International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2756-326X Vol. 12 (4), pp. 001-012, April, 2026. Available online at www.internationalscholarsjournals.org © International Scholars Journals

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Full Length Research Paper

Integrated use of Arbuscular Mycorrhiza Fungi, Biochar and Kale biofumigation against Fusarium Wilt of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract

Received 16 November; 2025; Revised 22 December, 2025; Accepted 24 December, 2025; Published 14 April, 2026

Tomato wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), is one of the most destructive and economically important fungal disease, resulting in significant yield loss ranging between 10%-90%. The disease is primarily managed using chemical fungicides, which are not always sustainable. The present study focused on evaluating eight treatments comprising of the individual and combined effect of Arbuscular mycorrhizal fungi (AMF), biochar, and kale biofumigation on the inhibition of two isolates of *Fusarium oxysporum* f. sp. *lycopersici in vitro.* The experiment was conducted in a split-plot arrangement using a Completely Randomized Design (CRD) with three replications in the Plant Pathology laboratory, Department of Seed, Crop, and Horticultural Sciences, University of Eldoret, Kenya. Potato dextrose agar (PDA) was used for fungal growth. Tomato tissues were surface-sterilized and plated to obtain pure cultures of FOL using the single-spore technique. Two isolates of *Fusarium oxysporum* f. sp. *lycopersici* were obtained from tomato plants showing wilt disease symptoms, namely FOL 1 and FOL 2. AMF was obtained from the commercial inoculant Arbuscular mycorrhiza fungi (RHIZATECH TM), biochar obtained from the seedballs company, Kenya, and the kale variety Ahadi was used for biofumigation. The interaction of AMF and FOL isolates was carried out using dual culture, food poison technique for biochar, sandwich-plate assay for kale biofumigation and modified assay for the combined applications. AMF, Glomus sp. was isolated by wet sieving and decanting technique. The antifungal efficacy of biochar was evaluated by mixing it with PDA media at 1% (w/w) prior to autoclaving. Kale leaves were macerated and incorporated as biofumigant. The inhibition was determined based on the reduction in the radial mycelial growth. The findings from *in vitro* experiments indicated that application of AMF, biochar, and kale biofumigation, alone and in combinations, suppressed the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* isolates. However, the degree of inhibition was isolate dependent. In the individual application, AMF alone had the highest inhibition in FOL1 with a value of 50.83%, followed by kale (43.74%). Triple combination of AMF, biochar, and kale biofumigation was the most potent, with the highest inhibition across the two isolates of 64.07% in FOL 1 and 52.12% in FOL 2, respectively. The findings of this study reveal the potential of integrating AMF, biochar and kale biofumigation as a viable and eco-friendly alternative to chemical fungicides in the management of tomato Fusarium wilt, contributing to a more sustainable integrated disease management, potentially reducing the reliance on chemical products and improving the agricultural productivity.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici, Tomato, Arbuscular Mycorrhiza Fungi, Biochar, biofumigation & in vitro.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most extensively cultivated and consumed vegetable crop in the world and is ranked the second most important vegetable after potato (Haleema *et al*., 2024; Terada *et al*., 2023). Globally, tomato production reached approximately 192 million metric tonnes in 2023, making it one of the top vegetable crops in terms of volume (FAO, 2024). In 2024, Kenya produced 482,951.50 MT of tomatoes from an area of 25,807.23 hectares (AFA, 2025). Tomato production in Kenya is a significant component of Kenya's agricultural sector, contributing to food security and rural livelihoods (Irina *et al*., 2024). Nutritionally, tomatoes contain good source of vitamins including vitamin A, B1, B2, and C and minerals such as phosphorus (P), potassium (K), calcium (Ca), zinc (Zn), sodium (Na), magnesium (Mg) and manganese (Mn) (Irina *et al*., 2024; Umeohia & Olapade, 2024). Additionally, tomatoes contain antioxidants like lycopene, which gives them their natural color and has been linked to health benefits, including the prevention of heart disease, a reduced risk of cardiovascular conditions, and various types of cancer (Panwar *et al*., 2024; Singh *et al*., 2024). Despite the strong demand from consumers, the current yield attained is still below the potential yield production of 30.7 t ha-^{1} (Mwangi *et al*., 2021; Ochilo *et al*., 2019). In addition, the tomato greenhouse production, which was touted as a game changer in the lucrative tomato subsector, has also largely failed to live up to expectations, with its production still accounting for only 5% of the total tomato production in Kenya. According to AFA (2025), the current tomato production trend is continuously decreasing, underlining a yield gap given the Kenyans potential production capacity. The decline in production has been liked to biotic and abiotic factors; among biotic factors, Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* is considered one of the most destructive tomato disease in the world; under warm soil conditions (Mugao, 2023).

Fusarium oxysporum f. sp*. lycopersici* is the agent of the scourge Fusarium wilt disease infecting tomato (*Solanum lycopersicum*) around the globe (López-Zapata *et al*., 2021). The colonization capability of the pathogen, which causes extensive soilborne inoculum and prolonged survival in most traditional control procedures, has made its management difficult. Fusarium wilt has been reported to extensively damage tomato plants, resulting in yield loss ranging between 10% and 55% (Haruna *et al*., 2024; Jimoh & Elizabeth, 2024), with yield losses exceeding 90% especially in greenhouses where monocultures and limited crop rotation are practiced (Kawicha *et al*., 2023; Madege *et al*., 2023; Vignesh *et al*., 2021). Recent trends in sustainable agriculture have drawn direct attention to the possibility of using biochar, arbuscular mycorrhizal fungi (AMF), and plant-based bioactive extracts as a supplementary tool to inhibit soil pathogens. The bio-stimulants and biocontrol agents use their direct antagonistic action as well as induced systemic resistance, providing a multidimensional approach to the concept of integrated pest management (IPM) (Maurya *et al*., 2019).

