Hungarian University of Agriculture and Life Sciences

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Combined application of biocontrol microbes and *Funneliformis mosseae* increases phenolic compound and resistance to grey mold infection on okra plants and increases Glutathione S-transferases (GST) enzyme activity and plant growth

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1. INTRODUCTION

The utilization of microbial control agents as alternatives to synthetic pesticides has emerged as a significant strategy for managing insect pests and plant diseases, offering outcomes comparable to those of chemical pesticides (Jha, 2023). This shift towards biological control agents and biofertilizers necessitates persuasive evidence and practical guidance to encourage adoption, thereby reducing reliance on harmful chemicals. Symbiotic interactions between microorganisms and crop plants have demonstrated the potential to enhance plant tolerance to pathogens and foster optimal growth and development (Kour *et al.*, 2020).

Arbuscular mycorrhizal fungi (AMF) play a pivotal role in promoting environmentally sustainable agriculture, with approximately 72% of the vascular plants associated with them (Brundrett, Brundrett and Tedersoo, 2018; Qin *et al.*, 2020). AMF aid in nutrient absorption, promote litter decomposition, and produce compounds beneficial for plant health, contributing to ecosystem biogeochemical processes (Chaudhary *et al.*, 2022; Elnahal *et al.*, 2022; Rajapitamahuni, Kang, and Lee, 2023). Additionally, incorporating *Trichoderma spp*. into compost extracts enhanced the biocontrol activity, further supporting the effectiveness of biocontrol measures (Poveda and Eugui, 2022).

Trichoderma asperellum, *Aureobasidium pullulans*, and *Streptomyces griseoviridis* are among the biological agents that have shown promise in enhancing plant resistance to pathogens and promoting growth (Subramaniam, Arumugam and Rajendran, 2016; Guzmán-Guzmán *et al.*, 2019; Zin and Badaluddin, 2020). These microorganisms modify the rhizosphere microbiome, produce bioactive compounds, and establish beneficial relationships with host plants, thereby contributing to improved plant health and stress management.

Tomatoes, okra, and sweet potatoes, which are essential crops, face challenges from various stressors, necessitating sustainable production systems (Ramírez-Cariño *et al.*, 2020; Gopalakrishnan *et al.*, 2021).

The problem stems from the necessity to reduce reliance on synthetic fertilizers and pesticides, aligning with the principles of sustainable agriculture (Vega, 2018). Understanding and leveraging plant-microbe interactions can lead to sustainable agricultural solutions that enhance plant development, mitigate infections, and increase resilience to environmental stressors, thereby addressing the multifaceted challenges facing global agriculture (Sambangi, Srinivas, & Gopalakrishnan, 2021). Embracing a multidisciplinary approach holds significant potential for devising environmentally sustainable and effective strategies to address these challenges.

In conclusion, the combined application of biocontrol microbes and AMF presents a sustainable approach to agriculture by enhancing plant resistance to pathogens, increasing phenolic compound levels, and promoting plant growth. This integrated approach offers potential benefits to agricultural and ecosystem health.

1.1 Research objectives :

1- Determination of the effect of microbial biocontrol agents (BCA) on the efficacy of mycorrhiza inoculation in okra, tomato and sweet potato.

2- Testing and comparison of the effect of combined BCA and mycorrhiza inoculation on the vegetative growth and yield of three vegetable species.

3- Testing the effect of combined treatments on the enzyme (GST) activity of okra is related to disease resistance.

4- Testing the effect of the combined treatment on phenolic compounds invokra fruits.

2. LITERATURE OVERVIEW

2.1 Okra

Okra, scientifically known as *Abelmoschus esculentus* (L.) Moench (formerly classified as Hibiscus esculentus L.), the common English name "okra or lady's finger, " is also known as gumbo bamia in Arabic nations. It belongs to the Plantae, Magnoliophyta, Magnoliopsida, and Malvaceae families (Kumar *et al.*, 2013). The plant is cultivated in almost all tropical regions, originating in Africa, and is an essential crop in tropical countries(Singh and Nigam, 2023). Okra is spreading around the globe as awareness of the crop grows. Okra has the distinction of being one of the oldest cultivated crops that is in demand for cultivation today. Its cultivation extends across different regions, including South America, North America, Africa, Asia, and southern Europe.

Ethiopia has made an inaugural discovery of okra, marking the first known presence of this crop worldwide (Al-Kanani, Al-Hilifi and Al-Kareem, 2019). Typically, self-renewal occurs in these organisms through self-fertilization, primarily because of their floral structure and absence of a self-incompatibility mechanism. However, up to 6% of outcrossings may exist in okra, depending on the species or variety, time of year, and region (Hossain, 2014). Okra is cultivated in various regions with distinct and unique differentiating traits; thus, it is vulnerable to low temperatures and drought conditions (Kumar *et al.*, 2013; Wen-xue *et al.*, 2018).

Okra, a perennial plant grown annually in temperate zones, grows to 2 m. The palmately lobed leaves had 5-7 lobes and were 10-20 centimeters (4-8 inches) wide. The flowers range in diameter from 4 to 8 cm and feature five white to yellow petals with a crimson or purple base patch. The pentagonal capsule-shaped fruit contains numerous seeds and measures up to 18 cm in size (Marak, Deepanshu and Niharika, 2023).

Figure 1: Okra fruit (finger lady) https://za.all.biz/okra-ladies-finger-g12930

Okra is frost sensitive and requires suitable climatic conditions for successful growth. Because of its tropical and subtropical nature, okra thrives in regions characterized by warm climates and ample sunlight (Wahyuningsih *et al.*, 2020). According to (Lachman-White, Adams and Trotz, 1992), the okra plant is an annual herbaceous plant with a shrub-like look that grows up to 1.5 m tall, with big leaves and solitary blooms. These are substantial, 5-7 lobed, spherical, or heart-shaped plates that may be either dark or bright green in color. Moreover, after pollination and flower wilting, a fruit called an elongated octagonal multi-seeded box with a pointed end (Figure 1), which externally resembles pepper pods, develops. The entire surface of the plant, including the fruits, is coated with tiny hairs. They all rise from leaf axils and are rounded or elongated on short stalks.

Okra is rich in essential vitamins (A and C), minerals, carbohydrates, proteins, dietary fibers, and antioxidants, which collectively contribute to reducing the risk of major chronic diseases, including type-2 diabetes, cardiovascular conditions, and digestive disorders. The consumption of diets incorporating okra has been linked to lower prevalence rates of these diseases (Elkhalifa *et al.*, 2021; Sonali *et al.*, 2023). Carbohydrates account for 6.4% of the overall composition of okra fruit, followed by protein (1.9%), fat (0.2%), dietary fiber (1.2%), minerals (0.7%), and water (89.6%). Owing to its delicate green fruits, okra is often consumed as a vegetable in several ways. The high content of unsaturated fatty acids in okra seed oil, particularly linoleic acid, is of significant nutritional value to humans. A comprehensive investigation explored the proximate chemical composition of wild and domesticated okra seeds (Badrie, 2016). Farmed seeds were

found to have higher oil, carbohydrate, and moisture contents (24.69, 51.69, and 6.51%, respectively), but wild seeds had higher protein, fiber, and ash levels. In addition, compared to the produced oil, wild oil has greater iodine, peroxide, and saponification values (Abdalwahab and Ahmed, 2020; Elnahal *et al.*, 2022).

Okra *Abelmoschus esculentus* (L.) Moench is a widely consumed vegetable that has gained attention owing to its high nutritional value and potential health benefits. Phenolic compounds, including flavonoids, phenolic acids, and tannins, are among the major bioactive constituents of okra. These compounds have been extensively studied for their antioxidant, anti-inflammatory, and other beneficial properties (Badrie, 2016).

The composition of okra phenols can vary depending on factors such as the cultivar, maturity stage, and processing methods. Flavonoids such as quercetin, kaempferol, and rutin are commonly found in okra, and are known for their antioxidant and anti-inflammatory effects (Wu *et al.*, 2020; Wang *et al.*, 2023). Okra contains phenolic acids, such as caffeic acid, chlorogenic acid, and p-coumaric acid, which are known for their potential health benefits. These phenolic acids have been associated with various positive effects on human health (Mounir *et al.*, 2021). Tannins such as catechins and epicatechins contribute to the astringent taste of okra and have shown potential antimicrobial and anticancer activities (Kaczmarek). Nevertheless, assimilation and processing of these substances may be affected by variables such as meal composition, gut microbiota, and individual differences in metabolic processes. (Bento-Silva *et al.*, 2020). Assessing the bioavailability of phenols present in okra is essential for understanding their potential health impacts and developing efficient delivery systems. Numerous studies have explored the biological activities of okra phenols (Agregán *et al.*, 2023). The antioxidant properties of these compounds have been well documented and attributed to their ability to scavenge free radicals and inhibit oxidative stress (Cañas *et al.*, 2023; Jabłońska-Trypuć *et al.*, 2023).

Okra phenols have also shown anti-inflammatory effects by modulating inflammatory pathways and reducing the production of proinflammatory mediators (Liu *et al.*, 2021; Winiarska-Mieczan *et al.*, 2023). Besides displaying antioxidant and anti-inflammatory properties, okra phenols have shown promising potential for various therapeutic applications (Elkhalifa *et al.*, 2021). These include their roles in cardiovascular health, gastrointestinal health, metabolic disorders, neuroprotection, and skin health (Elkhalifa *et al.*, 2021; Cañas *et al.*, 2023).

Diseases of Okra

In comparison to other crops, okra has received less attention and has a worse breeding record for resistance to pests and diseases, yet its output has grown significantly over time. Numerous causes, including the impact of production practices on plant physiology, notably in India and Nigeria, advancements in technology, and the crop's preference for warm climates, may explain this rise in warm climates. Nonetheless, climate change seems to have this benefit and has a detrimental impact on the crop's capacity to survive by bringing about new issues, such as an increase in pests and illnesses that threaten the crop. The most important diseases of the okra plant cause loss *of yield, quality and quantity of the fruits, Cercospora leaf spot, caused by Cercospora abelmoschi* and *C. malayensis*, damping-off, induced by Pythium species and *Rhizoctonia solani*, are the particular fungal diseases that affect okra. *Erysiphe cichoracearum* causes powdery mildew, *Sclerotium rolfsiii* causes Southern blight, *Verticillium alboatrum* causes Wilt disease, *and* Choanephora cucurbitarum causes wet rot (Raid and Palmateer, 2006).

Yellow vein mosaic disease (OYVMD)

Okra is resistant to pests, illnesses, heat, and drought, even if they may be damaged. Other difficulties include okra yellow vein mosaic disease (OYVMD), a very damaging biotic stress syndrome caused by the virus. This virus is persistently transmitted by the whitefly vector (*Bemisia tabaci*) (Umar *et al.*, 2023). Yellow vein mosaic virus infections, linked to climate change, cause severe crop loss and destruction. The mosaic virus may reduce okra production by 94% depending on when it is infected. Diseased plants produce small, deformed, difficult, pale yellow fruits (Sisodia and Mahatma, 2020). White-fly control and IPM are essential. Instead of timing-based techniques, need-based solutions that fit the current agroecosystems might improve management. This strategy would provide sustainable and efficient white-fly control and their repercussions (Dhole *et al.*, 2023).

Damping Off:

Damping off, which is produced by *Pythium spp*. and *Rhizoctonia solani* (Kühn), hinders okra cultivation. Okra root rot caused by *Rhizoctonia solani* is a major threat. Recent studies have focused on saprophytic microflora as a biological control for plant pathogenic fungi. Pseudomonas populations were abundant in the agricultural rhizospheres. Beneficial rhizobacteria, which help plants grow, create growth-promoting compounds (Muthukumar, Udhayakumar and Naveenkumar, 2016). Damping-off is one of the most widespread diseases caused by phytopathogenic agents. A variety of soil-borne pathogenic fungi have been linked to pre-emergence damping-off or post-emergence damping-off. That is the case with Pythium

spp.(Lamichhane *et al.*, 2017). The oomycete *Pythium spp*. is a significant soil-borne plant disease that causes damping-off in over 300 vegetable plant species (Kamoun *et al.*, 2015).

Fusarium Wilt:

Fusarium Wilt (*Fusarium oxysporum f. sp*. vasinfectum (Snyder & Hansen]) is a long-term soil fungus that causes this type of disease. The first sign is brief wilting that develops over time, causing lasting harm to more plants (Bellini *et al.*, 2023). Over time, these symptoms develop and kill the plants. Leaf withering and yellow or brown discoloration in the vascular bundles of the collar region occur suddenly toward the conclusion of the plant. The root and vascular systems are colonized by fungi. Thus, water mobility is hindered and fungal toxins affect cellular activities (Zulfadli *et al.*, 2023). Cutting of the stem base revealed a black woody region, suggesting disease severity. Biological control agents and resistance inducers may operate in integrated pest management (IPM) (Bellini *et al.*, 2023).

Verticillium wilt:

Verticillium wilt is one of the worst and most pervasive vascular diseases affecting vegetables, ornamental plants, and tree crops. Verticillium, a group of fungi, has a lengthy taxonomic history. To date, approximately 190 species have been described(Yan *et al.*, 2018). In addition, Verticillium wilt prefers cold, wet soils with temperatures between (70-81^{o}F). In the context of integrated pest management (IPM), alternative control methods such as applying biological control agents and using resistance inducers present promising solutions. These methods involve the incorporation of beneficial microorganisms such as Trichoderma spp., which can effectively contribute to disease management and prevention (Kowalska, 2021).

Powdery Mildew:

Powdery mildew caused by (*Erysiphe cichoracearum alphaendornavirus* (EcEV)): The fungus infects several plant species, causing serious production losses in economically important vegetables and crops(Rm *et al.*, 2022). By competing with host sinks for resources, powdery mildew fungi may affect the host photoassimilate synthesis and partitioning. The powdery mildew on crops and grains is growing due to climate change. Previously unaffected plants may become infected (Bhardwaj, Banyal and Roy, 2021). As plant-endophyte interactions might increase plant resistance to phytopathogens, microorganisms are utilized as a biological control for this disease. In particular, endophytic bacteria benefit plants (Jiao *et al.*, 2023).

Cercospora Leaf Spot:

Fungal infections from *Cercospora abelmoschi* and *C. malayensis* hinder okra development and output. These diseases severely restrict okra growth and productivity, and Cercospora leaf spot, leaf blight, and fruit rot hinder okra growth and yield. These diseases damage okra plant health and productivity by affecting its leaves and fruits (Na and Hipertensiva, no date). Small brown irregular dots surrounded by pink color appear on the infected okra (Kumar *et al.*, 2010; Hayamanesh, 2018).

Grey mold:

Grey mold caused by *Botrytis cinerea* Pers. Fr. Botrytis spp. includes various plant pathogenic species that harm crops (Orozco-mosqueda *et al.*, 2023). Grey mold, attributed to the fungal pathogen *B. cinerea* Pers., is a prevalent disease responsible for its development. belonging to the family Sclerotiniaceae and the class Leotiomycetes. Members of this genus are known to be morphologically diverse, and members of the same species can demonstrate morphological dissimilarity and plasticity (Garfinkel *et al.*, 2019).

Botrytis cinerea (Ascomycota) causes gray mold on over 200 plant types, such as fluffy mycelia. This has caused $10–100 billion in global damage. It resists several plant defense chemicals. It is one of the most researched necrotrophic plant infections (Robson, 2020). *Botrytis cinerea* releases many asexual spores that germinate on plant surfaces and develop an appressorium and a penetration peg that breaks the plant cuticle. Secreted enzymes include cutinases and lipases, and the penetrating peg tip generates H2O2. The penetration peg enters an epidermal cell and commonly develops into a pectin-rich cell wall perpendicular to the plant surface when the cuticle is broken. Low pectin plant species are poor hosts for *Botrytis cinerea*, which contains pectinolytic machinery (Boddy, 2016).

