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Integrated Management for Fusarium Oxysporum f. sp. Capsici of Capsicum using Biological Control and Fungicide

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HIGHLIGHTS

- Capsicum is a major vegetable, grown for high-value spices and industrial inputs.
- Fusarium wilt caused by Fusarium oxysporum is a major constraint of Capsicum species.
- Integrated disease management is viable, feasible, and most practical.
- The integrated approach reduced the wilt by 93%, and increased yield ha-1 by 406.9%.

Abstract

Fusarium wilt caused by Fusarium oxysporum f. sp. capsici is a major constraint of Capsicum species. This study aims to develop an integrated management approach for Fusarium wilt in Capsicum. The study was conducted in two consecutive Winter Pepper growing seasons. A randomized complete block design with three replicates resulted in eight treatments. The soil near the stem region of the pepper was drenched with a mixture of 10 g of Trichoderma harzianum powder in a liter of water, and 500 ml was applied to each plant immediately after transplanting. Pepper seedling roots were dipped in a Metalaxyl-M mixture of Metalaxyl-M and Mancozeb for 30 minutes during transplanting. Wilt incidence was recorded at 10-day intervals using a scale and converted to the percent disease index. All data were analyzed using SAS software. The combined effect of fungicide and bioagent significantly reduced wilt disease by 93% for the Bako local and 91.2% for Melka Zala in the first season, 75.3% for the Bako local cultivar, and 79.9% for the Melka Zala variety in the second season. As a result, marketable yield per hectare increased by 187.6% in the first and 406.9% in the second season. This study demonstrates that integrating T. harzianum, fungicide, and Capsicum cultivars can effectively manage the disease. This suggests the potential for large-scale formulation and commercialization of this bioagent. However, additional studies should be conducted across locations to validate the current findings. The combined effects of Trichoderma, effective bacterial bioagents, and compatible fungicides against Fusarium wilt under field conditions are also appreciated.

Keywords: Biocontrol, Capsicum, Fungicide, Fusarium Wilt, Trichoderma Harzianum.

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1. Introduction

Pepper (Capsicum spp.) is a major vegetable crop in Eastern Africa, where it is primarily grown by small-scale farmers, including in Ethiopia. The genus Capsicum contains over 30 species, five of which are domesticated and mainly grown for various purposes (C. annuum, C. frutescens, C. chinense, C. baccatum, and C. pubescens) (Tripodi and Kumar, 2019). In Ethiopia, the pepper crop is used as a high-value vegetable and spice, as well as in dishes, fresh "karia" and "berbere" processed products. Locally processed (coloring agent) and exported in the form of oleoresin (red pigment) and ground powder in various forms (Kahsay, 2018). Pepper is becoming increasingly important, and high-value spices and industrial raw materials for producing oleoresin commodities have been prioritized in vegetable research in Ethiopia (Dessie et al. 2019). The average dry (red) and green (fresh) hot pepper production in Ethiopia is 1.8 t/ha and 5.9 t/ha, respectively, which is significantly lower than the global average (CSA 2020). In 2020, Ethiopia's total cultivable area for green and red pepper was 185,872.63 ha, with an estimated total production of 380,318.87 metric tons. Surprisingly, this data shows that pepper covers 77.91% of all vegetable areas in Ethiopia (CSA 2020).

Despite the significance of the pepper crop in Ethiopia, disease-related yield failure is common, and farmers are frequently forced to stop production due to high disease pressure in the field (Mihajlovic et al. 2017; Mohammed et al. 2021), resulting in losses of 25-90% in some areas of the country, with up to 100%yield losses recorded due to different diseases in a conducive environment. Climate change and the current situation in the country indicate that fungal pathogens are devastating the crops at various growth stages, from nursery to harvesting, and significant yield loss is observed, limiting smallholder farmers' ability to produce the crops. Wilt-causing fungal pathogens were reported to be the leading problem of pepper crops, causing 86.4% or above in Ethiopia (Mohammed et al. 2021). Recent efforts have concentrated on developing economically safe, long-lasting, and effective biocontrol methods for Fusarium wilt disease management (Loganathan et al. 2013).

Among the fungi that are effective biocontrol agents, Trichoderma species are well-known as model organisms due to their ability to multiply, spread, isolate, and culture easily (Yedidia et al. 1999). The use of Trichoderma for Fusarium wilt biocontrol is not only safe for farmers and consumers, but it is also an environmentally friendly option. Trichoderma employs several biocontrol mechanisms to inhibit the growth of a variety of phytopathogenic organisms, including bacteria, nematodes, and fungi (Fusarium, Pythium, Phytophthora, Botrytis, and Rhizoctonia), either directly (e.g., hyper parasitism, competition for nutrients and space, and antibiosis) or indirectly by enhancing their ability to take up nutrients, increasing stress tolerance, promoting plant growth, bioremediation of the contaminated rhizosphere, and producing several secondary metabolites, enzymes, and pathogenesis-related (PR) proteins (Aydin 2015; Kredics et al. 2003; Kumar 2013). Several studies have shown that T. harzianum is an effective fungal bioagent against Fusarium oxysporum f. sp. capsici (Abdelaziz et al. 2023; Al-aamel and Al-maliky 2023; Al-Morad et al. 2018; De Corato 2020; Girma 2022).