Although a lot of research has been done on FOL, the disease is still an immense yield reduction agent, particularly when inoculum pressure is high and the soil poorly drained or high in soil moisture (Van Bruggen *et al*., 2016). The use of chemical fungicides as the primary form of control is being curtailed over time due to their overreliance, which significantly contributes to the development of resistant strains and also compromises environmental and human health (El-Aswad *et al*., 2023). Besides, their practicality is constrained as many smallholder and organic tomato producers incur a lot of costs on the chemical inputs, not to mention that they require accurate, timely application. As a result, there is an urgent need for alternative and greener options, which can be incorporated into various systems of cropping (Hassan, 2020).

Other management methods include cultural (crop rotation, soil solarization), biological (beneficial microorganisms, antagonistic fungi), and the use of resistant cultivars. Although biochar has been reported to be effective in the inhibition of pathogenic organisms such as *Phytophthora capsici*, *Pythium aphanidermatum* and *Fusarium oxysporum* f. sp. *lycopersici* in capsicum, cucumber and tomato, respectively (Akhter *et al*., 2016; Jaiswal *et al*., 2018, 2019; Wang *et al*., 2019), its compatibility with other biocontrol agents and its synergistic and antagonistic effects are not fully characterized (Bhatt *et al*., 2024). Likewise, AMF is reported to increase the plant vigor and resistance, such as Fusarium wilt in Salvia miltiorrhiza and cucumber, chickpea and tomato (Ahammed *et al*., 2020; Meddad-Hamza *et al*., 2023; Pu *et al*., 2022 and Shukla *et al*., 2015). However, the interactions between AMF colonization, FOL inhibition and the role of secondary metabolites in companion crops in this process are not clearly comprehended (Sonbol *et al*., 2025). *In vitro* and *in situ* testing of kale, which is highly concentrated in glucosinolates and phenolic compounds, reveals a potential new plant-based inoculant; its activity against FOL *in vitro* and *in vivo* has not been systematically tested. Although numerous studies have investigated the use of AMF, biochar, and kale, the underlying mechanisms behind their interactions remain unexplored. Therefore, this study embraced a holistic multi-layered experimental design to determine the effects of AMF, biochar and kale biofumigation, individually and in combination on FOL viability for sustainable production under *in vitro* conditions.

MATERIALS AND METHODS

Study Location

The study was conducted at the Plant Pathology laboratory, Department of Seed Crop and Horticultural Sciences, University of Eldoret, Kenya. The experimental site lies at a latitude of 0°34’ N and longitude 35°18’E with

an elevation of 2153 meters above the sea level, with a mean temperature of 17.3°C and an annual average rainfall of 2027 mm (https://en.climate-data.org/africa/kenya/uasin-gishu/eldoret-926351/ Climate-Data (2025).

Media preparation

Potato Dextrose Agar (PDA) medium was prepared by dissolving 39g of PDA in 1000 mL of distilled water. The media was sterilized by autoclaving at 1 P.S.I. and 121 ºC for 15 minutes. The molten medium was allowed to cool, after which 0.16g/L of Streptomycin sulfate was added to inhibit bacterial growth (Troni *et al.*, 2021). The molten medium was then mixed well before dispensing 20 ml into the sterilized petri plates and allowed to solidify (Troni *et al.*, 2021).

Collection, Isolation and Pathogen Identification

Samples of Fusarium wilt-infected tomato plants showing typical wilting symptoms were obtained from a greenhouse in Kajiado County, Kenya. Ten symptomatic plants were sampled, consisting of roots, stems and leaves. All samples were packed in zip-lock bags and delivered to the Plant Pathology Laboratory, Department of Seed, Crop and Horticultural Sciences, University of Eldoret, for isolation and identification. Samples were kept in a refrigerator at 4 ˚C in order to preserve the pathogen's viability during storage. To isolate wilt fungus, the collected samples were carefully washed with distilled water. Afterwards, the diseased roots, stems, and leaves were aseptically cut into small pieces approximately five (5) mm long. These pieces were surface sterilized by dipping in 70% ethanol and 1% sodium hypochlorite solution for 30 seconds and 1 minute, respectively (Adhikari *et al.*, 2020), after which, the sterilized pieces were rinsed three times in sterile distilled water to remove traces of ethanol and sodium hypochlorite. The sterilized pieces were blotted dry on a sterilized serviette under a laminar flow hood. The sterilized pieces of the tissues were aseptically plated on solidified Potato Dextrose Agar (PDA) medium, supplemented with streptomycin sulfate at a concentration of 0.16 mg per liter to inhibit bacterial growth. The petri plates were then sealed with parafilm M® (Bemis Company Inc., USA) and incubated at 25 ± 2°C for seven days. The cultures from the plated pieces were checked regularly for fungal development.