Botrytis cinerea produces several low-molecular-weight metabolites that kill hosts, including botrydial, oxalic acid, and HSTs. *Botrytis cinerea* causes an oxidative burst, free radical buildup, and hypersensitive cell death in plant cells during cuticle penetration and the first lesion development. Programmed plant cell death protects against biotrophic infections (Jeblick *et al.*, 2023), yet necrotrophs such as *Botrytis cinerea* feed on dead cells. This fungus suppresses host immunity by silencing genes with short RNA (sRNA) molecules (Boddy, 2016).

Botrytis cinerea generates cellulases, hemicellulases, and pectinases that degrade plant cell walls for nutrition. *Botrytis cinerea* is the primary species associated with bud rot (Punja and Ni 2021). The most damaging Botrytis species is *Botrytis cinerea* Persoon, which may cause damage to

economically significant crops (Wang *et al.*, 2022). Likewise, over 500 species of vascular plants are susceptible to infection by the opportunistic fungus *Botrytis cinerea* (Windram, Stoker and Denby, 2015). Botrytis-related fungi are significant pre- and post-harvest diseases of several commercially significant crops, including grapevines and many other crops (Windram, Stoker and Denby, 2015). Because it may live as mycelia, conidia, or sclerotia in crop waste for a long period, *Botrytis cinerea* is challenging to manage because it has a variety of attack mechanisms and various hosts as inoculum sources. Owing to these factors, it is doubtful that any control method would be effective. Hence, it is crucial to have a thorough knowledge of the host-pathogen relationship, the fungus's operating environment, and its microbial rivals on the host (Adnan *et al.*, 2019). Ahmed and El-Fiki (2018) found that *Trichoderma harzianum* antagonists increased strawberry total phenols, nitrogen percentage, and chlorophyll content compared to the control group. In vitro, linear growth suppression of *Botrytis cinerea* fruit rot pathogens was best achieved using *Bacillus sp., T. asperellum* (T34), and *T. viride*.

The tested treatments reduced fruit rot disease incidence (D.I.) in the field better than switching (synthetic fungicide) and tap water. Spraying strawberry fruits with *T. asperellum* (T34) before harvest is the best way to avoid fruit deterioration, decrease colour change, decay, and maintain reasonable shape, firmness, acidity, TSS%, and weight loss (Rashid, Adbelghany and Abd-EL-Hamed, 2022).

2.2 Tomato

Tomato (*Solanum esculentum* Mill, family Solanaceae) is a highly developed horticulture crop. It has been growing extensively worldwide. It covers an area of over 5 million hectares and yields more than 182 million tons of tomato annually. However, the combination of monoculture conditions, rigorous selection, domestication over recent decades, global trade of infected plant material, and climate change has greatly facilitated the emergence and rapid spread of numerous pathogens, enabling organisms to thrive in new and unfavorable environments (Caruso *et al.*, 2022).

Tomato is the consumable fruit of the *Solanum esculentum* plant, sometimes referred to as the tomato plant. This species originates in the western regions of South America, Mexico, and Central America (Hyman, 2019). The beginning of its domestication and cultivation as a food source may be traced back to the indigenous Mexican population, which introduced the plant to Europe as part of the extensive transfer of plants known as Columbian trade. Subsequently, tomatoes were disseminated to other regions of the European-colonized globe in the 16th century (Hyman, 2019).

It is commonly consumed in various forms, including salads, cooked vegetables, and as ingredients in processed items such as canned tomatoes, tomato juice, and ketchup, as well and they are often utilized as vegetables or side dishes and dehydrated pulp marketed as "sun-dried" tomatoes (Deribe, Beyene and Beyene, 2016; Kafle *et al.*, 2023) in addition, Tomatoes are umami-rich. Various tomato cultivars are cultivated worldwide in temperate areas, with greenhouses enabling year-round production (Ali *et al.*, 2020; Reddy, 2021). The average tomato plant height is 1–3 m. These vines have weak stems that spread and require assistance (Reddy, 2021), and native indeterminate tomato plants are perennials that grow as annuals. (Determinate, or bush, annuals cease growing at a set height and yield a crop immediately.) The breadth of tomatoes ranges from 1 to 10 cm depending on the variety (Bulgan, 2021).

Since tomato-based products are high in bioactive elements, such as carotenoids and antioxidant vitamins, they are thought to provide nutritional benefits (vitamins E and C). Minerals present in tomatoes are important for both human and animal diets (Gould, 2013). A decrease or inhibition of fruit set is now the most significant consequence of high temperatures, and water deficiency is another important factor influencing tomato production and quality.

Nutritional value of tomatoes

Tomato, scientifically known as *Lycopersicon esculentum* Mill, exhibits a notable water content ranging from 93% to 95% and a relatively low solid content. Within tomatoes, there is a considerable presence of antioxidants, including provitamin carotenes, ranging from 6 to 9 mg kg-1 and vitamin C content ranging from 160 to 240 mg kg-1. Moreover, the tomato also contains trace minerals such as copper, ranging from 0.1 to 0.9 mg kg-1, manganese from 1 to 1.5 mg kg-1, and zinc from 1 to 2.4 mg kg-1. These minerals play crucial roles in antioxidant enzyme function. Additionally, tomatoes contain 5–20 mg kg-1 vitamin E and 5–50 mg kg-1 flavonoids (Bilton *et al.*, 2001; Ali *et al.*, 2021; Collins *et al.*, 2022). In addition to various other health benefits, fruits and vegetables are exceptional sources of natural antioxidants in the human diet. The antioxidant components present in fruits and vegetables play a vital role in protecting the body against harmful free radicals.

Moreover, there is substantial evidence linking the consumption of antioxidant-rich fruits and vegetables to a reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-related functional decline (Wang, Cao and Prior, 1996; Liu *et al.*, 2000; Zhu *et al.*, 2018; Neysanian *et al.*, 2020).Tomatoes are rich in minerals, vitamins, proteins, essential amino acids (e.g., leucine, threonine, valine, histidine, lysine, and arginine),

monounsaturated fatty acids (such as linoleic and linolenic acids), carotenoids (including lycopene and β-carotenoids), and phytosterols (such as β-sitosterol, campesterol, and stigmasterol). Lycopene, the primary carotenoid found in tomatoes and tomato-based food items, protects against cancer, cardiovascular illnesses, cognitive decline, and osteoporosis when consumed by humans. Tomatoes contain phenolic chemicals that possess antioxidant properties and may effectively protect the human body from oxidative stress-related ailments.

Consuming tomatoes in one's diet enhances the body's antioxidant levels, effectively capturing reactive oxygen species and diminishing oxidative harm to vital biomolecules, including membrane lipids, enzyme proteins, and DNA. This helps to alleviate oxidative stress (Çolak *et al.*, 2023). In addition, among the different tomato varieties, red tomatoes exhibited the highest concentrations of total carotenoids and the most significant antioxidant activity. Following red tomatoes, purple, orange, pink, and yellow tomatoes display progressively lower levels of total carotenoids and antioxidant activity (Zhu *et al.*, 2018; Kurina *et al.*, 2021).

2.3 Sweet potato

The sweet potato, scientifically known as *Ipomoea batatas* (L.) Lam., which belongs to the family Convolvulaceae. It is ranked the ninth most significant food crop worldwide, with an annual output of over 100 million tons. It is cultivated across 8.62 million hectares of land in over 100 countries (FAO, 2020). Sweet potato is native to tropical regions of the Americas (Temmen *et al.*, 2022). It is sometimes known as sweet potato and is a root crop that is grown worldwide. It is abundant in various vitamins and minerals that are crucial for human growth and development. Sweet potato tubers were introduced to Europe by Columbus in 1492, and then to Africa, India, Southeast Asia, and East India by Portuguese explorers in the 16th century. In the sixteenth century, Mexican sweet potatoes were sent to the Philippines (Sawicka *et al.* 2021). In addition, this species was widely recognized before the potato (*Solanum tuberosum* L.) was discovered. It was originally reported in Spain in about 1564 (Zhang *et al.*, 2009); however, both biotic and abiotic conditions severely restrict the ability of the crop to grow. This plant is a perennial vine with herbaceous characteristics. It has either triangle-shaped leaves or is palmately lobed, and its blooms are medium-sized and sympetalous. The stems often creep over the ground, producing adventitious roots at nodes. The leaves were tightly spiraled around the stems. The petiole is between 5 and 20 in in length. The length of a stem may range from 0.5 to 4 m depending on the type. The flowers are hermaphroditic and consist of five petals and short stalks. They are either solitary or few in number, arranged in inflorescences that are stalked and zymously arranged (Nair, 2023).

The delicious tuberous root is elongated and gradually narrows, with a sleek outer layer that exhibits a spectrum of colors, including yellow, orange, red, brown, purple, and beige. The flesh

color varied from beige to white, red, pink, violet, yellow, orange, and purple (Figure 2). Sweet potato varieties with white or light-yellow flesh have lower sweetness and moisture levels than those with red, pink, or orange flesh(Buchmann, 2015; Nair, 2023).

Figure 2: Sweet potato tubers.

The species has remained a botanical curiosity for decades since the first sweet potato clones brought to Europe were of tropical origin and were unable to acclimatize to the temperate European environment. Soon after its introduction to agriculture in southern Europe, new and chosen sweet potatoes have spread rapidly around the globe (FAO, 2020). Many European nations, notably Portugal (24,000 tons), Spain (14,000 tons), Italy (13,000 tons), Greece (4,000 tons), and France (more than 5,000 tons), have previously farmed sweet potatoes (FAO, 2020). This is significant not only for Southwest Europe but also for Central and Central Europe, where there is a rising trend toward veganism and the need for secure gluten-free food. As a result, this area of Europe had the opportunity to diversify its agricultural production. The market for sweet potatoes in the EU is worth more than 350 million euros, and more than 300,000 tons are imported each year. In this part of the world, the demand for sweet potatoes is increasing by up to 12% annually (FAO, 2020).

The cooked sweet potato, which is baked with its skin, has a water content of 76%, carbs make up 21% of its composition, protein accounts for 2%, and it has little fat, according to the table. A 100-gram serving of baked sweet potato has 90 calories and is a rich source (20% or more of the Daily Value, DV) of vitamin A (120% DV), vitamin C (24% DV), manganese (24% DV), and vitamin B6 (20% DV). It provides a modest amount (10-19% Daily Value) of some B vitamins and potassium (Wang, Nie and Zhu, 2016).

2.4 Antagonistic microbes

Microorganisms can colonize surfaces, plant tissues, and organs. Plant genes and a gene module that supports plant-microbe symbiosis govern the involvement of microorganisms in plant defense (Delaux and Schornack, 2021; Dong *et al.*, 2021). Plants require accurate identification of symbiotic bacteria to avoid pathogens. Protein signals help plants to identify microorganisms. Mycorrhizal, endophytic, and rhizobial fungi generate effector proteins that regulate plant immunity and symbiosis (Miwa and Okazaki 2017). In salt, drought, and waterlogging, the rhizosphere produces more ethylene which limits root growth (Zapata *et al.*, 2003; Glick *et al.*, 2007). Inoculation with *Methylobacterium* and *Burkholderia* may reduce ethylene release and promote tomato growth by lowering nickel and cadmium toxicity. Metabolic exchange between microbial populations and hosts is essential. Photosynthesis provides carbohydrates to the rhizosphere microbial community, whereas fungi and bacteria assist plants in absorbing phosphate, nitrogen, and iron (Raaijmakers and Mazzola, 2012).

Plants may draw soil microorganisms to the rhizosphere during droughts, floods, insect pest infestations, or excessive salt levels. Stable microbial communities exhibit complicated nutritional competition, metabolic exchange, and interdependent interactions. This cohabitation may boost the plant stress resistance. A thorough analysis of rhizosphere microorganisms under stressed conditions is necessary to correctly design and employ synthetic microbial communities and to apply suitable ecological management (Ge *et al.*, 2023).

A diverse range of soil bacteria, primarily found in the rhizosphere of plants but also present in or near plant tissues, play a crucial role in promoting plant growth through various mechanisms. These microorganisms are commonly referred to as plant growth-promoting rhizobacteria (PGPR). Apart from positively influencing root development and morphology and enhancing nutrient availability in the rhizosphere, PGPR facilitates beneficial plant-microbe symbiosis, further contributing to plant growth and development. (Ge *et al.*, 2023)(Ge *et al.*, 2023). The use of plant growth-promoting rhizobacteria in agriculture and horticulture has received the most attention from researchers.

Several plant growth-promoting rhizobacterial formulations are being offered as commercial goods for agricultural productivity. By engaging in antagonistic activity, biofertilizers promote plant growth, boost fruit output, and decrease the tomato disease (nematode) population (10.66 was the highest amount of fruits produced by each plant), and there were notable variations from untreated plants. High yield and few nematode eggs were found in the soil of treated plants (Almaghrabi, Massoud and Abdelmoneim, 2013)(Aioub, Elesawy and Ammar, 2022). According to Chinese experts, the utilization of biofertilizers resulted in a significant reduction in tomato

disease incidence by 66.1% and 73.6%, respectively, compared with the control group. In addition, the use of bacteria-based biofertilizers led to substantial improvements in tomato yield, ranging from 49.5% to 70.8%(Guo *et al.*, 2004)(Aioub, Elesawy, and Ammar, 2022). The effect of plant growth-promoting rhizobacteria on reducing abiotic stressors in plants has been investigated over the last several decades (Dimkpa *et al.*, 2020)(Nadeem *et al.*, 2014)(Nadeem *et al.*, 2014). These bacteria trigger the plant's antioxidant defense mechanisms in response to salt stress to control the activity of several enzymes that scavenge reactive oxygen species (ROS)(Nadeem *et al.*, 2014)(Nadeem *et al.*, 2014). It has been shown that rhizobacteria promote drought tolerance by controlling the concentrations of crucial phytohormones, polysaccharides and proteins. Field tests are currently being conducted to improve tomato plant growth and promote rhizobacteria-mediated salt and drought resistance (Nadeem *et al.*, 2014; Singh *et al.*, 2018).

Cultivars of sweet potatoes with dark orange flesh have higher levels of beta-carotene, which is transformed into a greater amount of vitamin A after digestion. Owing to the prevalence of vitamin A insufficiency in Africa, there is a push to promote the cultivation of these sweet potato varieties (Ley 25.632, 2002).

Biofertilizers and microbial inoculants have become increasingly important, especially in the agricultural sector, as a reaction to the widespread problem of improper and excessive use of pesticides and artificial fertilizers. The use of biopesticides and biofertilizers in conventional and organic agricultural methods is promising (Rady *et al.*, 2020). The capacity of microbial inoculants to boost growth and inhibit plant diseases is two key factors that determine their utility in agricultural applications (Kamil *et al.*, 2018). Bioproducts offered to boost plant performance are known by numerous names, such as biostimulants, plant-strengthening agents, bio-fertilizers, and plant growth-promoting microorganisms (Bhardwaj *et al.*, 2018).

The word "biofertilizer" refers to soil microorganisms that boost plant mineral nutrient availability and absorption. These biofertilizers may promote plant development by suppressing plant diseases (Jaber and Enkerli, 2016; Kumar *et al.*, 2022). Both abiotic and biotic plant stresses can alter the interaction between plants and pests, making host plants more susceptible to pests and reducing their ability to compete against plant diseases. The detrimental effects of these stressors on plant growth and survival are further amplified by climate change, including shifts in precipitation patterns. Endophytic microbes have garnered attention because of their ability to stimulate plant growth and enhance plant responses to abiotic and biotic stress conditions. These microorganisms produce secondary metabolites that protect the host plant from stressful climatic conditions and phytopathogens (Kumar and Nautiyal, 2022).

Microbial control agents have replaced synthetic pesticides for insect and plant pathogen control. Bacteria, fungi, and protozoa may benefit, harm, or neutralize host plants. A study conducted by (Al Hamad *et al.*, 2021) examined the function of beneficial microorganisms as biofertilizers or biopesticides in crop productivity and protection. However, beneficial microorganisms must be promoted to persuade farmers to employ biological control agents and biofertilizers instead of hazardous chemical pesticides and fertilizers (Elnahal *et al.*, 2022). Microorganisms may help meet the nutritional needs of plants, ensure food safety, and prolong crop output. Microorganisms may help agricultural plants grow, develop, and fight pathogens. Their metabolites are used for plant growth stimulation, biocontrol, mass manufacturing, formulation, and commercial use. Bio-complexes, such as biofertilizers and biopesticides, produce plant growth regulators and siderophores, boost nutrient absorption, increase yield, and produce antagonistic substances, such as antibiotics, hydrolytic enzymes, hydrogen cyanide, and volatile organic compounds (Elnahal *et al.*, 2022).