Fungicides were used as seed treatment for seed-borne diseases because they eliminate seed-borne inocula, as well as soil treatment and/or seedling root dipping to suppress soil-borne pathogens such as Fusarium wilt (Attri et al. 2019; Suneeta et al. 2017). Besides, research has proven that Metalaxyl-M + Mancozeb fungicide, which contains systemic and preventative active ingredients, was compatible with Trichoderma species, including T. harzianum (Np and Km, 2020; Suneeta et al. 2017). They reported that among systemic fungicides, Metalaxyl-M + Mancozeb was the most compatible at all the concentrations evaluated in the study. Apart from the compatibility test, numerous investigations have verified that T. harzianum and Metalaxyl-M + Mancozeb, when used independently with a full dose (as per the recommendation of the manufacturer), are capable of managing the majority of soil-borne and/or seed-borne fungal plant pathogens, such as Fusarium oxysporum f. sp. capsici (Al-aamel and Al-maliky 2023; Al-Morad et al. 2018; Attri et al. 2019; Divya et al. 2023; El-kazzaz et al. 2022; Hasna 2020; Mannai et al. 2018).

Fusarium wilt, which is caused by Fusarium species in Capsicum, has become widespread and highly significant throughout Ethiopia in the past three years. There are national and annual variations in the prevalence and severity patterns of this disease in pepper because of the impact of climatic changes, including soil temperature, relative humidity, and precipitation. No cultivar, including hybrids, has been proven to be resistant or moderately resistant to Fusarium wilt, according to the research that has been reported so far in the country. The Melka Zala hybrid variety is high-yielding and widely grown (Teferi et al., 2015). A limited study revealed the response of this variety against the devastating Fusarium wilt disease epidemic outbreak in this disease hotspot area.

Many efforts have been made to quantify the disease in major pepper-growing regions in the field. Due to the outbreak of Fusarium wilt epidemics, small-scale farmers and other pepper producers are forced to use only synthetic fungicides to manage Fusarium wilt on Capsicum species. Using just synthetic pesticides to treat plant diseases in general and Fusarium wilt in particular has disadvantages, such as short-term effectiveness due to pesticide resistance and environmental or soil hazards (Corkley et al. 2022). Thus, it is critical to incorporate biological agents along with synthetic fungicides and develop integrated disease management systems, rather than be dependent solely on synthetic fungicides.

On the other hand, Trichoderma is one of the many potential microorganisms that could be employed for biological protection and plant growth enhancement. Trichoderma species are some of the most active competitors of Fusarium fungi, exhibiting antagonistic tendencies against fungal infections that cause diseases in Capsicum crops. Most published information on the use of Trichoderma against Fusarium diseases came from research based on dual cultures that were conducted in vitro. Still, the documented information on the biocontrol potential of this pathogen using Trichoderma fungi at the plant level in the field conditions is minimal, particularly in Ethiopia.

More than 8 Trichoderma species were molecularly identified and maintained in the Ethiopian Institute of Agricultural Research (EIAR) at Ambo Agricultural Research Center's (AARC) Mycology Laboratory. Because of AARC's excellence and mandate of coordinating plant pathology programs across the country, these Trichoderma species have been used in a variety of plant disease management applications nationwide. T. harzianum, T. viride, and T. asperellum have been effective and potential bioagents against diseases caused by such Fusarium species in diverse environments involving various crops, including Capsicum (Aliyi et al. 2018; Gabrekiristos et al., 2021; Menge et al. 2020). The center has been developing a formulation process for Trichoderma isolates using various organic and inorganic carriers, either through solid or liquid fermentation technology. Innovative scientific studies and viable disease management options for Fusarium wilt of Capsicum species must support such an initiative. In this regard, there is a lack of studies that revealed the potential of T. harzianum in integrated pepper wilt management in field conditions that contribute to the sustainable production of Capsicum.

A single technique for managing Fusarium wilt is challenging and inefficient; thus, an integrated approach is the best, feasible, and most practical choice. The country must investigate the development of management systems that consider agricultural ecology. Thus, the objective of this study was to develop an integrated management approach for the Fusarium wilt of Capsicum species.

2. Materials and Methods

2.1. Study Area and Experimental Design

The study was conducted on the experimental site of Ambo Agricultural Research Center (AARC), which is found between 8°57' 58'' N latitude and 37° 51' 33'' E longitudes and at an altitude of 2175 meters above sea level. The annual average temperature and rainfall were 27.54°C and 1077.68 mm, respectively (AARC, Metrology Station Database). It was conducted in experimental plots where, before the year, pepper had been devastated by Fusarium wilt caused by Fusarium oxysporum f. sp. capsici infection on the research station, marked as a hotspot for Fusarium wilt of pepper. The research was conducted over two consecutive pepper growing seasons in 2020-2021. This season is known as the Winter Season (December to April), the dry season in the study area. It was conducted at the same site (hotspots of Fusarium wilt in Capsicum) during the two experimental seasons using irrigation.

A randomized complete block design (RCBD) with three replicates was used, resulting in a total of eight treatments. Pepper cultivars (Bako local and Melaka Zala hybrid), and Management methods (Fungicide, bioagent, Fungicide + bioagent, control untreated), and treatments (a total of eight treatments) were arranged in an RCBD factorial experimental design. Details of the treatment descriptions and numbers are presented in Table 1 of the supplementary materials.