Colonies with the characteristics of fungi were subcultured on PDA to obtain pure cultures, using the single-spore technique. The hyphal tips of the actively growing mycelia were transferred from the colonies to petri plates containing PDA medium in the laminar airflow chamber (Sishuba & Ateba, 2022). The new inoculated petri plates were incubated at 25 ± 2°C for another 7 days under an alternating 12-hour light and dark cycle to promote sporulation. Two pure isolates were obtained and to confirm whether the isolates were *Fusarium oxysporum* f. sp. *lycopersici*, cultural characteristics and microscopic examination were conducted. Macroscopic identification was done through physical observation of the colony morphology and pigmentation of the pure culture plates of the isolates using morphological key as described by Leslie & Summerell, (2006). Microscopic examination was also performed using the same morphological key to determine the spore morphology (microconidia, macroconidia, and chlamydospores). The microscopic examination was done using a binocular compound light microscope (LEICA DM 500) under magnification 100x. To determine the isolates of the collected *Fusarium oxysporum* f. sp. *lycopersici* and to prove Koch's postulates, the two isolates of *Fusarium oxysporum* f. sp. *lycopersici* were tested for pathogenicity. On confirmation of pathogenicity by observing wilting diseased symptoms on artificially inoculated tomato plants, re-isolation was done and the two *Fusarium oxysporum* f. sp. *lycopersici* isolates were designated serially as FOL 1 and FOL 2. The pure cultures of the two isolates were tested in the present study.

Arbuscular Mycorrhizal Fungi

Arbuscular Mycorrhiza Fungal spores were isolated by wet sieving and decanting technique as described by Boyno *et al.*, (2025). Fifty (50) grams of soil inoculants were placed in a 500 ml conical flask and topped with 100 ml of distilled water. The mixture was shaken vigorously for 1 minute to free the AMF spores and then allowed to decant for 1 minute to enable heavier particles to settle, after which the supernatant was decanted through a 2 mm sieve. The supernatant was stirred once more to resuspend all particles and then decanted through a 45 µm sieve to obtain pure spores. The retained supernatant was washed and transferred into a petri dish for examination under a dissecting microscope. Under the microscope, spores were picked using a pipette and they were isolated based on spore color and shape and identified according to the descriptive features of the described species posted on the INVAM website (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html; Accessed: 31 July 2023). The isolated AMF spores were used to produce AMF inoculum through the single-spore inoculation technique (Selvakumar *et al.*, 2018). The spores were cultured on potato dextrose agar (PDA) and incubated in the dark in an inverted position, at 30°C for five days.

Biofumigant Preparation

A commercial kale variety (*Brassica oleracea L. var. acephala*) was used for biofumigation treatment. Three-week-old kale seedlings, var Ahadi F1, were purchased and transplanted into the University farm. The plants were carefully maintained until they were harvested at the vegetative growth stage for the experiment. Kale leaves were harvested at the vegetative stage, 60 days after planting. The harvested kale leaves were carefully rinsed

using tap water to remove dust and dried. The kale leaves were then disinfected in 10 % ethanol for 10 seconds and rinsed with sterile distilled water (SDW). The kale leaves were finely chopped into approximately 4 mm pieces using a kitchen knife before being macerated using a kitchen blender (Model: SGP22). Under sterile conditions, five grams (5g) of the freshly macerated kale was immediately transferred to the inverted lid (lower plate) of a Petri dish and sterile distilled water was added at 1ml/g tissue as described by Relevante & Cumagun (2013). A corresponding Petri dish of the same diameter containing PDA (upper plate) was centrally inoculated with a 5 mm mycelial disc taken from the edge of a seven-day-old culture of two isolates of *Fusarium oxysporum* f. sp *lycopersici*, serving as the lid. The two plates were paired and sealed together in a laminar airflow with Parafilm M ® (Bemis Company Inc., USA) to prevent leakage of volatile bioactive compounds.

Biochar

Biochar used was obtained from Seedballs Company, Kenya. The biochar was first sieved through a 100 µm sieve to obtain a uniform particle size before being added to PDA. A 1% Biochar Potato Dextrose Agar Medium (BPDA) was prepared by mixing one gram of sieved biochar with 100 mL of PDA in a 250 mL conical flask, ensuring thorough mixing for uniform consistency. The mixture was then autoclaved after adding streptomycin sulfate to prevent bacterial contamination.

Effect of AMF, biochar and biofumigation using kale on the radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*.

AMF, biochar, and kale biofumigation were evaluated individually and in various combinations under *in vitro* conditions against two isolates of *Fusarium oxysporum* f. sp. *lycopersici,* using potato dextrose agar as a basal culture medium. The following treatments were applied: individual application of AMF, biochar, kale biofumigation, Dual application of AMF x biochar, AMF x kale biofumigation, biochar x kale biofumigation and the triple application of AMF x biochar x kale biofumigation. Control treatment consisting of only Fusarium isolates was used for comparison. All the treatments were replicated three times.

The experiment was conducted in a split-plot arrangement in a Completely Randomized Design (CRD) with three replications maintained for each treatment. The two isolates of *Fusarium oxysporum* f. sp. *lycopersici,* FOL 1 and FOL 2, were the main plots, and the eight treatments formed the subplots.

Effect of biofumigation with kale (*Brassica oleracea* var *acephala*) on the radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici.*

The biofumigation potential of kale var Ahadi F1 on mycelial growth of two isolates of *Fusarium oxysporum* f. sp *lycopersici* (FOL) was evaluated using kale extract prepared as described under biofumigant preparation, using the sandwich plate assay method as described by Dassanayaka *et al.* (2023). Briefly, five grams (5g) of the freshly macerated kale was transferred to the inverted lid (lower plate) of a petri dish and sterile distilled water was added at 1ml/g tissue. A corresponding Petri dish of the same diameter containing PDA (upper plate) was centrally inoculated with a 5 mm mycelial disc taken from the edge of a seven-day-old culture of two isolates of *Fusarium oxysporum* f. sp *lycopersici*, serving as the lid. The control plate was inoculated with FOL isolates without plant tissue. The paired plates were incubated at 25 ± 2 °C for 7 days.