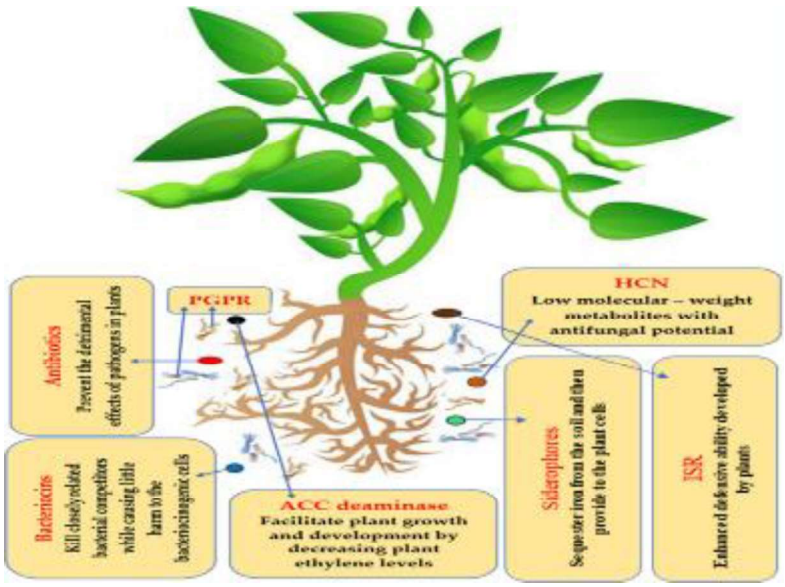


Figure 3: Mechanisms of plant growth-promoting rhizobacteria (PGPR) during the plant-microbe interactions. ACC 1-aminocyclopropane-1-carboxylic acid, HCN hydrogen cyanide, and ISR induce systemic resistance (Elnahal *et al.*, 2022).

Bacteria that inhabit the rhizosphere and demonstrate the ability to promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR). PGPR have been utilized in agriculture because of their capacity to enhance plant growth and control pests. Scientific evidence has shown that plants derive advantages from their interactions with PGPR (Figure 3). These interactions can result in improved plant health and growth promotion, suppression of disease-causing microorganisms, increased nutrient availability, and accelerated nutrient assimilation. In contrast, the eukaryotic domain, which encompasses filamentous fungi, yeasts, algae, protozoa,

and nematodes, exists at lower population densities in the rhizosphere. For instance, the density of fungi ranges from 105 to 106 cells per gram of root tissue, whereas algae and protozoa have densities of approximately 103 cells per gram of root tissue. These findings have been reported by (Beattie and Thomson, 2007; Vega, 2018). Furthermore, viruses and bacteriophages have been observed at a density of 108–109 cells/g of soil, as documented by (Ashelford, Day and Fry, 2003; Lefort *et al.*, 2016). Scientists have categorized studies of beneficial microorganism-plant interactions into three distinct groups. The first category involves microorganisms engaged in plant nutrition, directly or indirectly, and interacting with the plant. Nitrogen-fixing bacteria were used as examples. The second category is composed of biocontrol agents, microorganisms that indirectly boost plant growth by impeding the growth of plant pathogens. The third category encompasses microorganisms that directly affect plant growth via diverse mechanisms. These microorganisms generate plant hormones, aid phosphate solubilization, improve iron absorption, and emit volatile compounds. Studies conducted by (Nihorimbere *et al.*, 2011; Podile *et al.*, 2014; Vega, 2018; Koza *et al.*, 2022) have explored the roles of microorganisms within this category. Numerous microorganisms associated with plants offer significant advantages to both ecosystems and agricultural production. They serve as sustainable and environmentally friendly alternatives to chemical fertilizers and pesticides. These benefits were highlighted in studies conducted by (Gathage *et al.*, 2016; Al Raish *et al.*, 2021). The ecosystem is composed of a convoluted network of ecological niches and interdependent relationships between various creatures. Symbiotic relationships are common in the environment and play a significant ecological role, particularly when they involve both autotrophic and heterotrophic organisms (Arora 2013).

The environment comprises intricate ecological niches and interactions between various organisms. Symbiotic relationships are prevalent in the biosphere and are of significant ecological importance, particularly when they involve autotrophic and heterotrophic organisms. These associations can be categorized into three types of symbiosis: mutualism, exemplified by interactions such as bacterial root nodules supporting each other the nutrients or mycorrhiza to help the plant uptake water and some minerals such as phosphate, where both organisms derive benefits; commensalism, where one organism benefits without causing harm to the other; and antagonism, where one organism benefits at the expense of the other (Grgurina *et al.*, 2002; Mutalipassi *et al.*, 2021). These interactions can manifest as parasitism, wherein a parasitic organism benefits without causing substantial harm to its host (Grgurina *et al.*, 2002; Mutalipassi *et al.*, 2021; PulAveragety *et al.*, 2021). The interaction between microorganisms and plants, particularly microorganisms, constitutes an ideal system for fulfilling their requirements related to nutrition, water availability, defense against unfavorable environmental conditions (such as water

scarcity, radiation, and extreme temperatures), and competition (Koza *et al.*, 2022). Consequently, plants serve as significant habitats for numerous heterotrophic organisms, offering a nutrient-rich environment. Endophytism is a noteworthy example of microorganism/plant interactions. The definition of the term "endophyte" has evolved over the past decade, leading to substantial discord regarding its precise characterization (King, 2020). The term "endophyte" was coined by De Bary in 1866 to delineate the microorganisms that inhabit the inner tissues of stems and leaves (Rice, 2003). Subsequently, De Bary's original definition underwent multiple revisions and modifications.

Two extensively acknowledged definitions are as follows: endophytes establish residence within the living internal tissues of the host without inducing symptoms, despite the potential for the endophyte to provoke disease following an incubation or latency period (Bowes, 2020). Endophytes are fungi or bacteria that engage in inconspicuous and symptomless infections confined entirely within plant tissues throughout all stages of their life cycle (Rice, 2003). Currently, endophytic fungi are an important group of organisms in association with plants that are able to colonize all plant internal tissues and improve their fitness (Sohrabi, Samsampour and Bagheri, 2023).

Microorganisms have a dual influence, bearing both advantageous and detrimental effects on humans, plants, and the environment. The spectrum of plant-microbe interactions encompasses both mutually beneficial partnerships and pathogenic relationships. This dichotomy in microbial behavior hinges upon the interplay of abiotic and biotic factors in their surroundings (Sohrabi, Samsampour and Bagheri, 2023). Among these microorganisms, endophytes have emerged as plant symbionts that confer benefits to plant well-being. Conversely, certain microbes, known as pathogens, reside within plants and can cause harm. However, the intricacies of the interactions among endophytic communities have received limited attention. Environmental conditions substantially influence the behavior of plant-associated microbes. The dynamic nature of plant-microbe interactions has significant prospects for future research. The escalating global demand for resources derived from medicinal plants underscores their heightened exploitation for research and developmental purposes (Sohrabi, Samsampour and Bagheri, 2023). Colonization can have a major impact on plants, manifested by an increased tolerance to abiotic and biotic stresses, an increase in vigor, or an alteration in physiology. Research over the last 20 years has demonstrated extreme specialization of the endophytic colon (Ruiz *et al.*, 2014).

2.4.1 *Trichoderma asperellum*

Trichoderma spp. are well-known antagonistic fungi that have been marketed to control a variety of crop diseases (Zin and Badaluddin, 2020; Ferreira and Musumeci, 2021). Trichoderma was identified as the genus with the best biological control capability among the 25 fungal antagonists for controlling plant fungal infections (Rabiey *et al.*, 2019; Rai and Singh, 2023). A genus of filamentous fungi called Trichoderma has more than 260 species, 35 of which are economically significant as biological control agents (BCAs) in agriculture or as manufacturers of enzymes and antibiotics in industry (Shankar Naik, Abrar and Krishnappa, 2019; Sirikamonsathien, Kenji and Dethoup, 2023). Therefore, adding plant nutrients or biocontrol inoculants such as Trichoderma spp. could improve the biocontrol activity of compost extracts (Poveda and Eugui, 2022). Trichoderma can coexist with plants as an endophytic, epiphytic, or rhizospheric microbe without inhibiting the vascular bundles ('Poveda et al', no date; Rabiey *et al.*, 2019; Sirikamonsathien, Kenji and Dethoup, 2023). In addition, Trichoderma, when used in agriculture, can increase plant tolerance to abiotic stressors, promote plant growth, and function as a direct and indirect BCA (Guzmán-Guzmán *et al.*, 2019; Zin and Badaluddin, 2020).

Biostimulants, including plant growth-promoting rhizobacteria (PGPR) and BCAs such as *Bacillus thuringiensis* and *Trichoderma spp*., are the most common bioinoculants used in agriculture (Qiu *et al.*, 2019). In numerous host-pathogen systems, both in vitro and in situ, *Trichoderma spp*. alone or in combination with other advantageous microbes are the most prevalent and successful biocontrol agents for disease control (Ansari, Rizvi and Mahmood, 2020; Poveda and Eugui, 2022). Trichoderma fungi, through mycoparasitism, antibiosis, and competition for space and resources, help suppress pathogen growth and have a favorable impact on plant growth. Specifically, root system development is considered desirable during drought periods (Modrzewska *et al.*, 2022). Although the use of microbial bioinoculants in agriculture may help satisfy present and future production needs, it is crucial to create formulations that enable microorganisms to thrive in novel habitats and effectively colonize soil and plant tissues (Qiu *et al.*, 2019).

2.4.2 Aureobasidium pullulans

Aureobasidium spp. are widespread fungi found in various settings and are widely distributed in different environments, including lakes, oceans, rivers, rocks, soil, animals, and plant tissues. Suggesting that they can sense and respond to various extracellular signals and be adapted to different environments (Bahram and Netherway, 2022; Chiu *et al.*, 2022; Di Francesco, Zajc and Stenberg, 2023). However, Many fungi rely on melanin produced by *A. pullulans* as a key component of their resistance to a variety of stressful environments. Meanwhile, melanin and the

pathogenicity of fungi are strongly connected. However, under ideal growth conditions, melanin is not necessary for fungal growth and development (Jiang *et al.*, 2016). In addition, *Aureobasidium spp*. are dimorphic fungi with both primary yeast cells and small filamentous cells, because most strains of yeast-like fungi can synthesize melanin and deposit it in their cell walls (Li *et al.*, 2015). In addition, this observed abundance has led several scientists to investigate the possibility of biocontrol of a number of plant diseases, including Botrytis cinerea (. M. Yalage Don *et al.*, 2020).

Aureobasidium spp. are capable of producing a wide range of metabolites, several of which have practical uses in treating plant diseases (Di Francesco, Zajc and Stenberg, 2023). Aureobasidium species can produce and secrete significant amounts of liamocin, polymalate, siderophores, fumaric acid, and β-glycans (Zhao *et al.*, 2019). They also produce several extracellular enzymes that utilize a variety of substrates, including pullulan, starch, and glucoamylase, for hydrolysis (Liu *et al.*, 2008, 2018). (Shi *et al.*, 2022) demonstrated how the use of *Aureobasidium pullulans* affects bacterial and fungal populations, changing it in a way that can prevent plant pathogens and lower the occurrence of fruit diseases. However, Numerous microorganisms have been shown to be capable of biologically controlling infections; therefore, chemical fungicides may be replaced by antagonistic microorganisms because of their efficiency, affordability, and safety ((X. Zhang *et al.*, 2020). Aureobasidium spp. have the potential to be used as biocontrol agents and plant growth promoters because of their capacity to reproduce quickly, generate antibiotics and cell-wall-degrading enzymes, establish resistance in host tissues, and create plant growth regulators. Many yeast genera have been used to combat postharvest illnesses, particularly those affecting fruits. Strong evidence points to the possible biological management of illnesses caused by soil-borne fungal plant pathogens by yeast (El-tarabily and Sivasithamparam, 2006).

2.4.3 Streptomyces griseoviridis

Streptomyces spp. is the largest genus in the family Streptomycetaceae, belonging to the order Actinomycetales of the class Schizomycetes (Sharma, Gautam and Saxena, 2014; Jones and Elliot, 2017). The actinomycete *Streptomyces spp*. can control many fungal plant pathogens in vegetable crops (Hernandez-montiel, 2022; Haq *et al.*, 2023). Beneficial microorganisms have a significant positive impact on a variety of human and agricultural activities, with Streptomyces species being one of the most promising plant promoters because of their diverse roles in biofertilizers, biostimulation, biocontrol agents, and bioremediation (Al-tammar and Khalifa, 2023). The utilization of plant growth-promoting *Streptomyces sp.* provides an alternative technique for disease management that does not harm the environment, plants, animals, or human health (Art *et al.*, 2018). Owing to their environmental friendliness, low production costs, and decreased use of

nonrenewable resources, the use of bacteria with antimicrobial capabilities has emerged as one of the most attractive solutions for improving the sustainability of agricultural production. They make up a significant portion of the biomass of soil-dwelling microorganisms and can produce a broad range of secondary metabolites. It can also produce beneficial bioactive metabolites, including insecticides, fungicides, antibiotics, and herbicides (Subramaniam, Arumugam and Rajendran, 2016). In addition, Streptomyces species are prevalent in the soil and are significant rhizobacteria that aid in stimulating plant growth (Karimi and Noori, 2022). Several studies have established that Streptomyces strains can promote plant growth to enhance plant health, nutrient uptake, and defense-trigger responses. In addition, the rhizosphere microbiome is modified to support more significant growth and manage stress conditions (Song *et al.*, 2015; Ramírez-Cariño *et al.*, 2020; Gopalakrishnan *et al.*, 2021; Nah *et al.*, 2021; Sambangi, Srinivas, and Gopalakrishnan, 2021; Abbasi *et al.*, 2022). Moreover, it renders plants resistant to diseases (Nah *et al.*, 2021).

Actinomycetes have received increasing attention in several agricultural sectors as viable and profitable sources of proactive chemicals and biocontrol techniques. In addition, Streptomyces, which produces more than 70% of all antibiotics, has produced 60% of the new herbicides and pests in the past 30 years. Extensive research is being conducted worldwide to develop effective formulations containing actinomycetes as active ingredients and to increase our knowledge of the mechanisms activated by actinomycetes. This enhances plant nutrient uptake, stimulates and increases phytohormone production, and suppresses plant diseases (Pacios-Michelena, González and Alvarez-perez, 2021). Streptomyces sp. is a major natural source of several important bioactive secondary metabolites, including antifungals, antivirals, antitumors, antihypertensives, immunosuppressants, and most importantly, antibiotics (Emerson *et al.*, 2012).

2.5 Mycorrhiza

Mycorrhizal fungi live within the root tissues of the host plant either intracellularly (arbuscular) or extracellularly (ectomycorrhizal). The host plant facilitates fungal growth and reproduction, while mycorrhizal fungi enhance the nutritional status of the plant by providing minerals, facilitating water absorption, bolstering disease resistance, and promoting plant growth (Barea *et al.*, 2002; Rani *et al.*, 2023). Following an in-depth exploration of traditional knowledge surrounding mycorrhiza, the findings led to the conclusion that mycorrhiza predominantly adhere to and manifest through two primary approaches, as elucidated by (Ferlian *et al.*, 2018). The initial approach, identified as the ecological perspective, encompasses the determination that mycorrhizal symbiosis serves as a significant indicator of plant biodiversity and ecosystem variability. This symbiotic relationship plays a pivotal role in regulating essential nutrient cycles, specifically nitrogen and phosphorus, influencing plant productivity, soil aggregation, and enhancing seedling

survival, as highlighted by (van Der Heijden *et al.*, 2015). The second approach, denoted as the cellular, molecular, and physiological perspective, involves the utilization of a more streamlined biological system to generate data within controlled laboratory settings. This methodology aimed to unravel the intricate mechanisms underlying the complexity observed in in vivo studies, particularly in the context of in-field associations, as described by Rodriguez and Sanders (2015).

