 EC + Neem oil produced the highest fruit yield of  $70.42 \text{ t}\cdot\text{ha}^{-1}$  followed by *B. bassiana* AAUMB-29 + Hunter 40 EC ( $64.50 \text{ t}\cdot\text{ha}^{-1}$ ) on the Galilea tomato variety. This better yield of tomato could be due to the low level of insect infestation as a result of the combined application of the control agents with their multiple modes of action. A study also showed that the sequential application of chemical insecticides (Coragen and Emperor) and commercial formulation of Azadirachtin (Nimbecidine 3.5% EC) resulted in higher yields on super stain B tomato variety ( $28.25 \text{ t}/\text{fed}$ ) than uncontrolled treatment ( $8.4 \text{ t}/\text{fed}$ ) [67]. By the same token, the combined application of entomopathogenic fungi *B. bassiana* with *B. subtilis* increased the yield of tomato cultivar PKM1 ( $36.19\text{--}39.21 \text{ t}\cdot\text{ha}^{-1}$ ) by effectively reducing insect pests than individual treatment and untreated control

under field conditions [68]. These yields are much lower than the tomato yield found in this study. This may be due to variation in tomato cultivars, geographical location, exposure time, and concentration of biological control agents.

The data also showed that the marketable tomato fruit yield was from 39.88 to 77.39%, with the highest 77.39% displayed by IPM treatments (*B. bassiana* AAUMB-29 + Hunter 40 EC + Neem oil) over control (Table 8). This result was similar to the marketable fruit yield of 33.74 to 78.01% increase over control on L-37 tomato cultivar and the highest yield attained by the integrated treatment application [69]. In addition, the single-based application of neem leaf extract (2.0 ml/L) increased 77% yield over control on BARI tomato-09 tomato variety [70], whereas the *B. bassiana* (28.32%) and *M. anisopliae* (25.86%) increased Sonali cultivar of tomato yield over control [71].

## 5. Conclusions

A mass production study confirmed that sorghum substrate was the most suitable for high multiplication of *B. bassiana* and *M. anisopliae* isolates compared to wheat bran and millet substrates. The *B. bassiana* strain AAUMB-29 achieved the highest conidial yield, spore concentration, and conidia germination on sorghum substrate. The foliar spray applications of different single and combined bioinsecticide treatments have significantly reduced the population of whitefly as compared to control treatments under greenhouse and field conditions. The combination of entomopathogenic fungi *B. bassiana* and *M. anisopliae* with sublethal concentration of neem oil and chemical insecticide was an effective pest management option against whitefly species. The combined treatment application with the half dose of chemical insecticide increased tomato yield by 1.5–2.5 folds than the individual treatments. Among treatments, the integrated spray application of *B. bassiana* AAUMB-29 + Hunter 40 EC + Neem oil was found to be the most effective in lowering whitefly infestation and increasing marketable yields. Further validation under various agro-ecological conditions is required to determine the optimization and practicability of this integrated treatment application for the control of both sucking and chewing insect pests under field conditions.

## Data Availability

All data that support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare no conflicts of interest.

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