Effect of biochar on radial mycelial growth of *Fusarium oxysporum* f. sp *lycopersici.*

The efficacy of biochar on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* isolates was assessed using the poisoned food technique. Biochar Potato Dextrose Agar Medium (BPDA), prepared as described in the biochar section, was carefully poured into petri dishes and allowed to solidify. Using a sterile cork borer, a 5 mm mycelial plug of actively growing Fusarium oxysporum inoculum was taken from seven-day-old pure cultures and placed at the center of the PDA medium. Control plates were prepared without adding biochar. The inoculated plates were sealed with Parafilm M® and incubated at 25 ± 2°C for seven days. The radius of the colony was measured, recorded, and compared with the control (Akter *et al*., 2015; Manasa *et al*., 2024).

Effect of AMF on the radial mycelial growth of *Fusarium oxysporum* f. sp *lycopersici*

The dual culture technique was carried out to determine the antagonistic effect of AMF against the wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Five mm (5) mycelial discs of FOL isolates were taken from the edge of the seven-day-old culture and placed on one end of the petri dish with PDA using a sterile cork borer. A similar AMF disc was placed at the opposite end of the FOL mycelial disc. Petri plates inoculated only with FOL served as a control. The inoculated plates were sealed with Parafilm M® and incubated at 25 ± 2°C for 7 days (Dolatabadi, 2011).

Effect of combined application of AMF, biochar and kale biofumigation on radial mycelial growth of *Fusarium oxysporum* f. sp *lycopersici*

This experiment tested the synergistic effect of AMF and biochar, as well as kale biofumigation, on radial mycelial growth of Fusarium oxysporum f. sp. *lycopersici* isolates. To evaluate the effect of combinations of AMF, biochar, and kale biofumigation, dual culture, sandwich plate assay, and single culture methods with slight modifications were applied.

The combined effect of biochar and kale was carried out using the sandwich plate method with minor modifications. Five grams (5g) of finely macerated kale tissues were transferred into a Petri dish and the corresponding Petri dish of the same diameter containing PDA amended with biochar was centrally inoculated with a 5mm mycelial disc of the pathogen. The petri dish inoculated with the test pathogen was inverted over the petri dish with macerated kale and immediately sealed with parafilm and incubated at 25 ± 2°C for 7 days. Petri plates inoculated with only a FOL plug served as a control.

On the other hand, the dual culture method, with slight modification was used to determine the possible *in vitro* efficacy of the combined application of AMF and biochar. Mycelial discs (5 mm) of each Fusarium isolate and AMF were taken from actively growing edges, placed on opposite ends of the Petri dish with PDA amended with biochar, and incubated at 25 ± 2°C for 7 days. Petri plates inoculated only with the FOL plug served as controls.

A combination of dual culture and sandwich plate method was used to determine the possible *in vitro* efficacy of the combined application of AMF and kale biofumigation on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*. For biofumigation, five grams (5 g) of the freshly macerated kale tissues were placed into the inverted lid (lower plate) of a Petri dish. In a corresponding petri dish of the same diameter containing PDA (upper plate), a five (5) mm mycelial disc taken from the edge of a seven-day-old culture of two isolates of *Fusarium oxysporum* f. sp. *lycopersici* was inoculated on one end. A five (5) mm AMF disc was placed at the opposite end of the plate, opposite the FOL mycelial disc. The two plates were paired and sealed with parafilm and incubated at 25 ± 2°C for 7 days. Petri plates inoculated only with the FOL plug served as a control.

The integrated effect of AMF, biochar and kale on mycelial growth of the *Fusarium oxysporum* f. sp *lycopersici isolates* was assessed using *the* sandwich plate assay, food poison method and dual culture technique with a slight modification. Biochar potato dextrose agar medium was prepared. A 5 mm mycelial disc of each *Fusarium* isolate and AMF were taken from actively growing edges and placed on the opposite ends of a petri dish. Macerated kale was then added to an upturned petri dish, and sterile distilled water was added to induce the release of isothiocyanates (ITCs). The inoculated plates were immediately sealed with parafilm and incubated in an inverted position for 7 days at 25 ± 2°C. Petri plates that only had the FOL plug served as the control.

Data collection

Data on the inhibition of the mycelial growth of the two isolates of FOL was collected by measuring the radial mycelial growth in all plates every 24 hours using a ruler until the control colony completely covered the petri dish. The percent inhibition of the radial mycelial growth in each treatment was calculated using the formula proposed by Poussio *et al*., (2021).

$$PI = \frac{C - T}{C} X\,100$$

Where:
PI = Percent mycelial growth inhibition
C = Radial mycelial growth (mm) in the control plate**.**
T = Radial mycelial growth (mm) in treatment plates.

Data Analysis

The collected data was subjected to Analysis of variance (ANOVA) at a 5% level of significance using GenStat statistical software version 16.1; VSN International Ltd. The treatment means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

Effect of individual and combined application of AMF, biochar and kale biofumigation on radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*

The effect of AMF, biochar, and kale biofumigation, individually or in combinations, on radial mycelial growth of two *Fusarium oxysporum* isolates was investigated *in vitro*. In general, all individual and combined applications of AMF, biochar and kale biofumigation significantly inhibited the radial mycelial growth of the *Fusarium oxysporum* f. sp. *lycopersici* isolates when compared to the control (Figure 1).