These mycorrhizae may parasitize host plants in certain species or situations. Photosynthetic plants and fungi form beneficial mycorrhizae (Genre *et al.*, 2020). Photosynthesis produces sugars or lipids from the plant gives the fungus. The fungus offers plant water and soil-extracted nutrients such as phosphorus. Most mycorrhizae are found in vascular plant roots, although bryophytes have comparable relationships (Monika *et al.*, 2022). Additionally, fossils show that early terrestrial plants without roots formed arbuscular mycorrhizal connections. Most plant species form mycorrhizal fungi. An overview of association forms is presented below. Arbuscular mycorrhizal fungi dominate 70% of plant species, including grains and legumes (Kenrick and Strullu-Derrien, 2014)(Siddiqui, Akhtar and Futai, 2008).

Mycorrhizae are often classified into two main types: ectomycorrhizae and endomycorrhizae. These two forms are distinguished by the fact that the hyphae of ectomycorrhizal fungi do not enter individual cells inside the root, but the hyphae of endomycorrhizal fungi penetrate the cell wall and fold inward to the cell membrane. The user's text consisted of two references (Brundrett, 2004). Endomycorrhiza include arbuscular, ericoid, and orchid mycorrhiza, although arbutoid mycorrhizae might be categorized as ectoendomycorrhizas.

1. Ectomycorrhiza: The roots of 10% of plant families, predominantly woody plants, form symbiotic ectomycorrhizae. Occasionally, hyphae enter plant cells, creating ectomycorrhizae. In addition to the roots, ectomycorrhizal extramatrical mycelia run throughout the soil and leaf litter (Rice, 2003).
2. Endomycorrhiza: Variable endomycorrhizae include ericoid, arbutoid, orchid, and arbuscular (Chakraborty, Agrawala and Chakraborty, 2023).
3. Ericoid mycorrhizae: Ericoid mycorrhizae are the third most ecologically significant type. During their intraradical phase, they develop thick hyphae in the outermost root cell layer (Rice, 2003)
4. Arbutoid mycorrhiza: This differs from ericoid mycorrhiza and mimics ectomycorrhiza functionally and fungi-wise. Ectendomycorrhiza are different from ectomycorrhiza because some hyphae enter root cells(Wilson, 2003).

5. Orchid mycorrhiza: Orchids thrive solely in basidiomycete mycorrhizae because they are all mycoheterotrophic. Citation is required for nutrient exchange; hyphae enter root cells and form pelotons (Chakraborty, Agrawala and Chakraborty, 2023).
6. Mycorrhizal arbuscular: Arbuscular mycorrhizae (previously vesicular-arbuscular) produce dichotomously branched arbuscules to exchange nutrients with plant cells. Balloon-like vesicles are often used for storage. Fungal hyphae invade the cell membrane, generating a peri-arbuscular membrane, rather than the protoplast. Arbuscules enhance the contact surface area between the hypha and the host cell cytoplasm, making nutrition transfer easier. Arbuscular mycorrhizae are obligatory biotrophs that grow and reproduce in plants (Smith and Smith 2011).

2.5.1 Arbuscular mycorrhiza fungi (*Funneliformis mosseae*)

Arbuscular mycorrhizae (AMF), belonging to the phylum Glomeromycota, represent a pivotal category of mycorrhizae within the realm of plant-microbe interactions. These mycorrhizae establish an endomycorrhizal symbiosis with approximately 80% of angiosperms and nearly two-thirds of all plant species, underscoring their paramount significance in fostering mutualistic associations with a vast array of plant taxa (Mondal, Halder and Mondal, 2022). Within the rhizosphere of the host plant, arbuscular mycorrhizal (AMF) fungi form vesicles, known as vesicular-arbuscular mycorrhizae, representing a quiescent phase in the fungal life cycle. These vesicles enable fungi to transition into a dormant state and acquire the capacity to infect new plant roots. Certain AM fungi develop spores to endure the soil environment. It is noteworthy that (AMF) fungi display a strict dependency on their host plants, engaging in obligate symbiosis, and lack the ability to survive independently as saprotrophs in the soil. Instead, they remain dormant until they encounter a compatible host root (Cusant, 2019; Mondal Mondal, 2022). Recent investigations have shown that (AMF) fungal hyphae serve as a "highway" for bacteria to reach organic P patches, improving soil organic P consumption, and plants transmit atmospheric C into the soil, stabilizing terrestrial ecosystems (van der Heijden *et al.*, 2015; Jiang *et al.*, 2021; Keyes *et al.*, 2022). In addition to accessing macronutrients, mainly phosphorus (P) and nitrogen (N), as well as micronutrients such as zinc and copper, extraradical (AMF) hyphae have tremendous potential for use in soil micropores outside the rhizosphere zone (Adeyemi *et al.*, 2021; Gao *et al.*, 2021; Jiang *et al.*, 2021). There is evidence of an interaction between (AMF) fungus and plant growth-promoting rhizobacteria (PGPR) during the microbial processes of root colonization. Moreover, PGPR may affect the development and operation of (AMF) fungi, and as a result, mycorrhizas can impact PGPR populations in the rhizosphere (Barea *et al.*, 2002). Through encouraging spore germination and (AMF fungus mycelia extension, PGPR has been shown to

support (AMF) mycelial extension (Xavier and Germida, 2003; Chen *et al.*, 2023). This chemical alteration alters bacterial ecology, which causes the mycorrhizosphere effect. Similar to earlier research, PGPR are involved in the growth of the host plant's symbiotic relationship with (AMF) fungus (Abd Timonen, 2005; Muthukumar and Naveenkumar, 2016; Abd El-Azeem and Bucking, 2022). (AMF) enhance the development of secondary metabolites in medicinal plants, which are compounds that have healing properties for many ailments. Plant-derived secondary metabolites are used in the food, cosmetic, pharmaceutical, and agrochemical sectors (Thokchom, Gupta and Kapoor, 2023).

2.6 Glutathione S-transferases (GST)

Plant glutathione S-transferases (GSTs) are widely distributed and versatile enzymes encoded by several gene families. A distinctive characteristic of GST genes is their notable inducibility under a broad spectrum of stress conditions, including biotic stresses. Subsequent to numerous transcriptome-wide investigations, it has been established that specific GST clusters are prominently induced during the initial stages of bacterial, fungal, and viral infections (Cao *et al.*, 2022). Glutathione S-transferase (GST) enzymes constitute a vital component of plant biology and play pivotal roles in combating diverse stresses and interactions with pathogens. These enzymes serve as integral players in detoxification processes, defense mechanisms, and maintenance of cellular homeostasis (Gullner *et al.*, 2018). Evidence from numerous studies underscores the multifaceted functions of GSTs in plant physiology, including herbicide detoxification, alleviation of oxidative stress, hormone biosynthesis, and modulation of signalling pathways (Hussain *et al.*, 2022). Moreover, GSTs have emerged as key contributors to enhancing plant growth, conferring resistance against pathogens, and bolstering tolerance to abiotic stresses such as heavy metals and herbicides (Anjum *et al.*, 2012). The upregulation of GST genes in response to stress stimuli and their capacity to conjugate glutathione to toxic compounds exemplifies their pivotal role in shielding plant cells from damage and augmenting stress resilience.

Furthermore, the involvement of GSTs in facilitating flavonoid transport, orchestrating antioxidative reactions, and maintaining redox balance underscores their significance in orchestrating plant defense mechanisms and the biosynthesis of secondary metabolites (Rajput *et al.*, 2021). However, there are numerous transcriptome-wide variations, and it has been established that specific clusters of GSTs are prominently induced during the early stages of bacterial, fungal, and viral infections (Gullner *et al.*, 2018). Moreover, the induction of GST genes or heightened GST activities have frequently been observed in plants exposed to beneficial microbes (bacteria and fungi) that trigger a systemic resistance response (ISR) against subsequent pathogen infections.(Moons, 2005) .

Finally, the multifaceted benefits of GST enzyme activity in plants include detoxification, stress tolerance, defense responses, and metabolic regulation, underscoring their indispensable role in fostering plant resilience and adaptation to environmental challenges. Continued research efforts to unravel the intricate mechanisms underlying GST function hold promise for advancing our understanding of plant stress responses and devising novel strategies for enhancing crop productivity and sustainability(K, Moural and Zhu, 2022).

2.7 Phenolic compounds

Phenolic compounds are widely found in plants. Phenolic compounds are secondary metabolites that have no direct effect on plant growth and development. however, their effect Through signal transduction, controls other related pathways to carry out their role. These substances give plants their colour, taste, and scent while also shielding them from diseases and plant stress (Pandey and Rizvi, 2009; Kumar, Debnath and Singh, 2023).

Phenolic compounds are the most common secondary metabolites in plants. These compounds typically exhibit a common chemical structure, characterized by an aromatic ring with one or more hydroxyl substituents. These compounds can be classified into various classes including flavonoids, phenolic acids, tannins, stilbenes, and lignans (Gupta and Pandey, 2020). In addition, the phenolic compounds and phytochemicals present in a variety of marine plant species hold significant promise for prevention and therapy. This was attributed to their notable antioxidant, anti-inflammatory, and anticancer properties (Franco, Arazo and Benavides, 2023). However, phenolic compounds, recognized as bioactive phytochemicals, are well documented for their antimicrobial activities, toxicity against microbial agents, and inhibitory effects (Microorganisms *et al.*, 2020).

Free radicals generated through biological oxidation represent a category of reactive oxygen species, including superoxide, nitric oxide, and hydroxyl free radicals (Phaniendra and Babu, 2015). These radicals have the potential to assault DNA, proteins, and lipids within the organism, triggering cellular damage and internal perturbations, and subsequently contributing to the destruction of a spectrum of diseases such as cancer, atherosclerosis, malaria, and conditions related to nerve regeneration (Pham-huy, He and Pham-huy, 2008). Furthermore, antioxidants are known for their capacity to neutralize free radicals, thereby mitigating oxidative damage (Medicine, 2022). Nevertheless, the utilization of synthetic antioxidants is limited by concerns regarding their potential toxicity and carcinogenic properties. Consequently, there is a growing preference for exploring plants rich in natural antioxidant compounds. Polyphenols, a significant category of plant secondary metabolites, have been verified as effective natural antioxidants, as demonstrated by their ability to scavenge free radicals, chelate metals, and enhance the expression

of metabolic enzymes (Zhu *et al.*, 2021). Okra fruits contain polysaccharides and phenolic compounds, which are commonly known to have preventive properties against antioxidant, anti-hyperglycemic, and obesity. Furthermore, they exhibit diverse bioactivities, including antihyperlipidemic and neuroprotective activities. Polysaccharides and phenolic compounds are generally recognized as the primary bioactive constituents in okra fruits and serve as sources of the various biological activities attributed to them (Wu *et al.*, 2020).

Several researchers have investigated nutraceutical polyphenols in microorganisms through metabolic engineering interventions. One such example is kaempferol, a flavonoid belonging to the flavonol subclass found in various vegetables such as tea, cabbage, broccoli, and tomatoes. Kaempferol has garnered growing attention owing to its diverse bioactivities, including antioxidant, anticancer, anti-inflammatory, and neuroprotective effects. (Madhavan *et al.*, 2023).

Enhanced production of polyphenols usually occurs during abiotic stress or biotic stress conditions. Thus, eliminating competing trails directs the microbial cell into the production of polyphenols and hence results in a higher yield of kaempferol in plants.

Phenolic acids are the largest group of polyphenols, with a typical structure. Caffeic acid and gallic acid are examples of this polyphenol subclass. They are well known for their ameliorative effects on chemotherapy and protective effects against cardiovascular diseases and diabetes. Phenolic compounds (especially phenolic acids) represent the most extensive category of polyphenols and are characterized by a typical structural framework. Examples of this polyphenolic subclass include caffeic acid, gallic acid, and coumaric acid. These compounds are renowned for their therapeutic contributions, demonstrating ameliorative effects in chemotherapy as well as protective effects against cardiovascular diseases and diabetes(Ti *et al.*, 2006; Cheng *et al.*, 2016; Yang *et al.*, 2024).

3. MATERIALS AND METHODS

3.1 Microbes used

The microbes tested in the experiment were selected to be environmentally friendly. They are listed below:

1. Arbuscular mycorrhiza (AM), identified as *Funneliformis mosseae* (Glomerales, Glomeraceae) and indicated as AM, was procured from the Biotechnology Department., which has been registered in the EU as a plant protection substance (http2).
2. *Trichoderma asperellum* T34 strain (CECT No. 20417), a commercial product of a filamentous fungus selected for this study, stands out for its notable significance in agricultural and biotechnological applications. Registered in the EU as a plant protection substance (http2).
3. *Aureobasidium pullulans*, strain DSM 14940 has been registered in the EU as a plant protection substance (http2).
4. *Streptomyces* K61 strain (DSM 7206), previously *known as S. griseoviridis*, was used in this study. registered in the EU as a plant protection substance (http2).
5. Pathogens used to test the pathogenicity resistance of okra seedlings: *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* were obtained from the Institute of Plant Protection.

3.2 Test plants

The three cultivars used in the experiment were as follows.

1. The seeds of okra (Betera), scientifically identified as *Abelmoschus esculentus* L. (Moench), were acquired from Agrimax Products, signifying the source of the germplasm for the experimental investigation. This meticulous acquisition of plant material from Agrimax products establishes a foundational element in this study, ensuring the botanical authenticity and traceability of the okra seeds utilized in this study.
2. The Tomato cultivar, Moneymaker, was identified as *Solanum lycopersicum* L.. The selection of Moneymaker as the cultivar aligns with its widespread utilization in scientific research owing to its well-defined genetic characteristics and agronomic traits, contributing to the reliability and reproducibility of the experimental results.
3. The sweet potato (*Ipomoea batatas* (L.) Lam.) were obtained from the Biotechnology Department of MATE. This deliberate selection ensures the botanical authenticity and traceability of sweet potatoes.

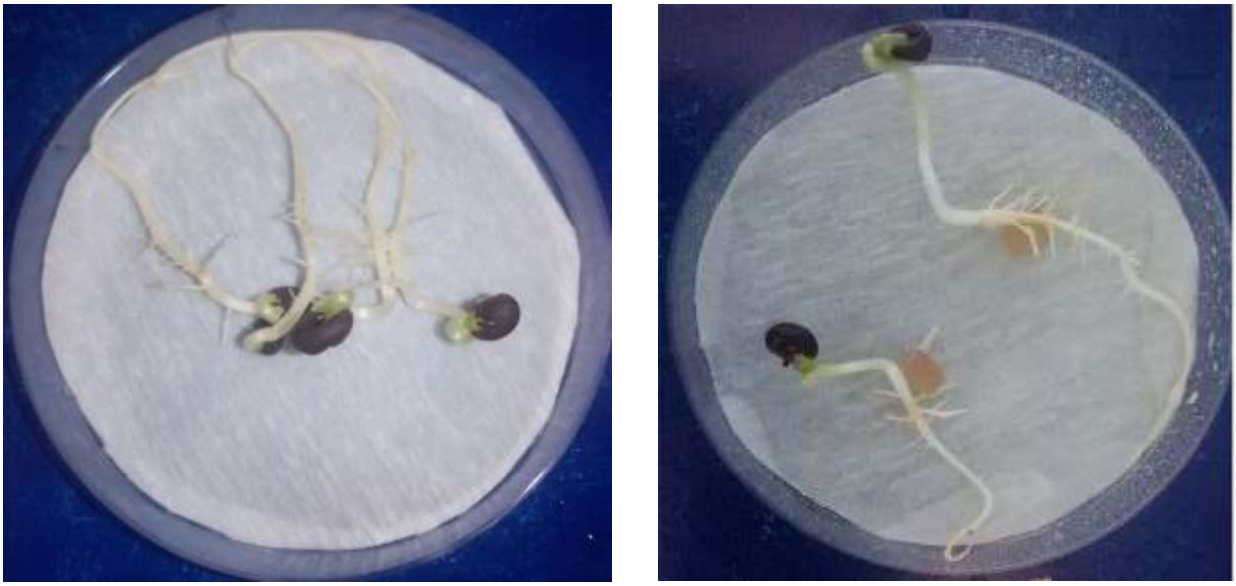
Media used for culturing the microbes

Potato dextrose Agar (PDA), medium containing 20 g dextrose, 15 g agar, and 4 g potato starch in 1 L of distilled to subculturing of fungus *T. Asperellum*, *A. Pullulans* and *S. griseoviridis* as BCAs*, S. sclerotiorum*, *R. solani*, and *M. Phaseolina* as pathogen.