2.2. *Experimental Materials and Treatments*

Seeds of the Capsicum species were collected from Bako and Melkassa Agricultural Research Centers. The seeds of the cultivars were sown on the seedbed at AARC. Pepper seedlings of a local cultivar and a hybrid variety, two months old, were transplanted into the permanent experimental field. The pepper seedling roots were dipped in Metalaxyl-M + Mancozeb fungicide and waited for 30 minutes. The dosage of the fungicide was halved from the manufacturer's recommendation. The soil was drenched around the rhizosphere, near the stem region of seedlings, with 10 g of formulated T. harzianum powder mixed in a liter of water, and 500 ml was given to each plant immediately after transplanting seedlings. Twelve (12) pepper seedlings were planted in each of the five rows, comprising a total of 60 plants per experimental unit. Space between rows and plants was 70cm and 40cm, respectively, and the plot size was 4.8m x 3.5m. The fungicide Ridomil Gold MZ 68 WG with an active ingredient of 40 g/kg Metalaxyl-M and 640 g/kg Mancozeb was purchased from the market, and it was manufactured by Syngenta Agro Services Agency in Ethiopia. The T. harzianum used in this study was previously isolated from Ethiopian indigenous soil samples, molecularly identified, and has been maintained in the EIAR/Ambo Agricultural Research Center's Mycology Laboratory. T. harzianum isolate was initiated on Potato Dextrose Agar (PDA) and allowed to sporulate. Then it was harvested from the PDA petri plate, diluted in sterile distilled water, and adjusted to a suspension containing conidia to 1 × 10^8 spores/ml of sterile distilled water using a hemocytometer. Then it was inoculated on flasks and/or beakers containing sterilized sorghum seeds and sporulated for three weeks. After the spores fully grew in all flasks, it was air-dried on a tray and pulverized into powder (Figure 1C). Carboxymethyl cellulose (CMC), which is used as a sticker, is mixed with ground sorghum and formulated as a powder form so that the final formulation contains 1 × 10^8 CFU/g as per the standard (Figure 1) (Komala et al. 2019).

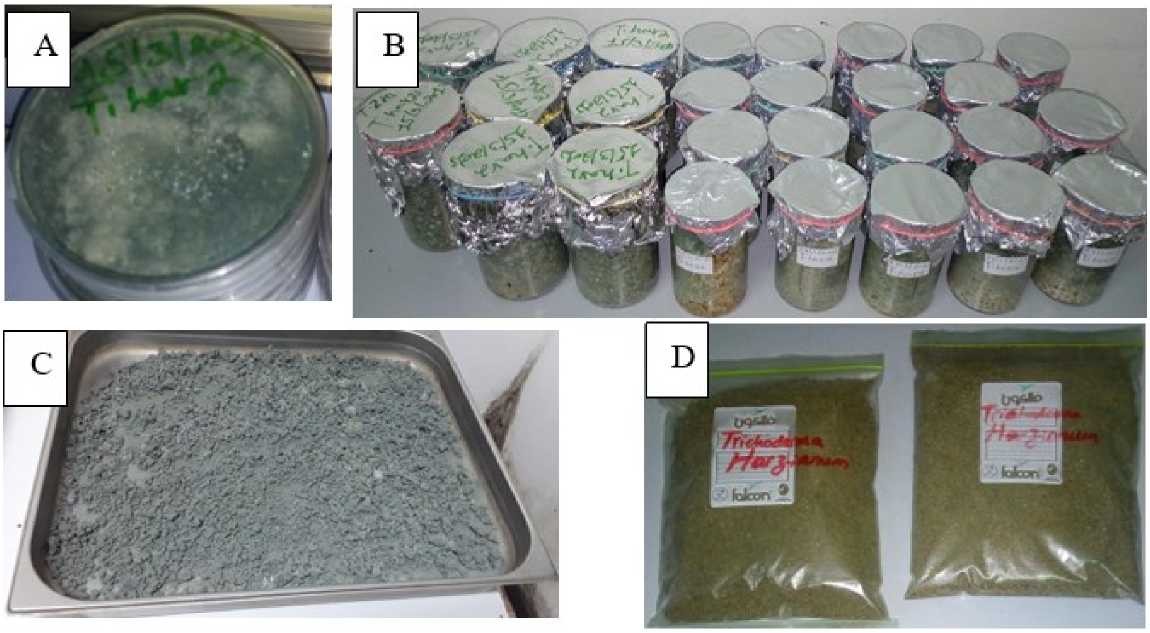

Figure 1. Trichoderma harzianum mass production and formulation in laboratory: (A) Spores on a petri plate with PDA, (B) Beaker containing sterilized sorghum seeds with spores, (C) Air-dried on a tray, and (D) ground into powder and packed in a plastic bag.

2.3. *Isolation and Identification of Fusarium from Infected Capsicum Plant*

Potato Dextrose Agar medium was used to isolate Fusarium oxysporum from plants with typical symptoms of Fusarium wilt (dark, necrotic lesions, and the stems and roots turned brown and rotted). Segments of stem and root samples with the characteristic lesions were thoroughly washed under running tap water, cut into small pieces (2mm x 2mm size) with half healthy and half diseased tissue, and surface sterilized with a 2% NaOCl for 2 minutes for 30 to 60 seconds based on the surface roughness before washing three times in sterile distilled water. The samples were dried on filter paper in a laminar flow cabinet before placing them on potato dextrose agar (PDA) plates (9 cm diameter), then treated with streptomycin (30 g/l) to avoid bacterial contamination and incubated at 25 ± 2°C in an incubator.

The single spore technique was adopted from Leslie and Summerell (2006) to obtain a pure culture. A small fungal plug (4 mm culture) was cut with a sterile scalpel, placed on a petri plate containing PDA medium, and kept under a dark condition at 25°C in an incubator for 12 days. Using a sterilized loop, very small amounts of conidia will be suspended in a 1500 µL Eppendorf tube containing sterilized water for 1 to 3 min and shaken gently on the vortex several times. A 100 µL of the spore suspension was spread onto a Water Agar (2%) medium 9 cm plate using a glass rod spreader and incubated for 24-48 hours at 20°C under 12 h dark and 12 h light conditions. A tiny scalpel was used to label and find germinating spores under a compound microscope. Spore or conidia was precisely marked and cut off under a compound microscope.