Among the individual treatments, AMF application showed the greatest inhibition of the two FOL isolates (42.84%), followed by kale biofumigation, which had moderate inhibition (29.16%), whereas biochar showed the least inhibition effect on the mycelial radial growth of FOL isolates (12.27%) (Figure 1). The treatment combination slightly increased the inhibition compared to individual treatment, suggesting a synergistic effect. The highest inhibition was recorded in the triple combination of AMF, biochar and kale biofumigation (58.09%), followed by dual application of AMF and biochar (44.67%), then AMF and kale biofumigation (44.03%), whereas biochar and kale biofumigation combination recorded the least inhibition (22.73%). (Figure 1, Plate 1, Plate 2).

In vitro* effect of AMF, biochar and kale biofumigation on isolates of *Fusarium oxysporum* f. sp. *lycopersici

The present study's results (Figure 2, Plate 2) showed that all the treatments, either individually or in combination, were effective in inhibiting the radial mycelial growth of wilt-causing fungus *Fusarium oxysporum* f. sp. *lycopersici.* However, the rate of inhibition was isolate dependent (Figure 2). In FOL 1, the highest radial mycelial growth inhibition was recorded in the treatment combination of AMF, biochar and kale biofumigation (64.07%), followed

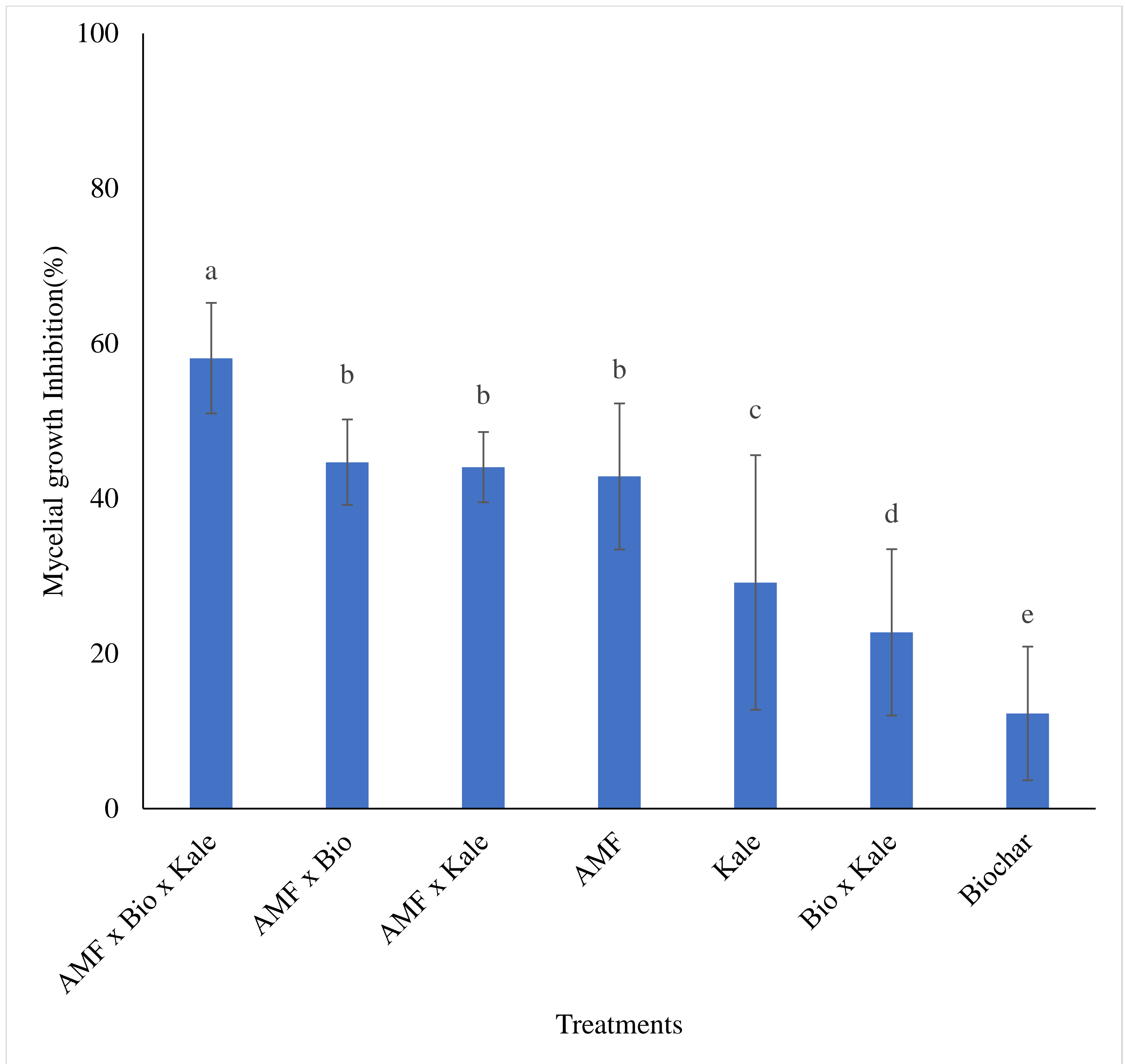

Figure 1: *In vitro* effect of AMF, biochar and kale biofumigation on radial mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici* isolates.
AMF; Arbuscular mycorrhiza fungi, Bio; Biochar. The error bars indicate ± SD (Standard deviation). Different letters above the error bars denote significant differences across the treatment means.

by a single application of AMF (50%) (Figure 2, Plate 2). In contrast, the minimum radial mycelial growth inhibition was recorded in the biochar treatment (4.48%). In FOL 2, the highest radial mycelial growth inhibition was recorded in treatment combination of AMF, biochar and kale biofumigation (52.12%), followed by a treatment combination of AMF and biochar (48.36%), then AMF and kale biofumigation (40.52%) whereas the least radial mycelial growth inhibition was recorded from individual treatment of kale biofumigation (14.58%) (Figure 2, Plate 2). Among the individual treatments, AMF recorded the highest radial mycelial growth inhibition, resulting in 50% and 34.85% inhibition in in FOL 1 and FOL 2, respectively.