Bacterial media, Nutrient Agar (NA) Five grams of peptic digest of animal tissue, 3 g of beef extract, and 15 g of agar in 1 L of distilled water were used to subculture *S. griseoviridis*.

3.4 In vitro pathogenicity test

Okra plant germination in the laboratory of the Plant Integrated Institute Department, the seedling of four okra seeds in a Petri dish (9-inch diameter) using filter paper to cover the seeds, incubated at room temperature and testing the resistance for three pathogens(*Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*). Seed germination was checked daily and pictures were taken; the germination of the seeds was 100% (Figure 4).



(A) (B)

Figure 4: Testing the resistance for three pathogens: *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* (A: okra seed germination B: Okra seedling infection).

3.5 In vitro Antagonism test

T. asperellum and *A. pullulans* as fungi were subcultured on a Petri dish (9-inch diameter) by an added disk of the pathogen with one of the BCAs for the opposite one to another on the PDA medium at the same time and incubated for 5 days at 20–28 °C. Data were collected after 24 h of growing the mycelium and daily measurements of mycelium growing both fungal bioagents and pathogens and taking pictures. Bacterial media was used to culture *Streptomyces K61* on nutrient agar (NA) for 5 days at 20–28 °C. Collecting data during mycelium Figure 5.

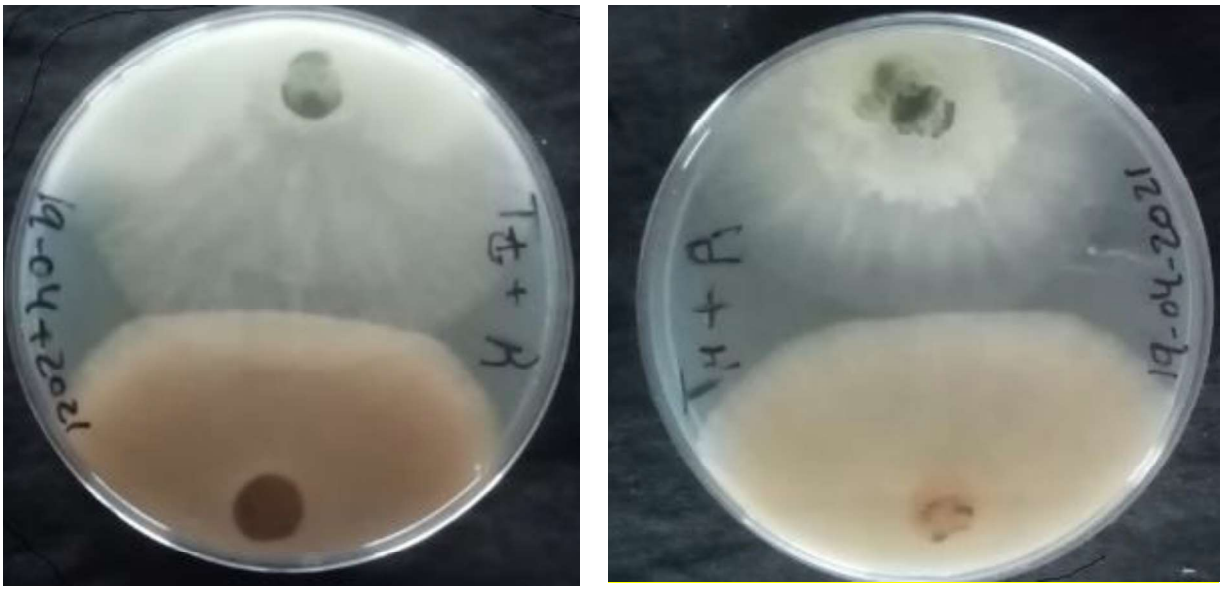


Figure (5): A: *Trichoderma asperellum* antagonism test

3.6 In vivo experiment

The research methodology employed in this study pertains to the systematic approach and techniques utilized for designing, conducting, and analyzing a research investigation. The provided statement delineates the sequential actions, methodologies, and instruments employed in the collection and examination of data, with the aim of addressing research inquiries or validating hypotheses. An appropriately structured research methodology guarantees dependability, validity, and credibility of the study, thereby ensuring the reliability and generalizability of the findings to a wider population.

The laboratory and field experiments were conducted in the experimental labs and fields belonging to the Plant Protection Institute – Hungarian University of Agriculture and Life Sciences, located at 2100 Gödöllő, Pest, Hungary (coordinates: 47.594315, 19.368984).

Field experiments were conducted in a controlled environment in a greenhouse using MATE. Greenhouses, renowned for maintaining optimal temperature conditions typically ranging between (18-35) °C (65-95 °F), Illumination Duration: 12-14 hours of light per day in a greenhouse, maintaining optimal growth rates, and flower production, provide an instrumental setting for precise experimentation and observation in agricultural studies. This controlled environment ensured that the experimental conditions closely mimicked the natural habitat of the plants, offering a reliable platform for comprehensive investigations into the intricate dynamics of plant growth and responses to various treatments. Soil type: sandy soil mixed with soil horticulture (3/1) (v/v); pots containing 2 kg of dry sandy soil mixed with the soil horticulture with one plant per pot. Pots were arranged on plastic benches in a greenhouse for two months and transferred to the field. The pots were placed in glasshouse benches in a completely randomized design for each treatment.

The soil pH ranges between 6.0 and 7.0. Fertilizer: Provides adequate soil fertility every four weeks for the healthy growth of okra plants. Plant irrigation: Plants require consistent soil moisture and irrigation every two days to support their growth and prevent water stress. They were performed on three tomato, okra, and sweet potato plants treated with microorganism agents to enhance plant growth and pathogen resistance. Different plants were treated separately with the three microorganism agents and mycorrhiza. The experiment set up 12 replacats of tomato plants, 11 replacats of okra plants, and one sex replicat of sweet potato plants.

Okra plant seedling

The flora under investigation was initially cultivated as juvenile plants or seedlings within small pots containing Figuer (6) as a growth medium. Subsequently, the seedlings were transferred to 2-liter plastic pots, and to transfer seedlings, it was essential to transplant them into the pots and fill them in mixed sand soils with horticulture soil (a proportion of approximately 1:3). The seedlings were planted at the correct depth, watered gently, and placed in an environment with appropriate lighting, temperature, and humidity levels. It is important to regularly monitor moisture levels and effectively manage pests to nurture healthy growth and establish an optimal environment for the initial stages of plant growth.

Figure 6: Okra seedlings in the MATE greenhouse

Figure 7: Transplant okra plants to 2 kg of soil in the greenhouse.

Sweet potato

The experiment consisted of eight treatments, arranged in a random design. The treatments in the experiment involved the inoculation of microorganisms, resulting in five treatment groups: inoculated (AM), non-inoculated, and cooperation of AM with each of the microorganism agents (*T. spirillum*, A. pullulans, and *S. griseoviridis*). A sweet potato plant (Ipomoea batatas) was used in this study. The plants were initially grown as seedlings in small 3 L plastic pots filled with horticultural soil (Figure 7). The plants were transplanted into large pots. Each treatment was replicated in seven pots, resulting in a total of 56 pots, including the control group. Each pot contained 10 kg of horticultural soil and was planted with a single plant (Figure 8).

Figure 8: The sweet potato shoots were initially grown by planting (shoot rooting) in small plastic (pots 3 L).

To maintain consistency and uniformity, the seedlings were transplanted into 2-litre plastic pots containing a sand and horticultural soil mixture with peat moss, and sand (1-3) was positioned on plastic dish benches in a greenhouse environment. This acclimatization phase lasted for approximately one month before their transfer to the field. The experimental treatments were conducted separately for each plant species, with 14 pots assigned to each plant, and microorganisms acting as bioagents. The pots were arranged in a completely randomized design within the greenhouse, ensuring that any potential confounding factors were evenly distributed across the experimental groups.

Figure 9: Sweet potato was transplanted into a pot (10 kg) of horticultural soil with a single plant.

3.6.1 Botrytis infection of okra

Botrytis cinerea was obtained from infected okra fruits (naturally infected) grown in a greenhouse. The fungus was isolated and subcultured on potato dextrose agar (PDA) at room temperature. Detached okra plant infections were carried out using leaves of comparable age and size (leaves at nodes 3 and 4, counted from the top) selected randomly from the okra plant. To study the pathogenic effects of *B. cinerea* on okra plants, mycelium discs with PDA were placed at the base of the leaves or flowers of okra plants (Figure 9).

Figure 10 : Okra plant infection of *Botrytis cinerea*

3.6.2 Soil inoculation

A- Arbuscular mycorrhiza fungi (*Funneliformis mosseae*)

Arbuscular mycorrhizae were introduced to okra and tomato plants through a meticulous inoculation process involving the addition of (1 g) of mycorrhizal spores per plant. This inoculation was performed by incorporating spores into the top 10 cm of the soil adjacent to the root rhizosphere of okra plants. The plant is characterized by the presence of nine or more unfolded leaves on its main shoot, as illustrated in Figure 10. These experimental conditions were meticulously maintained within the controlled environment of a greenhouse, where temperatures ranged between (18-35) °C. This greenhouse setting ensures an ideal milieu for the growth and observation of plants, fostering an environment that closely mimics their natural habitat, and facilitates the systematic exploration of the impacts of arbuscular mycorrhiza on plant development and responses under regulated conditions.

(A)

(B)

Figure 11: A: Okra inoculated with arbuscular mycorrhiza *(Funneliformis mosseae)*. B: Tomato plants inoculated with arbuscular mycorrhiza *(Funneliformis mosseae)*.

1. *Trichoderma asperellum* strain (T34) was cultured on PDA medium at 25 C (room temperature) for a week; after that a suspension of *T. asperellum* spores was added (20 ml) 9*10^7 spoors to the top of the soil around the plant equally to all the treatments after 20 days of inoculation with mycorrhiza during vaccination the treatments nine or more leaves on the main shoot unfolded of the plant.
2. *Aureobasidum pullulans* was grown on a PDA medium at 25°C (room temperature) for a week; after that added suspension of *A. pullulans* cells (25 ml), $22*10^7$ spoors to the top of the soil around the plant equally to all the treatments after 20 days of inoculation with mycorrhiza during vaccination the treatments nine or more leaves on the main shoot unfolded of the plant.
3. *Streptomyces griseoviridis* (K61) was cultured on nutrient agar medium at 25°C (room temperature) for eight days and the suspension was added to the top of the soil around the plant equally to all treatments. After treatment with mycorrhiza, 20 ml of spore solution containing $22,48*10^7$ spores was applied to each plant until 25 ml was utilized.

3.7 Evaluation of Botrytis infection (gray mold)

The onset of *Botrytis cinerea* was detected within a 24-hour timeframe, as evidenced by the emergence of characteristic symptoms, as depicted in Figure 11. To monitor disease progression, plant symptoms were documented at three distinct time points throughout the infection. This was achieved by employing a standardized evaluation method, which involved quantifying the extent of leaf or stem infection; specifically, a numerical scale was utilized, whereby a score of 1 indicated leaf infection only, a score of 2 indicated both leaf and stem involvement, and a score of 3 indicated stem death. The effects of *B. cinerea* infection on different plant components are shown in Figure 11.

A B

C D

Figure 12: Shows Okra plant symptoms of infection with Botrytis cinerea on different treatments:(A) control treatment, (B) mycorrhiza treatment (*Funneliformis mosseae)*, (C) mycorrhiza+A. *pullulans* treatment, (D) mycorrhiza+ *S. griseoviridis* treatment.

3.8 Evaluations of plant growth

1. Okra leaf size and leaf number

To assess the impact of *T. asperellum* and mycorrhiza species on okra plant growth, leaf size measurements and leaf number were recorded for individual plants at eight weeks post-planting. The third fully open leaf from the top of each plant was selected for analysis to ensure that the leaves were at a comparable developmental stage. The leaf area was measured using a ruler and the results were recorded in centimeters (cm). This methodology was employed to provide a standardized and consistent approach for evaluating the effects of microorganisms on okra plant growth.

2. Plant height

To measure the height of an okra plant using a ruler, the measurement starts from the base of the stem at the soil surface and continues up to the highest point of the plant, which is typically the tip of the apical bud. This measurement was repeated four times during the growth period (for 10 days between each measurement), which is crucial for accurately tracking the growth and development of okra plants. The height of the okra plant can be recorded in centimeters to monitor its progress

and health throughout its growth stages. This parameter applies to the okra plant tomato and sweet potato.

3. Fruit number

During the growing season, the assessment of fruit numbers in okra plants under different production treatments involved meticulous data recording. Data collection was performed four times, with the averages computed for each treatment. The initial harvest generally takes place around two months post-okra planting.

4. Fruit weight measurement

The yield of okra and tomatoes can be measured in terms of the quantity of produce harvested per treatment, which is typically expressed in grams. In the case of okra fruit, the yield was collected during the three weeks of fruit production and fruit weighing each time. However, tomato yield was harvested at the end of the season. However, each crop can vary based on planting ratio and cropping density. The measurement of yield in such intercropping systems is crucial for assessing the productivity and economic viability of okra and tomato cultivation.

5. Tuber weight of sweet potato

To accurately determine the weight of the sweet potato roots, a standardized methodology was employed. Sweet potatoes were harvested from the designated plots, typically when they reached maturity, which was typically approximately 100 days post-planting. Following harvest, the sweet potatoes were meticulously cleaned to remove any extraneous soil or debris that may have become attached to the roots during the growth process. This cleaning process was essential to ensure that the weight of sweet potato roots was accurately measured without any confounding factors that could potentially skew the results. Figure 13.

Figure 13: Harvesting sweet potato.

This was performed using gentle brushing, ensuring minimal damage to the roots, and each sweet potato root was weighed using a reliable and calibrated weighing scale. The scale was sufficiently sensitive to accurately measure root weight. The weight was recorded in grams (g) for consistency, and the measurement process was repeated for multiple sweet potato roots to obtain a statistically representative sample. This helped reduce any potential bias or variability in the data. The recorded weights were analyzed using appropriate statistical methods to determine the mean, standard deviation, and other relevant parameters. This analysis provides insights into the overall distribution and variability of sweet potato root weights.

6. Okra and tomato plants dry weight measurement

Fresh weight refers to the precise weight measured during the immediate harvest of plants. This parameter encompasses the moisture content inherent in the plant; To determine the fresh weight of a vegetative plant component, it is necessary to collect the specific organ or tissue of interest through the process of harvesting, Extract all the plants including the roots from the soil and cleanse them to eliminate any loose soil particles then gently dab the plants using a soft paper towel to eliminate any excess surface moisture. It is essential to perform this step promptly to prevent water loss from plants. The plants were removed from the soil to measure the wet weight of the roots, and any loose dirt was gently washed off. Next, the plant was carefully blotted to remove excess surface moisture. The roots were then separated from the top portion by cutting on

the soil line. Finally, the roots and upper part (vegetative) of each treatment were immediately weighed using a scale to obtain the correct measurements.

The process of determining the dry weight of plant roots and vegetative plants by leaving the plant in the laboratory for 4-6 weeks This procedure involves carefully removing all moisture from the plant samples using controlled drying methods until a constant weight is achieved. Dry weight provides an accurate biomass assessment, eliminating the influence of fluctuations in water content. Overall plant biomass directly indicates the performance of our plant experiment in relation to factors such as photosynthetic capacity, nutrient uptake, and environmental conditions. To obtain the dry weight of the vegetative plant component, it was necessary to separate the root from the top portion by cutting at the soil line. Subsequently, the roots were removed from the plant and dried by placing them in an environment at room temperature, after which the weight of the plants was measured following the same process for the roots.