Morphological identification of Fusarium oxysporum was carried out based on macro- and micro-conidial morphology, mycelial characteristics, and pigmentation on PDA medium using keys in the Fusarium Laboratory Manuals (Burgess et al., 1994; Leslie and Summerell, 2006).

2.4. *Data Collection and Analysis*

In the first experimental season, disease data and marketable and unmarketable yield were recorded. Still, in the second experimental season, yield-related data such as pod count per plant and marketable yield were recorded in addition to disease measurement data. The incidence and/or severity of wilt was recorded at 10-day intervals up to maturity of the crop, starting from disease onset, converted to a percent severity index. The number and weight of fruits for each treatment were recorded from three central rows. Every agronomic package recommended for the pepper crop was applied consistently to every plot. The symptoms determined for scoring were leaf wilting, chlorosis, and/or stunting. Score 0 = no symptom, 1 = lower height compared to control, 2 = lower height and chlorosis, 3 = 10% chlorosis and/or 10% wilting, 4 = 11-25% wilting, 5 = 26-50% wilting, 6 = 51-100% wilting and dead. The Disease Severity Index (DSI) or present disease index was determined according to the following formula (Elmer and McGovern, 2004; Wongpia and Lomthaisong, 2010).

$$\text{PSI} = \frac{\Sigma(\text{Disease scale} * \text{Number of plants infected})}{(\text{Highest scale} * \text{Total number of plants})} * 100 \quad (1)$$

Where, PSI, Percent severity index (=Wilt incidence)

Both the preventive and/or control effects were calculated using the formula explained by (Song et al., 2004).

$$\text{PDC} = \frac{\text{PSI of the untreated control} - \text{PSI of treated}}{\text{PSI of the untreated control}} * 100 \quad (2)$$

Where PDC=percent disease control, PSI = Percent severity index.

The collected data were subjected to the mixed effect using the PROC MIXED statement to determine the treatment effect, combining fixed and random effects in a model, using SAS software version 9.4® (SAS, 2019). All the disease reactions for each treatment combination were evaluated by averaging data from the individual and the terminal or final disease scores in both seasons. Pairwise comparisons of least squares means

(LSMEANS) and the least significant difference (LSD) values were used to separate differences among treatment combinations' means at a 5% probability level by adjusting to Tukey's test.

3. Results

3.1. Characterization of Fusarium oxysporum f. sp. capsici

The characterization of the wilt causal agent was performed to ensure the inoculum in the pepper wilt-infected site of study. The Fusarium oxysporum that causes Fusarium wilt was isolated and identified from the infected pepper plants in the study area before this study. It was then morphologically characterized in the laboratory using a compound microscope at 40x magnification to confirm the inoculum in the sick plot. The macroconidia were short to medium in length, slightly curved to straight, thin-walled, and typically 3-5-septate (Figure 2E). The apical cell was small and slightly hooked. The basal cell was notched (Figure 2E and G). Conidia were developed abundantly in false heads on short monophialides (Figure 2F). Colony morphology on PDA varies among isolates from white to pinkish. Mycelia were abundant, ranging in color from pinkish to purple pigments (Figure 2C and D). Some isolates developed abundant light orange or pale violet macroconidia in the center of the spore masses. Chlamydospores in couples and in a chain of two units (Figure 2G).

3.2. First-year 2020 pepper growing season

Trichoderma species bioagents and fungicides against Fusarium oxysporum f. sp. capsici were assessed and screened in the lab before this study. Effective and promising fungicide (Redomil Gold, a.i. Metalaxyl-M + Mancozeb), and bioagent (T. harzianum) from in-vitro level were selected for field study. Thus, two cultivars, four control methods, and cultivar x control methods were integrated to manage the pepper wilt intensity. The mixed effect analysis using proc mixed indicated that there was a considerable difference among cultivars, Fusarium control methods, and cultivar x Fusarium control method interaction on PSI and yield t ha-1. The means of disease and yield parameters varied significantly($p\leq0.05$) across treatments, i.e., cultivars, control methods, and the interaction (Table 1). The better yield ton per hectare (6.76) obtained from the interaction of Bako local cultivar with Trichoderma harzianum + Fungicide. Whereas the maximum percent disease index (59.7%) was recorded in the untreated control with the Bako local cultivar combination (Table 1). The significant difference among cultivars might be due to genetic potential, but the significant difference among the interaction of cultivars and control methods was due to both factors on the PSI and Yield.

As a result, the untreated control of the Bako local cultivar recorded the highest terminal percent disease index up to 59.7%, while the Melka Zala variety treated with Metalaxyl-M + Mancozeb had the lowest (1.67%). This figure, however, did not differ significantly ($p\leq0.05$) from the effect of T. harzianum alone or when combined with Metalaxyl-M + Mancozeb in either cultivar. The combined effect of fungicide and bioagent reduced wilt disease by 93% for Bako local and 91.2% for the Melka Zala variety (Table 3). The untreated Bako local cultivar and Melka Zala hybrid variety had the highest wilt percent disease index, whereas the integrated management approach using Metalaxyl-M + Mancozeb and T. harzianum had the lowest disease index.