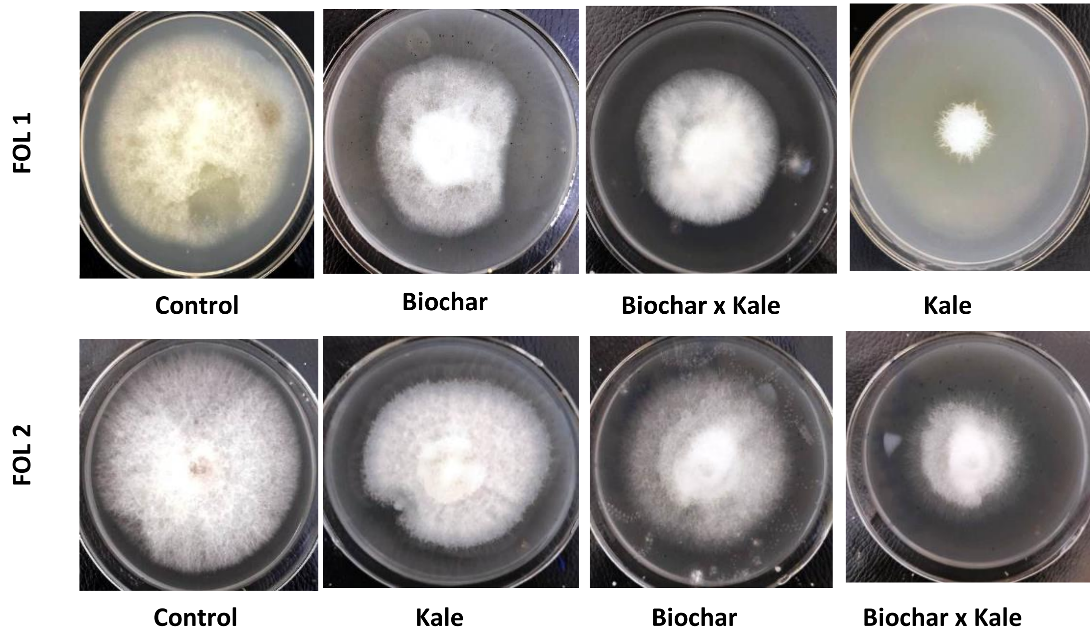

Plate 1: Inhibitory effects of AMF, biochar and kale biofumigation against isolates of *Fusarium oxysporum* f. sp. *lycopersici* mycelial growth on 7th day using Single culture assay and Sandwich assay: Fol 1& Fol 2; *Fusarium oxysporum* f. sp. *lycopersici* isolates.

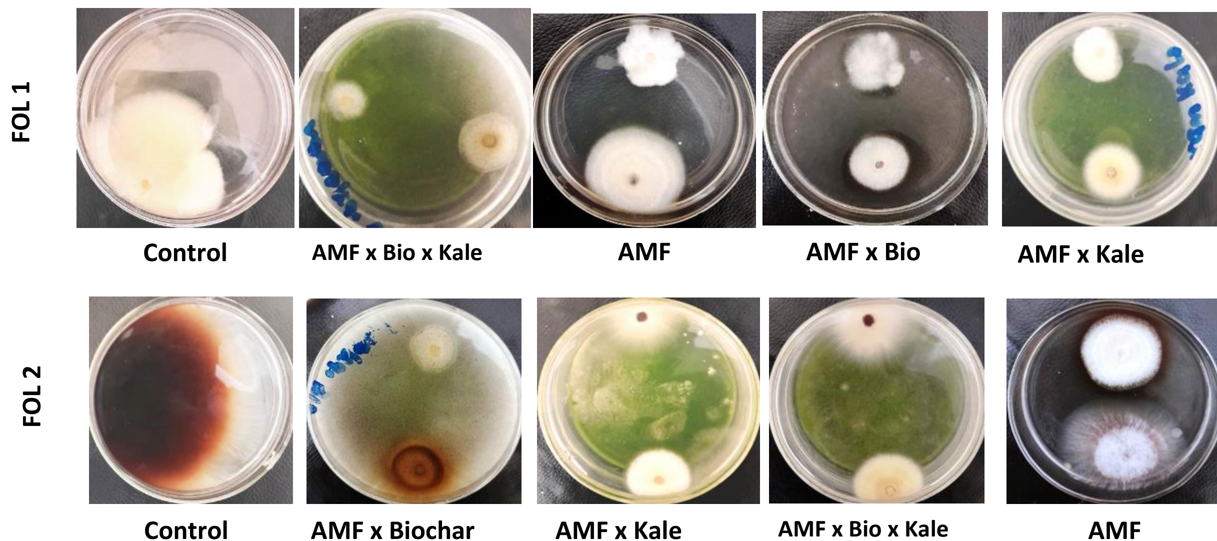

Plate 2: *In vitro* effect of AMF, biochar and kale biofumigation against isolates of *Fusarium oxysporum* f. sp. *lycopersici* mycelial growth on 7th day using Dual culture assay and modified method: FOL 1& FOL 2; *Fusarium oxysporum* f. sp. *lycopersici* isolates. AMF; Arbuscular Mycorrhiza Fungi; Bio: Biochar.