3.9 Determination of root colonization

1- Trichoderma *asperellum*

Root samples were collected after the plants were inoculated with mycorrhiza species, and the samples were introduced into the top 10 cm of soil near the roots of the plant (Okra, Tomato, and sweet potato) rhizospheres (Figure 12). They were inoculated with the following microbial agents: *T. asperellum* strain 34. After three weeks, root samples were examined to investigate the cooperation of *T. asperellum*. At room temperature, chopped roots were placed on PDA in Petri dishes (9-inch diameter). After two days, Trichoderma colonization of the root was examined by accounting for Trichoderma.

To investigate *T. asperellum* colonization in okra roots, soil samples were collected from the uppermost 10 cm of soil surrounding the root rhizosphere of Okra and Tomato plants in the field. These samples were obtained after the inoculation of the roots with the aforementioned microorganisms. Following a three-week incubation period, the interaction between T. asperellum and okra roots was collected from the sample, and the results of this analysis are presented in Figure 14.

Figure 14: Root sample taken to check Trichoderma mycelium cooperation with the roots.

2- Mycorrhiza (*Funneliformis mosseae)*

Root samples of the plants were randomly taken to identify mycorrhiza colonization in the roots of the plants (Okra, Tomato, Sweet potato). Internal and external mycorrhizal mycelia in the root were evaluated by taking the root to the laboratory and staining it.

Arbuscular mycorrhiza (*Funneliformis mosseae*) in the roots of the okra plant

Before microscopic examination, the roots were stained to facilitate assessment of root colonization. Root colonization was evaluated through visual observation using a stereomicroscope at a magnification of 100x. Root samples of okra plants were washed thoroughly with tap water and then cut into 1 cm long pieces. Fresh, rinsed, or fixed roots were placed in 10 % aqueous solution of KOH (w/v). We routinely used ca. 3 g (f.wt) of roots in 55 cm3 test tubes (25 × 150 mm) containing 40 ml of 10% KOH. Roots and KOH are heated in a water bath at 90 C' for 60 minutes; then decant the solution and wash the roots with running water; after that, wash the root with 5% of vinegar(acetic acid) for 1-2 minutes and put the roots in 5% of ink (Pelikan blue) than boil the root - ink 2 minutes in the presence of the dye when finishing wash the roots with running water finely stoor the root sample in the glycerol solution for a long time in the laboratory and used The gridline intersection method was employed to estimate mycorrhizal colonization. This involved observing the presence or absence of mycorrhizal structures at the intersections between root fragments and gridlines. gridlines (Phillips and Hayman 1970).

Arbuscular mycorrhizal colonization (*Funneliformis mosseae*) identification in the roots of tomato and sweet potato plants

Before microscopic examination, the roots were stained to facilitate assessment of root colonization. Root colonization was evaluated through visual observation using a stereomicroscope at a magnification of 100x. Root samples of okra plants were washed thoroughly with tap water and then cut into 1 cm long pieces. Fresh, rinsed, or fixed roots were placed in 10 % aqueous solution of KOH (w/v). We routinely used ca. 3 g (f.wt) of roots in 55 cm^3 test tubes (25 × 150 mm) containing 40 ml of 10% KOH. Roots and KOH are heated in a water bath at 120 C' for 5 min; then decant the solution and wash the roots with running water; after that, wash the root with 5% of vinegar(acetic acid) for 1-2 minutes and put the roots in 5% of ink (Pelikan blue) than boil the root - ink 2 minutes in the presence of the dye when finishing wash the roots with running water finely stoor the root sample in the glycerol solution for a long time in the laboratory and used The gridline intersection method was employed to estimate mycorrhizal colonization. This involved observing the presence or absence of mycorrhizal structures at the intersections between root fragments and gridlines (Phillips and Hayman, 1970).

3.9.1 Glutathione-S-Transferase (GST) activity

1. Sampling

Plant leaves (the third leaf from the top of the plant, which is healthy) collected from the field experiment were placed in aluminum foil immediately following collection and in liquid nitrogen until transferred to the laboratory, and then placed in a resealable plastic bag and held at approximately -80 C to prevent leaf stress and enzyme change concentration.

2. Enzymes activity measurement

After measuring the leaf (0.5 g), the tissue was resuspended in 100 µL of cell lysis buffer, homogenized with a laboratory jar very quickly, centrifuged for 10 min at 4°C at top speed using a cold microcentrifuge to remove any insoluble material, and the supernatant was collected and transferred to a clean tube and kept on ice. Based on the Sigma-Alddrich protocol of Glutathione-S-Transferase (GST.N CS0410) assay kit and Habig, et al. 1974 used to measure GST activity in the plant.

3.9.2 Polyphenol content measurement

7. Sampling

To conduct phenol analysis, the okra fruit was physically harvested by handpicking it every two to three days, commencing with the commencement of the flowering and fruiting period. It was then preserved in a biotechnology laboratory for MATE at -80 °C.

8. HPLC determination of phenol compounds in okra

Three hundred milligrams (300 mg) of lyophilized (freeze-dried) whole okra fruit were crushed in a crucible mortar in the presence of 1-2 grams of quartz sand. Phenolic compounds were extracted by the addition of methanol containing 2% orthophosphoric acid. The macerate was then transferred to a centrifuge tube and subjected to ultrasonication for 15 min at 40°C in a water-bath ultrasonic device (model RK-165-BH Bendelin Sonorex, Germany), followed by mechanical shaking at room temperature for 20 min. The extract was centrifuged for 5 min at 5000 rpm (M-Universal, MPW Med. Instrument, Poland). The supernatant was decanted into a round-bottom flask and the solvent was evaporated to dryness under vacuum at 45°C. The residues were redissolved in 5 ml of 1:1 methanol and 1% orthophosphoric acid and finally purified through a 0.45µ, 25 mm Cellulose acetate syringe filter before injection into the HPLC apparatus.

HPLC instrument and conditions

A Chromaster Hitachi HPLC instrument containing a Model 5160 gradient pump, Model 5260 autosampler, Model 5310 column oven, and Model 5430 diode-array detector was used with the parad_KB0_2dat software for operation and data processing.

The separation of phenolic compounds was performed on an Ascentis phosphor-conditioned C18 phase (C18-PCP, Supelco, USA) with gradient elution of 1% ortho-phosphoric acid (A) and acetonitrile (B) according to a recently developed protocol (under publication). The gradient elution started with 1% B in A, changed to 20% B in 20 min, remained isocratic for 10 min, changed to 30% B in 5 min, remained isocratic for 10 min, and finally changed to 1% B in 5 min. DAD detection was performed between 190 nm and 700 nm. The quantification was based on recording the area at the maximum absorbance wavelength of each compound and relating it to that of the standard solution.

Stock solutions of different phenolics (Sigma-Aldrich, Merck, Budapest, Hungary) were prepared by dissolving 2-3 mg in 10 ml absolute ethanol or methanol and diluting 10 times with 40% ethanol in 1% ortho-phosphoric acid. The working solutions were used for the calibration curves, identification, and quantification of phenolic compounds. When no standard was available, the compounds were tentatively identified based on a comparison of their spectral characteristics and

chromatographic behavior with those reported in the literature (Romdhane et al. 2020; Yang *et al.*, 2022; Wang *et al.*, 2023).

4. RESULTS

4.1 Pathogenicity test - plant pathogens on okra seedling

To evaluate the susceptibility of okra (*Abelmoschus esculentus*) seedlings to *Sclerotinia sclerotiorum*, a germination assay was conducted. During this assay, okra seedlings were inoculated with *S. sclerotiorum*. The results of this assay revealed a pronounced impact of *S. sclerotiorum* on okra seedlings, wherein all exposed plants became infected and subsequently died. This finding suggests that okra plants are highly sensitive to *S. sclerotiorum*, and that this pathogen may pose a significant threat to the growth and development of okra crops. A graphical representation of the germination assay results is shown in Fig. 15.

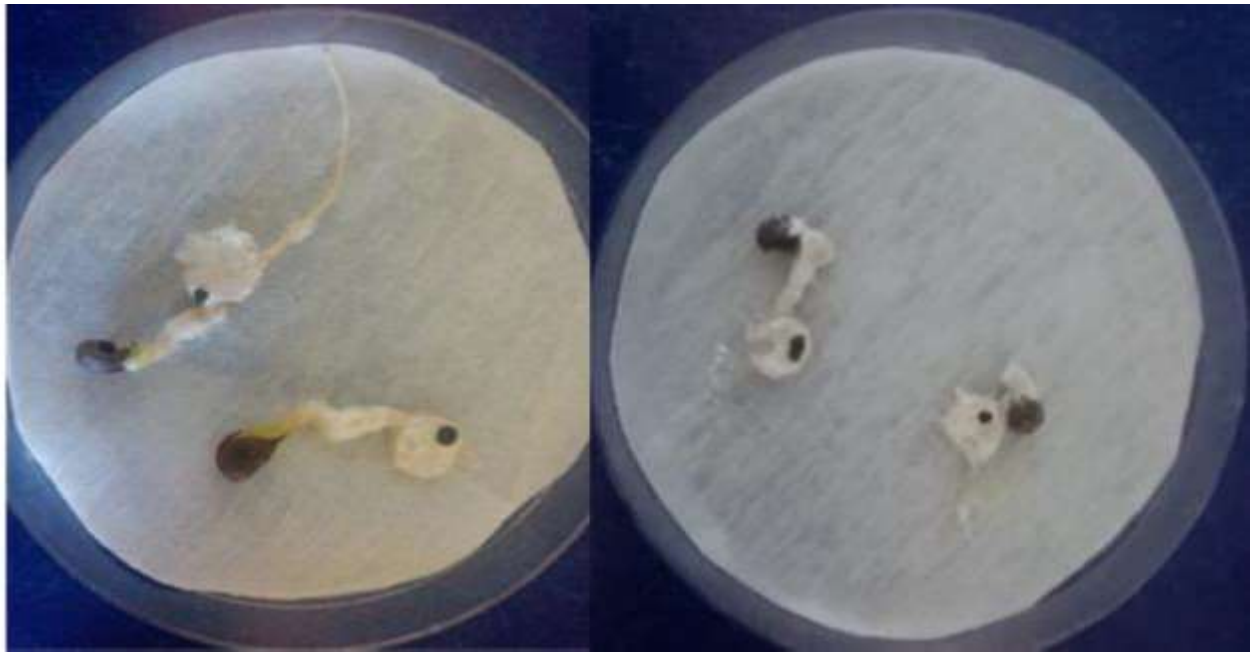


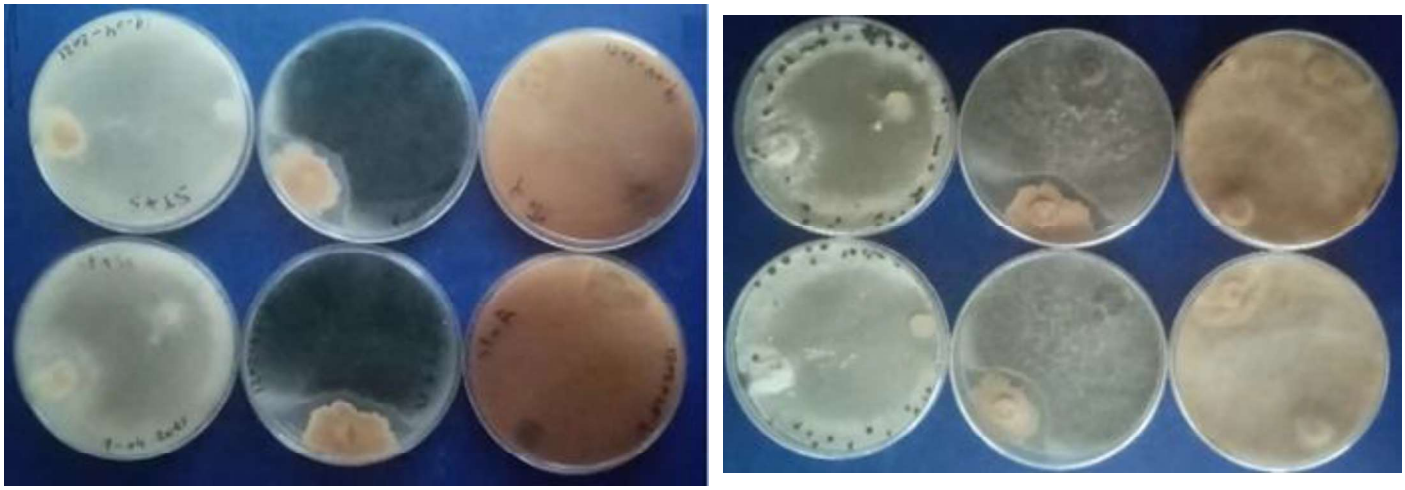
Figure 15: Okra plants infected with *S. sclerotiorum.*

4.2 In vitro antagonism test

1- *Streptomyces griseoviridis*

The efficacy of *Streptomyces griseoviridis K61* as a biocontrol agent against various pathogens, including *Sclerotinia sclerotiorum, Macrophomina phaseolina*, and *Rhizoctonia solani*, was assessed. The results demonstrated significant inhibition of *M. phaseolina* growth by *S. griseoviridis*, suggesting its potential as a biocontrol agent for managing *M. phaseolina* infection. However, their effectiveness against *S. sclerotiorum* and *R. solani* is limited. Although *S. griseoviridis* initially inhibited both *M. phaseolina* and *S. sclerotiorum*, it did not exhibit the same inhibitory effects against *R. solani (Figure 16)*. While previous studies support the inhibitory capabilities of *Streptomyces spp*., the coexistence of multiple fungi under field conditions

necessitates biocontrol treatments capable of targeting multiple pathogens simultaneously (Vurukonda, Giovanadri and Stefani, 2021; Díaz-Díaz *et al.*, 2022)..



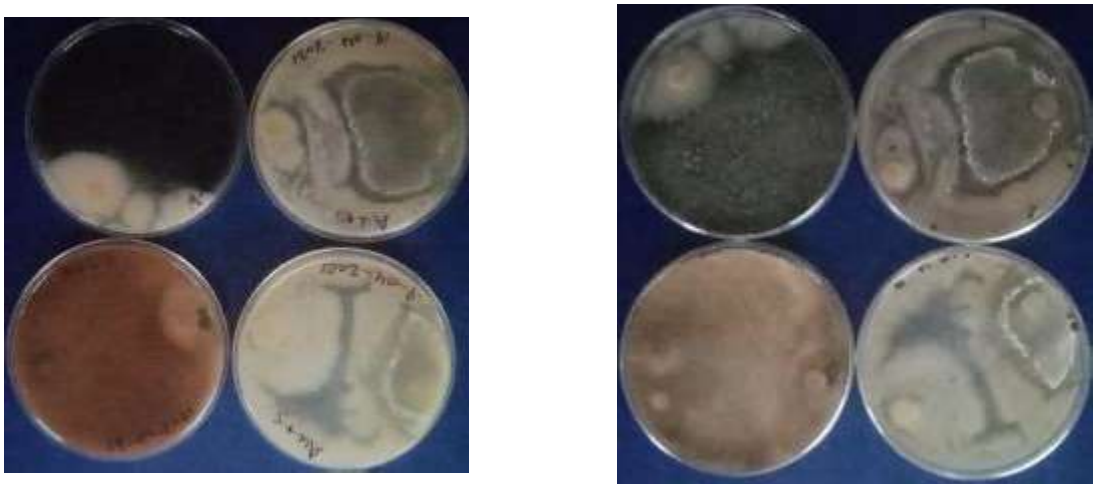
A1 B1 C1 A2 B2 C2

Figure 16: Shows antagonistic activity of Streptomyces against three pathogens. A: *Streptomyces K61* against S. *sclerotiorum*, B: *Streptomyces* K61 against *M. phaseolina*, C: *Streptomyces K*61 against *R. solani* Infront pictuer (A1,B1,C1); Back pictuer (A2,B2,C2).