In terms of yield, the significant maximum yield (6755.95 kg ha-1 or 6.76 t ha-1) was recorded from the Bako local cultivar treated with Trichoderma and fungicide, and the minimum (3601.19 kg ha-1 or 3.6 t ha-1) was obtained from an untreated control of the same cultivar. In this pepper growing season, the integrated management approach increased marketable yield per hectare by 170.5% on the Melka Zala variety and 187.6% on the Bako local cultivar when compared to the untreated control (Table 3). In addition to the genetic potential that made the cultivars differ in yield, biological and fungicide treatments play a great role in managing the Fusarium wilt on Capsicum species and contribute to the increment of yield per hectare (Table 3).

3.3. Second experimental year 2021 pepper growing season

The analysis of mixed-effect results for the 2021 growing season showed highly significant ($p\leq0.01$) differences between Factor A, Factor B, and the interactions (Supplementary material, Table 1). Proc mixed effect analysis revealed significant differences in PSI and yield tons per hectare for cultivars, Fusarium control methods, and cultivar × Fusarium control method interaction (Table 2). Trichoderma harzianum + Fungicide combined with the Melka Zala cultivar gave a higher yield ton per hectare (up to 12.6). Similarly, the minimum terminal percent severity index (13.2%) was noticed in the same treatment combination. In contrast, the untreated control with the Bako local cultivar combination had the highest disease index (88.4%) (Table 4). As a result, the integrated effect reduced Fusarium wilt by 75.3% on the Bako local cultivar and by 79.9% on the Melka Zala hybrid variety in the 2021 pepper growing season (Figure 3 and Table 4The integrated wilt management on Melka Zala and Bako local recorded a terminal percent wilt index of 13.2 and 21.8%, respectively. The green pods per plant showed significant variation across the treatments. As a result, the Melka Zala hybrid variety with fungicide + biocontrol agent produced the most (23.7) green pods per plant, while the untreated Bako local cultivar produced the fewest (5.5) green pods per plant (Table 4). Regarding yield produced t/ha, the Melka Zala variety had the highest marketable yield (12.6 t/ha) from the integrated management of biocontrol and fungicide, followed by Bako local, which had (9.6 t/ha) from the same wilt management approach. Untreated control plots of Bako local and Melka Zala, on the other hand, had the lowest marketable yield, tons per hectare, with 2.4 and 3.7 t/ha, respectively. This result confirmed that the integrated management approach increased marketable yield per hectare by 340.8% for the Melka Zala hybrid variety and 406.9% for the Bako local cultivar compared to the control (Table 4). Genetic potential may be the reason for the notable variation between cultivars, although both variables on PSI and yield were responsible for the large variation between cultivars and disease control methods.

Alternatively, the mixed effect analysis using proc mixed indicated that there was a considerable difference among cultivars, Fusarium control methods, and cultivar x Fusarium control method interaction on PSI and yield t ha-1 (Table 1). The significant difference among cultivars might be due to genetic potential, but the significant difference among the interaction of cultivars and control methods was due to both factors on the PSI and Yield. The better yield ton per hectare (6.76) obtained from the interaction of Bako local cultivar with Trichoderma harzianum + Fungicide. Whereas the maximum percent disease index (59.7%) was recorded in the untreated control with the Bako local cultivar combination (Table 1).

Table 1. Mixed effects on cultivar, control method, and cultivars x control methods interaction on percent severity index and yield tons per hectare in the 2020 growing season

Factor A (Bako local, Melka Zala)	**Factor B (Fungicide, *Trichoderma harzianum*, *Trichoderma harzianum* + Fungicide, Untreated)**	PSI (%) **Mean**	**Standard Error**	MYLD (t/ha) **Mean**	**Standard Error**
	Effect=Cultivar, Method=**LSD(*P<0.05*) Set=1**				
Bako Local		18.383[a]	2.6404	5.506[a]	0.3481
Melka Zala		8.958[b]	2.6404	5.409[a]	0.3481
	Effect=Control method, Method=LSD(*P<0.05*) Set=2				
	Untreated	44.017[a]	3.7341	3.7647[b]	0.4923
	Trichoderma harzianum	5.000[b]	3.7341	5.7438[a]	0.4923
	Trichoderma harzianum+fungicide	3.333[b]	3.7341	6.7262[a]	0.4923
	Fungicide	2.333[b]	3.7341	5.5953[a]	0.4923
	Effect=Cultivar*Control method, Method=LSD(*P<0.05*) Set=3				
Bako Local	Untreated	59.700[a]	5.2808	3.601[c]	0.6963
Melka Zala	Untreated	28.333[b]	5.2808	3.9283[abc]	0.6963
Bako Local	*Trichoderma harzianum*	6.667[c]	5.2808	5.833[ab]	0.6963
Bako Local	*Trichoderma harzianum* + Fungicide	4.167[c]	5.2808	6.7560[a]	0.6963
Melka Zala	*Trichoderma harzianum*	3.333[c]	5.2808	5.655[abc]	0.6963
Bako Local	Fungicide	3.000[c]	5.2808	5.833[ab]	0.6963
Melka Zala	*Trichoderma harzianum* + Fungicide	2.500[c]	5.2808	6.696[a]	0.6963
Melka Zala	Fungicide	1.667[c]	5.2808	5.357[abc]	0.6963

*Means with the same letters are not significantly different. PSI, Percent severity index, MYLD, marketable yield

Table 2. Mixed effects on cultivar, control method, and cultivars * control methods interaction on percent severity index and yield tons per hectare in the 2021 growing season