DISCUSSION

The present study observed that individual and combined application of AMF, biochar, and kale biofumigation could enhance control effectiveness against *Fusarium oxysporum* f. sp. *lycopersici* through additive or synergistic effects. Single, dual, and triple applications of these treatments effectively inhibited *Fusarium oxysporum* f. sp. *lycopersici* isolates, although the level of inhibition varied among the isolates.

Among the treatments, the triple application of AMF, biochar, and kale showed the highest inhibition in FOL1

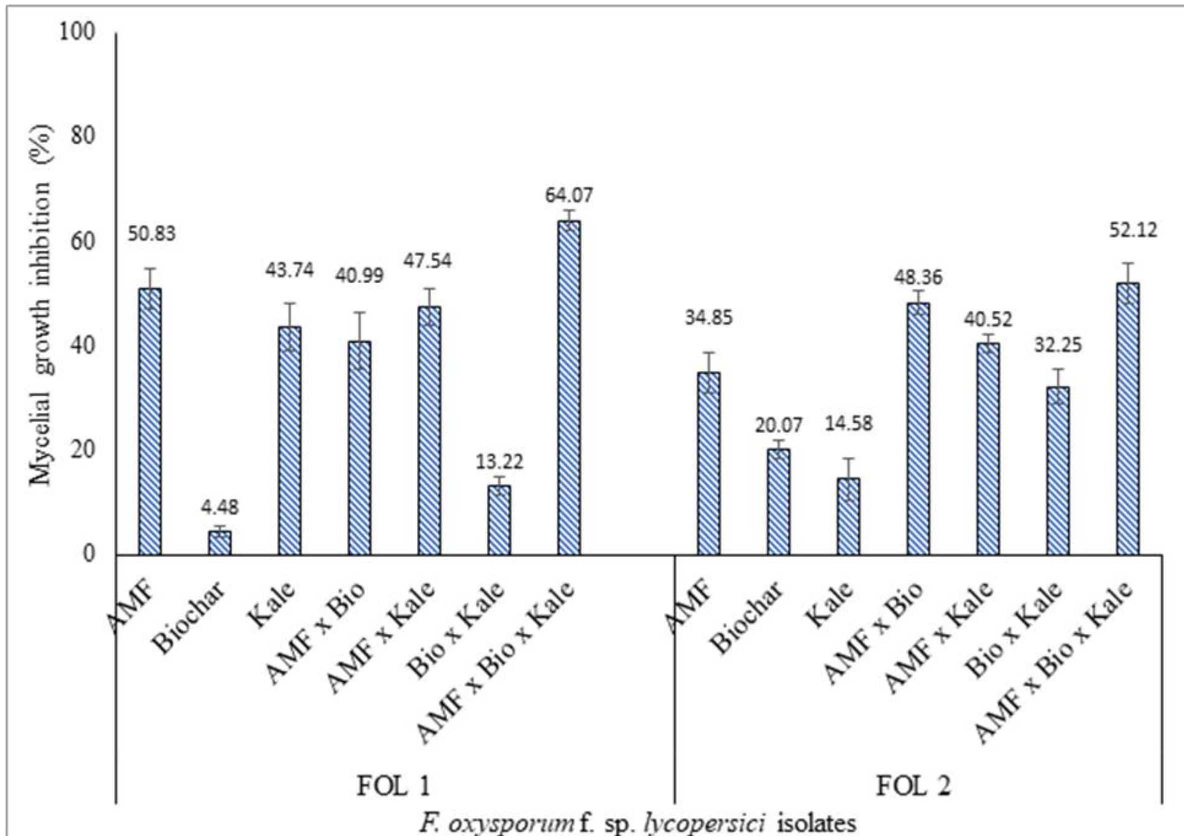

Figure 2: Interaction effect of AMF, biochar and kale biofumigation and isolates of *Fusarium oxysporum* f.sp. *lycopersici* on mycelial growth inhibition under *in vitro* conditions. The error bars indicate ± SD (Standard deviation). Different letters above the error bars denote significant differences across the treatment means.

and FOL 2, whereas biochar and kale recorded the least inhibition in FOL1 and FOL2, respectively.

The present study demonstrated the antifungal potential of volatile compounds released from kale (*Brassica oleracea* var *Acephala*) on the growth of *F. oxysporum* mycelium. These results are in agreement with the findings from other researchers on the suppressive effects of Brassicaceae on plant soilborne pathogens. The radial mycelial inhibition observed is attributed to the toxicity of isothiocyanates released by macerated kale during glucosinolate hydrolysis (Plaszkó *et al*., 2021). These compounds are hydrolysed by the action of the myrosinase enzyme and produce isothiocyanate (ITCs) and nitrile gases, which can decrease or inhibit the action of soil phytopathogens due to their biocidal effect (Meng *et al*., 2018) as cited by Vieira *et al*., (2022). Additionally, the abnormal hyphal growth observed in this study is consistent with findings by Li *et a*l., (2020) who reported that application of allyl isothiocyanate causes distortion of hyphal morphology and destruction of mycelial stuctures. According to Calmes *et al*., (2015), ITCs primarily target the mitochondria, leading to mitochondrial depolarization, which alters mitochondrial function. Fusarium infections indirectly contribute to the accumulation of reactive oxygen species (ROS) such as singlet oxygen, superoxide radicals, and hydrogen peroxide. The increased oxidative stress potentially disrupts the vital metabolic processes such as the absorption of water and nutrient uptake, transpiration, and chlorophyll biosynthesis (Abdelaziz *et al*., 2024). Despite the inhibition observed, the extent of mycelial growth varied among the FOL isolates when subjected to Kale ITC. The effect of Kale was found to be more toxic, showing highest inhibition in Fusarium oxysporum isolate one (FOL1) compared to isolate two (FOL 2). The variation in mycelial inhibition is consistent with findings by Smolinska *et al*., (2003) who reported different response among F. oxysporum isolates of the same species when exposed to isothiocyanates (ITC). The varying response among the isolates could be attributed to the variation in cellular permeability and also sensitivity of the target isolates to isothiocyanates (ITC).