2- *Aureobasidium pullulans* activity

The effectiveness of *Aureobasidium pullulans* as a biological control agent against three plant pathogens, *R. solani*, *M. phaseolina*, and *S. sclerotiorum*, was evaluated in this study. *A. pullulans* has been recognized as a promising alternative to fungicides for reducing the incidence of plant diseases (Di Francesco and Baraldi, 2021). These results indicate that *A. pullulans* demonstrates positive behavior as a biological control agent against S. sclerotiorum. However, its efficacy against *M. phaseolina* was limited and it showed no effect against *R. solani* (Figure 17). While some studies have reported *the ability of A. pullulans* to control *R. solani*, differences in results may stem from variations in the experimental conditions (Di Francesco *et al.*, 2021). This study suggests that the effectiveness of *A. pullulans* as a biological control agent may vary depending on the specific pathogen, with a promising potential against certain plant pathogens.

A1 A2

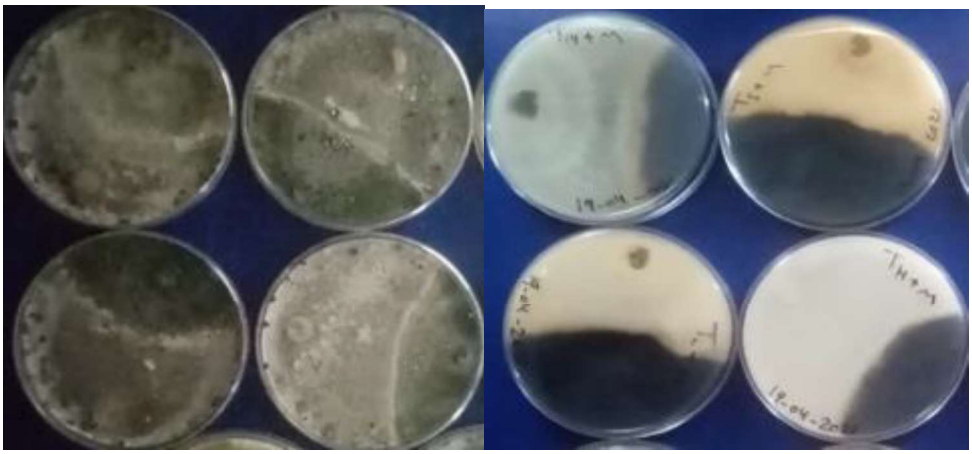


B1 C1 B2 C2

Figure 17 Shows the antagonistic activity of *Aureobasidium pullulans* against three pathogens. A: *M. Phaseolina*, B: *R. solani*, C: *S. sclerotiorum* . Infront pictuer of petri dish (A1,B1,C1); Back pictuer of petri dish (A2,B2,C2).

3- *Trichoderma asperellum*

The efficacy of *Trichoderma asperellum* (T34) as a biocontrol agent against various plant pathogens was also investigated. The results showed that simultaneous introduction of both the pathogen and *T. asperellum* yielded growth rates of S. sclerotiorum, M. phaseolina, and R. solani comparable to those of *T. asperellum* alone. However, direct interaction between *T. asperellum* mycelia and pathogens led to effective suppression of pathogen growth, with *T. asperellum* outcompeting them. This observation is consistent with previous research (A, D and Y, 2017; Stracquadanio *et al.*, 2020). In summary, *T. asperellum* (T34) shows promise as a biocontrol agent against *S. sclerotiorum, M. phaseolina*, and *R. solani* infections, as illustrated in Figures (18,19), which demonstrating its inhibitory effects on pathogen growth.



A **B**

Figure 18: *Trichoderma asperellum* antagonistic activity against two pathogens: A: *S. sclerotiorum* and B: M *Phaseolina.*

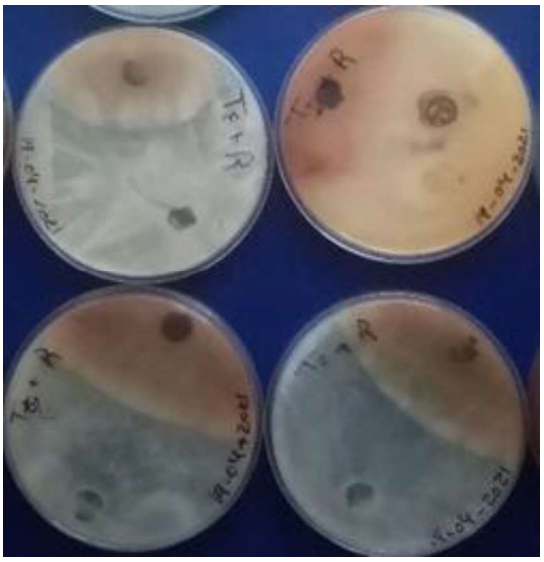


Figure 19: Shows *Trichoderma asperellum* antagonistic activity against *R. solani.*

4.3 Effect of arbuscular mycorrhiza (*Funneliformis mosseae)* and antagonists treatment on Botrytis infection okra plant

In this study, the efficacy of microbial inoculants, namely *S. griseoviridis*, *T. asperellum*, and *A. pullulans*, as biological control agents (MBCA), was assessed against *B. cinerea* infection in okra plants. Volatiles produced by *T. griseoviridis* and *A. pullulans* have been found to inhibit *B. cinerea* growth. The results demonstrated significant differences in okra plant resistance to *B. cinerea* infection among various treatments. The combination of arbuscular mycorrhiza fungi (*Funneliformis mosseae)* AMF with *T. asperellum* (M+T34), AMF with *A. pullulans* (M+Ap), and mycorrhiza with *S. griseoviridis* (M+Sg) showed the highest plant tolerance, with infection rates reduced to 89%, 89%, and 75% respectively, compared to the control group (Figure 20).

Combining AMF with *T. asperellum, S. griseoviridis*, and *A. pullulans* resulted in a significant enhancement in plant tolerance to *B. cinerea* infection. This enhanced tolerance can be attributed to the mechanisms that promote systemic plant resistance.

The combination treatments of AMF with antagonistic agents (M+T, M+Ap, and M+Sg) resulted in a statistically significant reduction in gray mold infection compared to the control group. These combination treatments demonstrated superior efficacy in mitigating grey mold infection compared to single treatments involving *T. asperellum alone*.

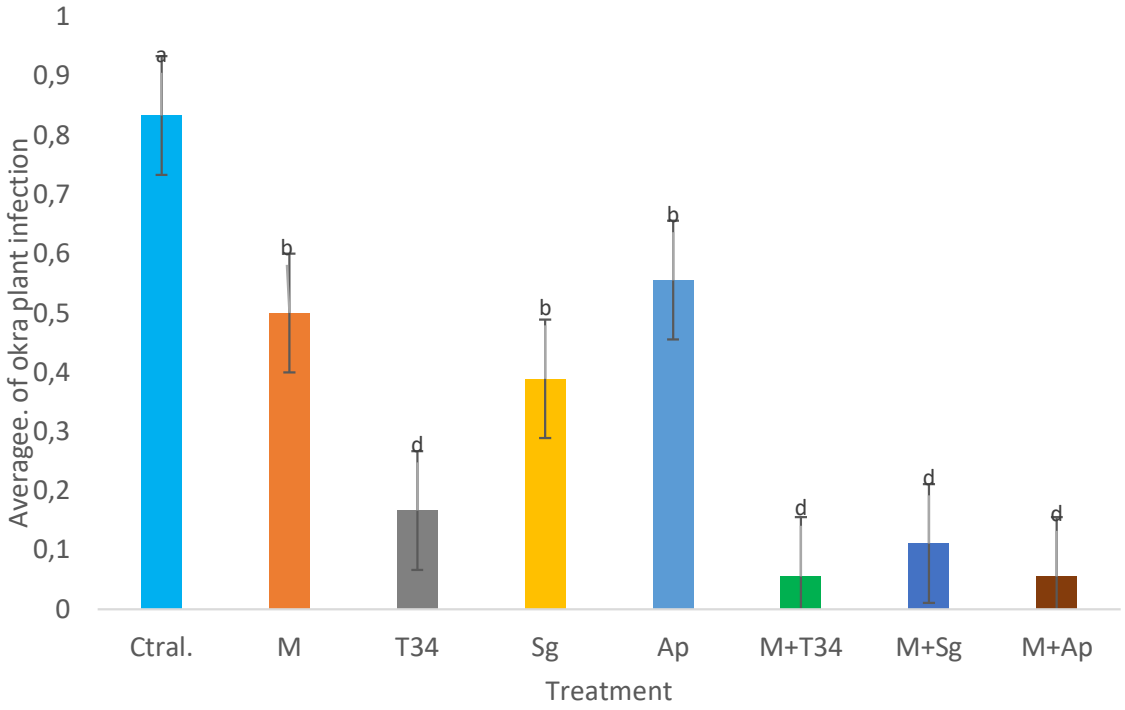


Figure 20 shows the infection rate of okra plants treated with biocontrol agents and their combination. Ctral: Control, M: Mycorrhiza (*Funneliformis mosseae)*, T34: *Trichoderma asperellum* strain 34, Ap. *Aureobasidium pullulans Sg. Streptomyces griseoviridis*).

4.4 Effects of mycorrhiza and antagonists on plant growth.

4.4.1 Effects on okra leaf size and leaf number

Leaf size measurements and leaf number counts were acquired from okra (*Abelmoschus esculentus*) plants as part of the data collection protocol. This data-gathering endeavor was executed on individual plants precisely eight weeks after their initial planting. The third fully expanded leaf from the apical meristem of each plant was carefully selected for subsequent analysis. Subsequently, leaf area was quantified using a ruler, with measurements recorded in centimeters (cm). The outcomes of this empirical investigation are delineated in Figures 21 and 22, encompassing data derived from diverse treatments, notably Mycorrhiza + *A. pullulans* (M +

Ap), arbuscular mycorrhiza + *T. asperellum* strain 34 (M + T34), and arbuscular mycorrhiza + *S. griseoviridis*.

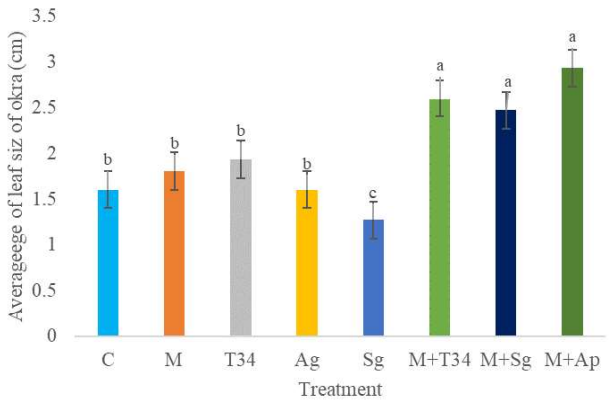


Figure (21) shows average of okra leafe size measurements control: C, Mycorrhiza (Funneliformis mosseae M), T. asperellum strain 34: T34, streptomyces griseoviridis Sg, and Aurobasidum pullulans: Ap

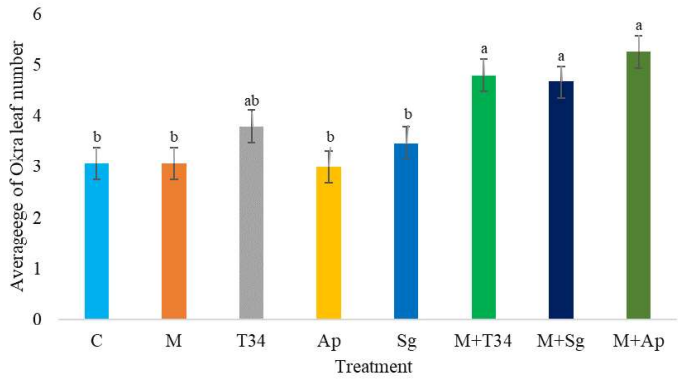


Figure (22) shows average of okra leafe number measurements control: C, Mycorrhiza (F. mosseae M), T. asperellum strain 34: T34, S. griseoviridis: Sg, and A. pullulans: Ap.

4.4.2 Effect plant height:

1- okra plant height

Figure 23 illustrates the findings suggesting a positive influence of arbuscular mycorrhizal AMF (*F. mosseae*) symbiosis, characterized by the integration of biocontrol agents with AMF on the vertical growth of okra plants. Additionally, the introduction of biocontrol agents had varying effects on plant growth, indicating a potential modulation of mycorrhiza-induced growth promotion affecting the height of okra plants. The results revealed discernible disparities among treatments with respect to the height of the okra plants. Specifically, treatments involving the application of AMF combined with *T. asperellum* (M+T34), AMF combined with *S. griseoviridis* (M+Sg), and AMF combined with *A. pullulans* (M+AP) exhibited significantly higher significance in comparison to the control treatment. Conversely, treatments with *T. asperellum* (T34), mycorrhiza alone (M), and S. griseoviridis (Sg) consistently demonstrated a slight increase in plant height compared to the control condition. This study is the first to report a combination of biological agents with AMF. The increase in plant height was as follows: (M+Sg)=52%, (M+Ap)=42.5%, T34= 15.%, M=11% and Ap=11% respactelly. Hence, it suggests that combining treatments significantly increased the plant height as well as antagonistic agents treatments alone slightly enhanced the plant height

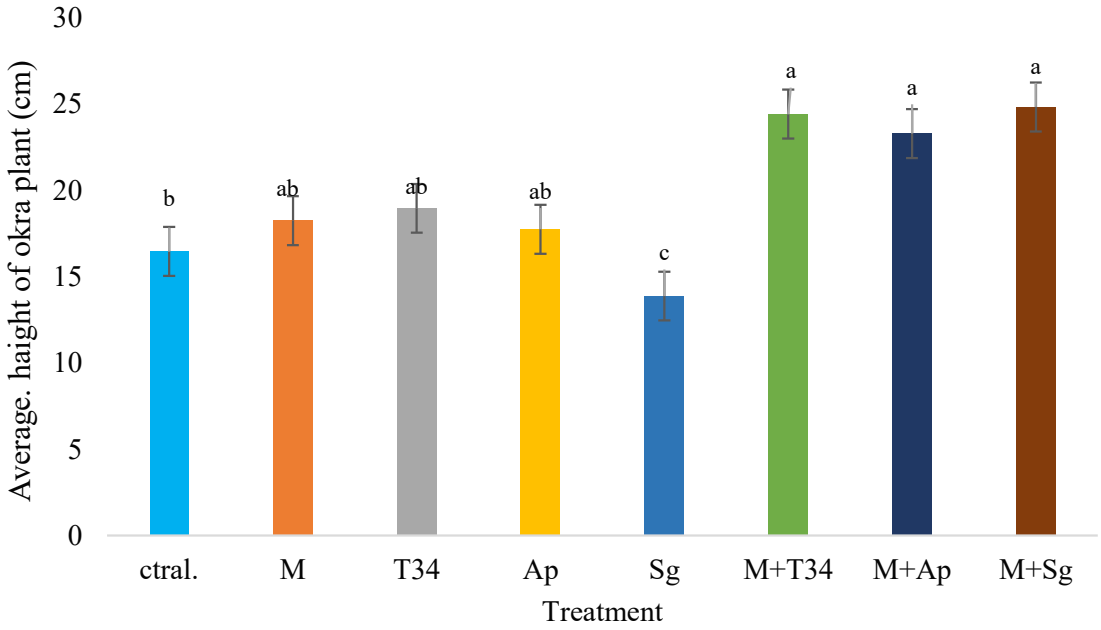


Figure (23) shows the average plant height of okra plants: Control: Ctral, Mycorrhiza (*F. mosseae*) M, *Trichoderma asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

2- Tomato plant height.

The data acquired from the experimental trials revealed discernible patterns in the height of tomato plants influenced by mycorrhiza (*F. mosseae*) and antagonist agents. Quantitative assessments revealed fluctuations in the height parameters, with a particular focus on the stimulation or inhibition of growth induced by the applied treatments. Statistical methodologies were used to ascertain notable disparities among the experimental cohorts. Figure 24 illustrates the disparity in tomato plant height between treatments involving arbuscular mycorrhiza and other treatments. The introduction of antagonist agents yielded diverse effects, as evidenced by the mycorrhiza (*F. mosseae*) (M) alone or in combination with biological agents: M + *T. asperellum* (M+T34), M + *S. griseoviridis* (M+Sg), and M + *A. pullulans* (M+Ap) treatments. Additionally, the *T. asperellum* (T34) treatment displayed a slight enhancement in plant height compared to the control treatment..