Factor A (Bako local, Melka Zala)	Factor B (Fungicide, *Trichoderma harzianum*, *Trichoderma harzianum*+ Fungicide, Untreated)	PSI (%) Mean	PSI (%) Standard Error	MYLD (t/ha) Mean	MYLD (t/ha) Standard Error
Effect=Cultivar, Method=**LSD(*P<0.05*) Set=1**					
Bako Local		45.952ᵃ	1.5518	5.958ᵇ	0.3155
Melka Zala		33.657ᵇ	1.5518	7.035ᵃ	0.3155
Effect=Control method, Method=LSD(*P<0.05*) Set=2					
	Untreated	77.125ᵃ	2.1946	11.133ᵃ	0.4461
	Trichoderma harzianum	35.138ᵇ	2.1946	6.000ᵇ	0.4461
	Trichoderma harzianum + Fungicide	29.467ᵇ	2.1946	5.817ᵇ	0.4461
	Fungicide	17.488ᶜ	2.1946	3.037ᶜ	0.4461
Effect=Cultivar*Control method, Method=LSD(*P<0.05*) Set=3					
Bako Local	Untreated	88.417ᵃ	3.1036	2.367ᵈ	0.6309
Melka Zala	Untreated	65.833ᵇ	3.1036	3.707ᵈ	0.6309
Bako Local	Fungicide	43.083ᶜ	3.1036	5.967ᶜ	0.6309
Bako Local	*Trichoderma harzianum*	30.500ᵈ	3.1036	5.867ᶜ	0.6309
Melka Zala	*Trichoderma harzianum*	28.433ᵈ	3.1036	5.767ᶜ	0.6309
Melka Zala	Fungicide	27.193ᵈ	3.1036	6.033ᶜ	0.6309
Bako Local	*Trichoderma harzianum* + Fungicide	21.810ᵈᵉ	3.1036	9.633ᵇ	0.6309
Melka Zala	*Trichoderma harzianum* + Fungicide	13.167ᵉ	3.1036	12.633ᵃ	0.6309

*Means with the same letters are not significantly different. PSI, Percent severity index, MYLD, marketable yield

Table 3. Summary of **the** Integrated effect of pepper cultivars and Fusarium wilt control method on mean percent disease index and yield in the 2020 growing season.

Factor A Cultivars	Factor B Control methods	PSI (%)	Disease reduction over control (%)	MYLD, Green Fruit Weight (kg/ha)	Increment MYLD per hectare (%)	UN MYLD, Green Fruit Weight (kg/ha)
Bako Local	Metalaxyl-M + Mancozeb	3.0ᶜ	94.9	5833.33ᵃᵇ	161.98	267.86ᵇ
Bako Local	*Trichoderma harzianum*	6.67ᶜ	88.8	5833.33ᵃᵇ	161.98	297.62ᵇ
Melka Zala	Metalaxyl-M + Mancozeb	1.67ᶜ	94.1	5357.14ᵃᵇᶜ	136.4	267.86ᵇ
Melka Zala	*Trichoderma harzianum*	3.33ᶜ	88.2	5654.76ᵃᵇᶜ	143.9	505.95ᵃᵇ
Bako Local	*Trichoderma harzianum*+ Metalaxyl-M + Mancozeb	4.17ᶜ	93	6755.95ᵃ	187.6	476.19ᵃᵇ
Melka Zala	*Trichoderma harzianum*+ Metalaxyl-M + Mancozeb	2.5ᶜ	91.2	6696.43ᵃ	170.5	654.76ᵃᵇ
Bako Local	Untreated	59.0ᵃ	0	3601.19ᶜ	0	863.10ᵃ
Melka Zala	Untreated	28.33ᵇ	0	3928.57ᵇᶜ	0	327.38ᵇ
	Mean	13.67		5457.589		457.589
	CV (%)	70.1		23.28		66.7
	LSD (0.05)	22.63		2225.1		534.75

* Means with the same letter are not significantly different. MYLD, Marketable Yield, UN MYLD, Unmarketable Yield, PSI, percent severity index

Table 4. Summary of **the** Integrated effect of pepper cultivars and Fusarium wilt control method on mean percent disease index, Number of pods per plant, and marketable yield in the 2021 growing season.

Factor A Cultivars	Factor B Control methods	PSI (%)	Disease reduction over control (%)	Number of pods per plant	MYLD (t/ha)	Increment MYLD per hectare (%)
Bako Local	Metalaxyl-M + Mancozeb	43.08ᶜ	51.3	17.81ᵇ	5.967ᶜ	252.1
Bako Local	*Trichoderma harzianum*	30.5ᵈ	65.5	14.983ᵇᶜ	5.867ᶜ	247.9
Melka Zala	Metalaxyl-M + Mancozeb	27.19ᵈ	58.7	11.763ᶜᵈ	6.033ᶜ	162.7
Melka Zala	*Trichoderma harzianum*	28.43ᵈ	56.8	11.593ᶜᵈ	5.767ᶜ	155.6
Bako Local	*Trichoderma harzianum*+ Metalaxyl-M + Mancozeb	21.81dᵉ	75.3	16.143ᵇᶜ	9.633ᵇ	406.9
Melka Zala	*Trichoderma harzianum*+ Metalaxyl-M + Mancozeb	13.17ᵉ	79.9	23.67ᵃ	12.633ᵃ	340.8
Bako Local	Untreated	88.42ᵃ	0	5.477ᵉ	2.367ᵈ	0
Melka Zala	Untreated	65.83ᵇ	0	7.120ᵈᵉ	3.707ᵈ	0
	Mean	39.80		13.57	6.497	
	CV (%)	14.11		23.168	17.03	
	LSD (0.05)	9.836		5.506	1.938	