In vitro, It was observed that the mycelial growth of the two FOL isolates was inhibited when biochar was added to the PDA. These agree with the findings of Yu *et al*., (2023), who reported that mixed hardwood biochar inhibited the mycelial growth of *Pythium aphanidermatum,* causing root rot in poinsettia. Similarly, Kumar *et al*., (2024) observed that biochar suppressed the growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum,* the causal agent of root and stem rot disease of cucumber. The inhibition effect of biochar observed in isolate two could relate to the direct effect of

compounds released from biochar, which have been reported to suppress the growth of harmful pathogenic microbes (Rogovska *et al*., 2017) Among the compounds that have been identified in biochar to prevent the growth of harmful pathogenic microbes are ethylene glycol, propylene glycol, hydroxy propionic, hydroxybutyric acids, benzoic acid, quinones, and 2-phenoxyethanol (Graber *et al*., 2010).

The *in vitro* efficacy of AMF on *Fusarium oxysporum* f. sp. *lycopersici* resulted in the reduction of the mycelial growth of the fungal pathogen. Findings of the current study are concurrent with those of Kumari & Prabina (2019), who investigated the inhibitory effect of arbuscular mycorrhizal fungus *Glomus* sp. on fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* in tomato plants. Results revealed that using the arbuscular mycorrhizal fungus Glomus sp. led to a clearing zone surrounding the mycorrhizal roots. The results of the study are also in line with those reported by Hare Krishna *et al*., (2016), who reported that use of AMF-inoculated maize roots resulted in inhibition of *Dematophora necatrix,* white root rot of Apple. The reduction in *Fusarium oxysporum* f. sp. *lycopersici* mycelial radial growth could be explained by the fact that AMF produces some metabolites that inhibit the mycelial growth of the fungal pathogen. Among the compounds involved in plant defense studied in relation to AMF formation are phytoalexins, enzymes of the phenylpropanoid pathway, chitinases, peroxidases, callose, hydroxyproline-rich proteins (HRGP), and phenolics.

Despite the inability of these volatile bioactive compounds to completely inhibit mycelial growth, it became apparent that the biofumigant treatment may have weakened the fungal integrity, hastening their loss of viability. Furthermore, this enhances their susceptibility to other control strategies, such as AMF and biochar, suggesting that combining biofumigation with other control techniques may improve pathogen suppression in the soil. The *in vitro* evaluation of biochar exhibited a fascinating trend. Contrary to the initial expectation, biochar exhibited low inhibition on *F. oxysporum* f. sp. l*ycopersici* isolates. Despite the relatively low inhibition against *F. oxysporum* f. sp. *lycopersici* isolates, its effect is in agreement with previous reports on the potential of biochar in suppressing soilborne pathogens (Araujo *et al*., 2021; Kumar, Meena, *et al.,* 2024; Makram-allah *et al*., 2025). Similarly, this result is in agreement with the findings of Reddy *et al*., (2023), who reported that biochar resulted in 25.5% and 42.8% inhibition of *F. oxysporum* f. sp. *ciceris* in chickpea at 1% and 5% application rate, respectively. The inhibitory effect exhibited by biochar can be attributed to phenolic compounds such as ethyl acetate and 6-2, dimethoxyphenol and organic acids found in biochar that have been recognized to have antifungal properties (Bouket *et al*., 2022). In the presence of AMF and kale biofumigation, the inhibiting effects of biochar on *Fusarium oxysporum* f. sp *lycopersici* mycelial growth were enhanced due to the impact of AMF and kale biofumigation on suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici*. The *in vitro* test showed that using AMF synergistically with biochar and kale biofumigation could positively control *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes.

The current study revealed that the *Fusarium oxysporum* f. sp *lycopersici* isolates responded differently to either individual or combined application of AMF, biochar and kale biofumigation. These indicate that the antifungal effectiveness of the selected treatments may depend on the sensitivity of the target isolate to the treatments. Despite the variability observed in the single and dual applications of AMF, biochar, and kale biofumigation, the triple application consistently resulted in the highest inhibition of mycelial radial growth across the two Fusarium isolates. The scientific rationale behind the synergistic effect lies in the complementary mechanisms of each of these treatments. These clearly demonstrate that combining AMF, biochar, and kale biofumigation can overcome isolate-level resistance / sensitivity, thus providing a reliable suppression effect on controlling the pathogen *Fusarium oxysporum* f. sp. *lycopersici* in tomato**.**

CONCLUSION

This study demonstrates the inhibitory effectiveness of AMF, biochar, kale biofumigation and their combinations in controlling *F. oxysporum* f. sp. *lycopersici* isolates. The degree of inhibition varied between the two *F. oxysporum* f. sp. *lycopersici* isolates, implying isolate-specific response. The differences in how the two isolates responded to the treatments emphasize the potential for isolate-specific sensitivity. However, their effects were more evident when AMF, biochar, and kale biofumigation were used together and consistently offered the strongest inhibition against both isolates. Therefore, developing the use of AMF, biochar, and kale biofumigation strategy can be a promising, sustainable, and eco-friendly approach to control Tomato Fusarium wilt.

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