* Means with the same letter are not significantly different, MYLD, Marketable Yield, PSI, Percent severity index

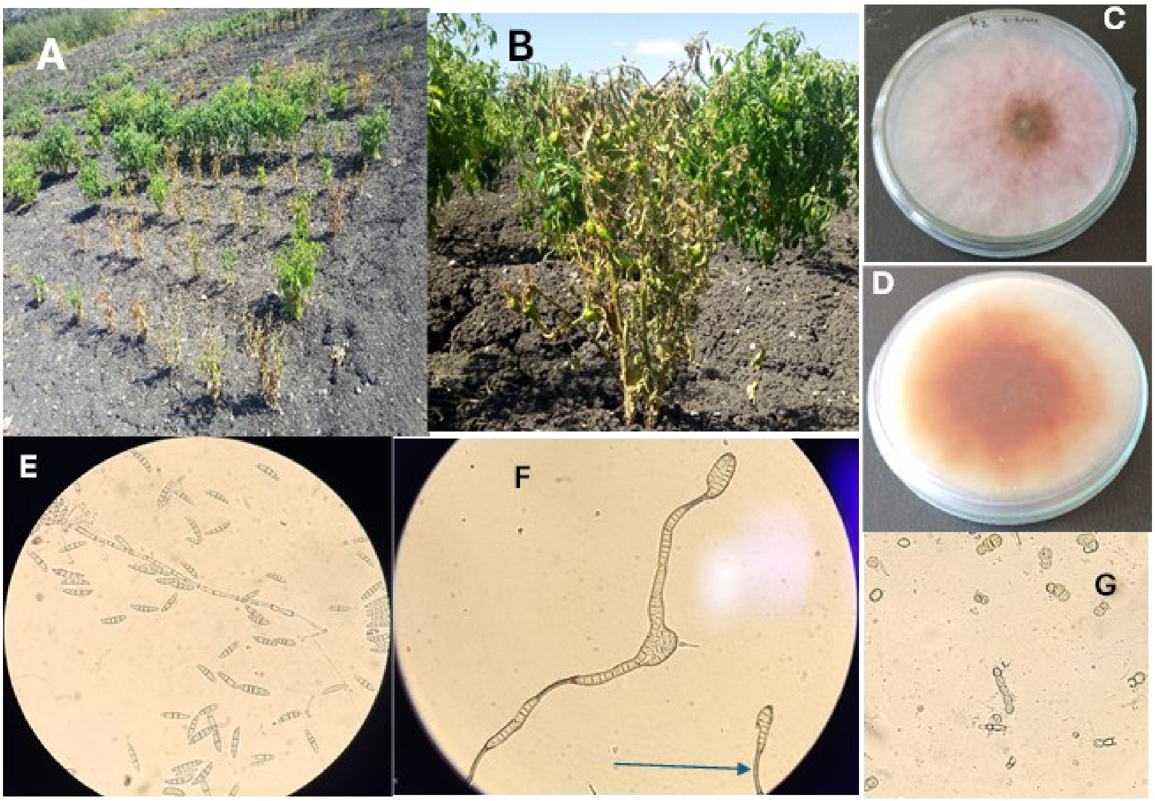

Figure 1. Devastated field with Pepper wilt and typical symptoms of Fusarium wilt (A) and (B), Colony morphology on PDA, abundant mycelia, pinkish and purple pigments of mycelia in color (C) back side, (D) front side, Macroscopic and microscopic characteristics of *F. oxysporum* f. sp. *capsici* (E) and (F), 3-5 septate macroconidia (E), False head(F), Chlamydospores in couple and in chain of two units (G).

Figure 2. Integrated management of *Fusarium oxysporum* f. sp. *capsici* pepper using *Trichoderma harzianum* and Metalxyl-M+ Mancozeb in the 2021 pepper growing season: treated plot (A) and (D), and Untreated plot (B) and (C) of Bako local cultivar.

4. Discussion

The current study aims to develop the integrated management of Fusarium wilt of pepper crops. Bioagents, fungicides, and cultivars were used in the study. Two Capsicum growing season experiments revealed that the developed integrated management for Fusarium oxysporum f. sp. capsici was effective under field conditions. The first season investigation indicated that Trichoderma harzianum, as a biological agent against pepper Fusarium wilt, performed well even in the field, although many of the studies so far reported its effectiveness only in in vitro and/or in greenhouse (Al-aamel and Al-maliky 2023; Divya et al. 2023; El-Mohamedy et al. 2010; Girma 2022; Gveroska and Ziberoski 2011; Natsiopoulos et al. 2022; Prasad et al. 2023). The application of T. harzianum alone significantly reduced (up to 6.67%) the percent severity index compared to the control. These results are consistent with (Al-aamel and Al-maliky 2023), who reported that the T. harzianum alone for management of Fusarium oxysporum f. sp. capsici reduced the final percent severity infection to 10%. They also stated that the combination of T. harzianum with other Fusarium wilt control options minimized the percent severity of infection to 5%. In the current study, the untreated control of the Bako local and Melka Zala hybrid variety showed significant variation in the final percent wilt index (59.7 and 28.33%, respectively), implying that the Melka Zala hybrid variety is more disease-tolerant than the Bako local cultivar. The untreated Bako local cultivar and Melka Zala hybrid variety had the highest wilt percent disease index, whereas the integrated management approach using Metalaxyl-M + Mancozeb and T. harzianum had the lowest disease index. The integrated effect of fungicide and bioagent minimized wilt disease pressure by 93% for the Bako local and 91.2% for the Melka Zala variety. These findings are more or less in line with the findings of Duc (2017), who stated that when multiple factors are present, the reduction in disease incidence and infection severity is caused by their synergistic interaction, which promotes plant growth and increases systemic resistance, both of which help to suppress the pathogen.

In this study, the combined effect of T. harzianum, fungicide, and cultivars resulted in a significantly maximized yield in kilograms per hectare. Whereas the untreated control plots gave a minimum yield regardless of cultivars. It was noticed that integrated disease management resulted in an increase in the marketable yield per hectare compared to the untreated control, but differed among cultivars. When compared to the untreated control, the integrated management method increased marketable yield per hectare by 187.6% on the Bako local cultivar and 170.5% on the Melka Zala variety in the 2019/20 pepper growing season. The difference in yield might be due to the genetic difference of the cultivars, but the control methods applied play a crucial role.

These findings agreed with what (AL-Surhanee et al. 2017) stated, T. harzianum causes weight increases in crops because of its ability to stimulate the production of growth regulators, which are essential for plant growth. Additionally, they observed that the fungus helps to nourish and support plants, which in turn promotes crop growth and productivity.

This study demonstrated that integrated management with cultivars, Trichoderma harzianum, and Metalaxyl-M + Mancozeb effectively manages Fusarium wilt on Capsicum species. Similarly, the integrated effect reduced Fusarium wilt by 75.3% on the Bako local cultivar and by 79.9% on the Melka Zala hybrid variety in the 2021 pepper growing season.

According to (Choudhary et al. 2023) the combination of T. harzianum and fungicide was the best combination for Fusarium wilt control, resulting in 70.21% to 70.02% wilt disease reduction. There is a lot of research that supports the use of Trichoderma harzianum as a biological control agent. Trichoderma spp. are capable of producing a wide range of secondary metabolites (De Corato 2020), such as antibiotics and phytohormones (El Komy et al. 2015), which have a direct impact on the pathogen. Some Trichoderma spp. strains have been shown to produce indole-3-acetic acid (IAA) and gibberellins (Awad-Allah et al. 2022), which can stimulate plant growth and improve plant resistance to disease (Awere et al. 2021).

Fungicide application prevented the development of symptoms by specifically reducing the incidence of Fusarium oxysporum wilt. Jamil and Ashraf (2020) found that the efficacy of fungicides in controlling the disease increased as their dosages increased, regardless of the method of application (soil application, seed treatment, or seedling dipping). However, the active ingredients in Ridomil gold fungicide (the Mancozeb), which is effective in reducing the Fusarium when applied once and a half dose, were discovered to be valuable for seedling dipping in the current study at transplanting. This is also another method of reducing fungicide resistance in the production of vegetable crops. It was confirmed in this study that the integrated management approach increased marketable yield per hectare compared to the untreated control. This showed that despite the single disease control method, the integrated method minimizes the pressure of the pepper Fusarium wilt disease (i.e., the most devastating disease of this crop currently across the globe, including Ethiopia) and enhances the yield per hectare.

In modern agriculture, Trichoderma species are the most effective bio-fungicides. Among the many biological controls used in agriculture to manage soil-borne diseases are Trichoderma-based biological control agents (BCAs). Trichoderma not only prevents diseases but also promotes plant growth, increases nutrient utilization efficiency, improves plant resistance, and reduces agrochemical pollution. Trichoderma spp. additionally, it acts as a safe, low-cost, effective, and environmentally friendly biocontrol agent for various crop species (Pokhrel et al., 2022; Yao et al., 2023).

Under stressful conditions, plants are thought to mobilize nutrients and redirect metabolism to support active defense mechanisms, at the expense of growth and final yield (Agrios 2005). However, by killing the inoculum before infection and/or attempted tissue colonization, by preventing the penetration of the pathogen, Metalaxyl-M + Mancozeb may have prevented the activation of active defense mechanisms. This would allow the crop to be allocated to the sink organ, resulting in a higher yield.

Various fungicides, both contact and systemic, have been tested and found to be effective against Fusarium wilt of pepper (Choudhary et al. 2023; Elmer and McGovern 2004; Madhavi and Bhattiprolu 2011; Song et al. 2004). Carbendazim (0.2%), carbendazim + mancozeb (0.25%), and tebuconazole + trifloxystrobin (0.1%) soil drenching were found to be effective in eliminating the disease and increasing fruit yield (Attri et al. 2019). The current study revealed the effectiveness of the Metalaxyl-M + Mancozeb application method, root dipped during seedling transplant for 30 minutes, and the integrated effect of this fungicide in field application against Fusarium oxysporum f. sp. capsici with formulated T. harzianum. To the best of our knowledge, this is the first report of an integrated management option for this destructive Fusarium wilt disease of Capsicum species at an in vivo level.

5. Conclusions

In conclusion, this study successfully developed an integrated management approach for the Fusarium wilt of the pepper crop. The combination of Trichoderma harzianum and Metalaxyl-M + Mancozeb fungicide significantly reduced the percent disease index and/or disease pressure and increased marketable yield per hectare. The findings highlight the importance of integrated disease management strategies for sustainable pepper production. Further research is warranted to investigate the large-scale formulation and commercialization of T. harzianum. Studies regarding the combined effects of multiple bioagents, potential fungal and bacterial bioagents, integrated with compatible fungicides to control Fusarium wilt on pepper under field conditions, likewise, should be given due attention.

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Data Availability Statement: This study's dataset, which was utilized to develop the integrated Fusarium management option, is available at: **https://www.kaggle.com/datasets/tam0912/raw-data**

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