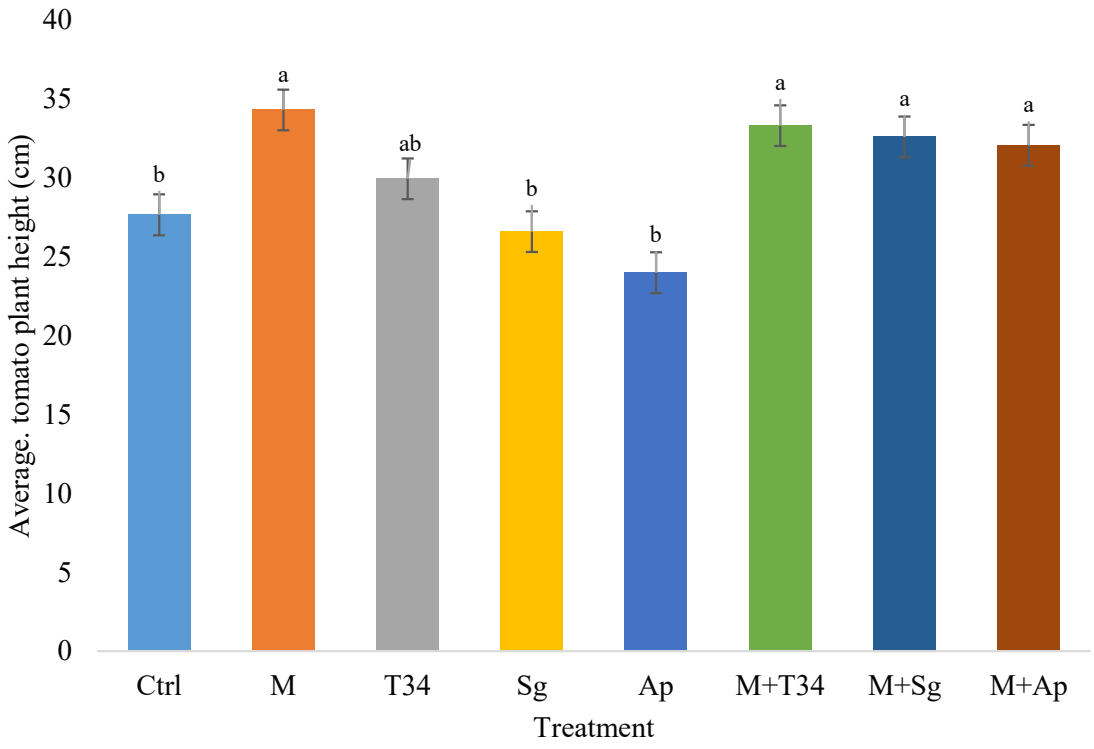


Figure (24) shows the average plant height measurement of tomato plants: control: Ctrl, Mycorrhiza (*F. mosseae):* M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

3- Sweet potato plant shoot length

The findings depicted in Figure 25 illustrate the mean shoot length of sweet potato plants subjected to diverse treatments involving microbial agents in combination with arbuscular mycorrhizal fungi (*F. mosseae*). The treatment with arbuscular mycorrhiza in combination with *T. asperellum* strain 34 (M + T34) exhibited the highest average shoot length compared to the other treatments and control group. Additionally, treatment with arbuscular mycorrhiza (*F. mosseae*) alone (M) and in combination with *S. griseoviridis* (M + Sg) increased shoot lengths relative to the control treatment. These findings imply that the synergistic application of arbuscular mycorrhiza (*F. mosseae*) and *T. asperellum* (34) may be effective in fostering sweet potato growth. Moreover, the utilization of arbuscular mycorrhiza (*F. mosseae*) alone and in combination with *S. griseoviridis* also had favorable effects on plant growth.

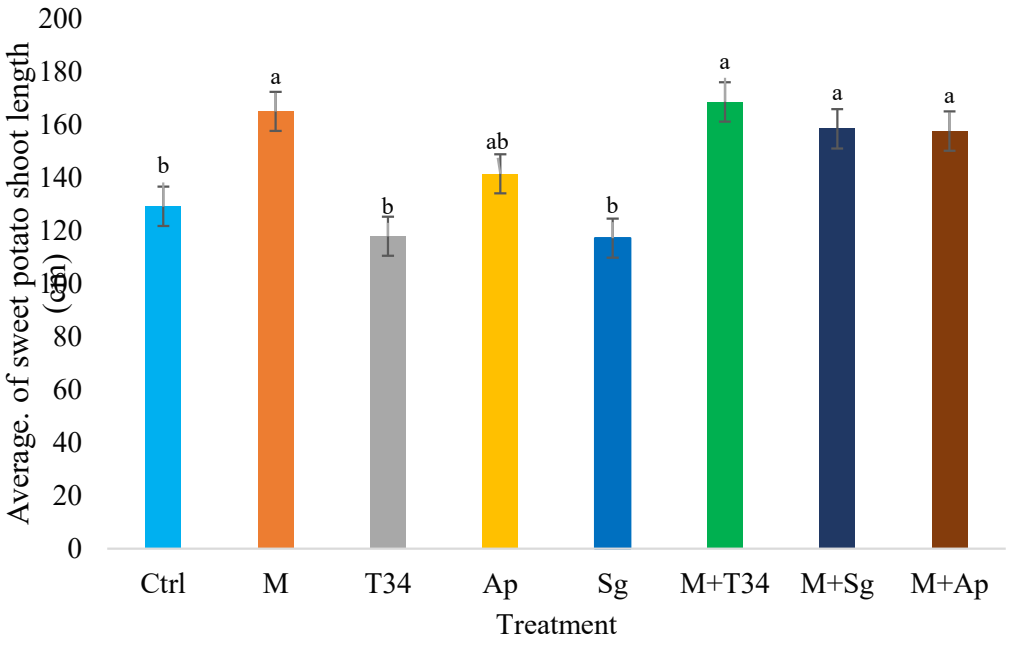


Figure (25) shows the average plant shoot length of sweet potato: control: Ctrl, Mycorrhiza (*F. mosseae):* M, *T. asperellum* strain 34: T34, *A. pullulans*:Ap. *S. griseoviridis*: Sg.

4.4.3 Effect on the Fruit Number of Okra

The data depicted in Figure 26 suggest a positive correlation between mycorrhizal (*F. mosseae)* symbiosis, involving mycorrhiza (*F. mosseae)* alone or a combination of mycorrhiza with biological agents, and the number of okra fruits per plant. Specifically, inoculation of plants with a combination of mycorrhiza and microbes, either collectively or individually, excluding antagonist agents, yielded varied effects. Variability in significantly increasing fruit weight percentage for the combination treatment was Mycorrhiza +*T. asperellum* (M+T34) =130%, Mycorrhiza+*A. pullulans* (M+Ap) = 96%, mycorrhiza (M) = 94%, and Mycorrhiza+*S. griseoviridis* (M+Sg) = 85% high specificity. However, the inoculation of plants with microbes (Ap = 41%, Sg = 37%, and T34 = 27%) was less significant than that of the control. The outcomes M+T34, M+Ap, M, M+Sg, Ap, Sg, and T34, respectively , demonstrated a significant increase in fruit numbers as a result of combination treatment. Fruit production was quantified on the basis of the number of fruits produced per treatment.

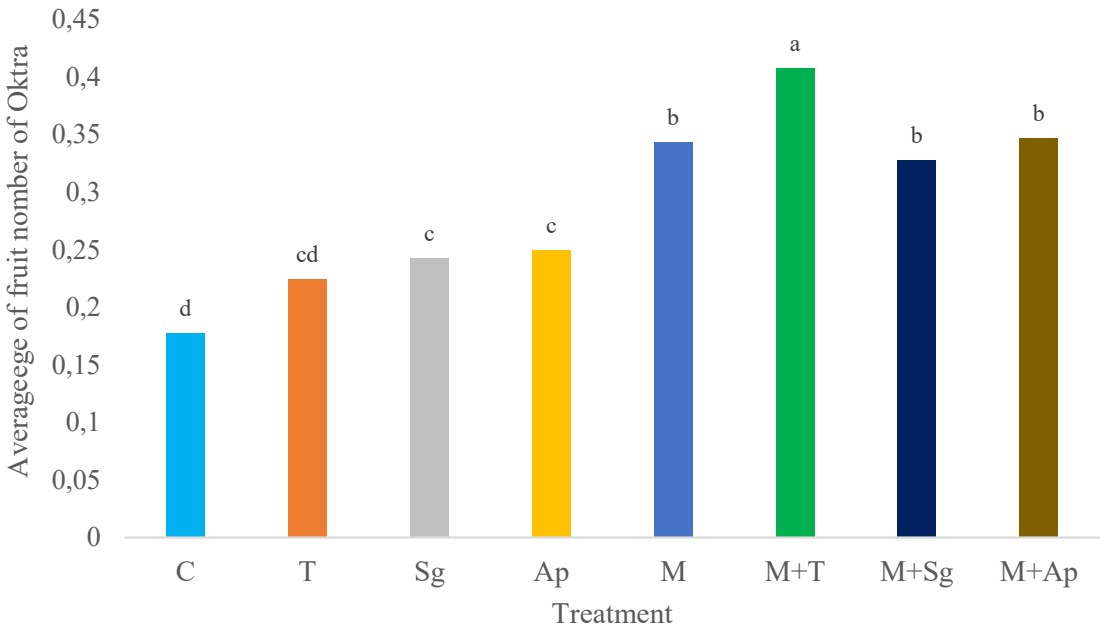


Figure (26) shows the average fruit number of okra plants. C: Control, M1: Mycorrhiza (*F. mosseae)* with perlite; T: *T. asperellum* strain 34; Ap: *A. pullulans*; Sg: *S. griseoviridis K61*.

4.4.4 Effect on the Yield:

1. Okra yield :

The data obtained from the experimental trials revealed discernible patterns in the yield weight of okra plants subjected to Mycorrhiza (*F. mosseae*) inoculation and combination with biological agents: Mycorrhiza (*F. mosseae)* (M), *T. asperellum* T34 (T34), *A. pullulans* (Ap), *S. griseoviridis* K61 (Sg), and combination of Mycorrhiza (*F. mosseae*) with biological agents. (M+T 34), (M+A p), and (M+S g). Specifically, the weight of the okra fruits ranged from combined and single treatments, M+T34 = 75%, M+Sg =70%, and M+Ap = 53%, (Sg) = 43%, (M) = 41% (T34) = 39%. The results offer M+T34, M+Sg, Sg, M, T34, and M+Ap insights into the potential influences of mycorrhizal associations and antagonist agents on the overall yield production of okra and Figure 27 shows combinations and individual treatments significantly increased okra yield compared to contro Howeverl., it is noticeable that the fruit weight of combining microbes with mycorrhiza treatment was highly significant compared with the control fruit weight. This indicates that the combined weight of the fruits and plants can vary considerably. The differences in fruit weights of okra plants when combining *F. mosseae* with microbial agents can be attributed to the complex interactions between these microorganisms and okra plants. Quantitative measurements were conducted to ascertain the variations in fruit weight, and statistical analyses were performed to elucidate the significance of these variations.

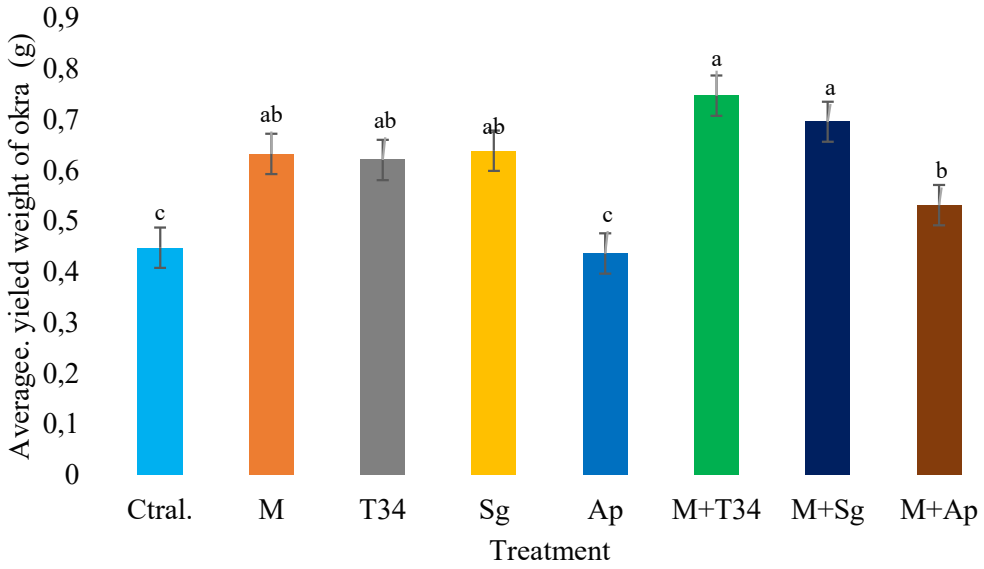
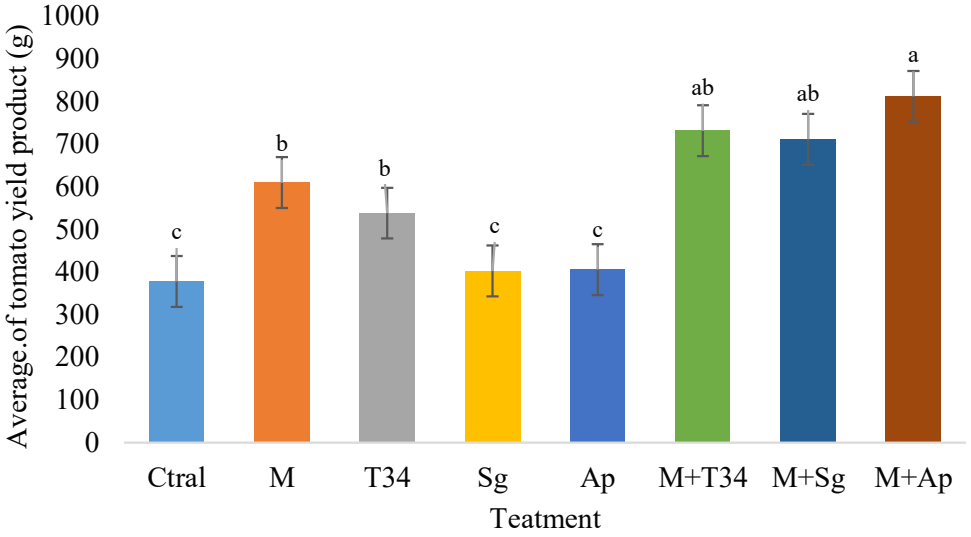


Figure (27) shows the average fruit weight of the okra. Ctral: Control, M1: Mycorrhiza (*F. mosseae)* with perlite; T34, *T. asperellum* strain 34; Ap, *A. pullulans*; Sg, *S. griseoviridis*.

2. Tomato yield.

This study investigated the effect of combining various microbes with mycorrhiza (*F. mosseae*) (M) treatment on okra plant yield. The combinations examined included M+T34 (T. asperellum), M+Sg (S. griseoviridis), and M+Ap (*A. pullulans*). The results indicated significant variability in okra fruit weight across the treatments. M+Ap, M+T34, and M+Sg, respectively, showed significantly increased yield compared to the control, as well as with individual treatments (M and T34), showing improvement in tomato yield. However, Ap and Sg did not exhibit yield enhancement (*F. mosseae)* and fungi were found to positively influence tomato plant growth and yield, suggesting that fruit weight variations in tomato plants with combined microbe and

mycorrhiza treatments may be influenced by the specific characteristics and interactions of these



microorganisms.

Figure (28) Show average of the average tomato yield (g) Control: Ctral, Mycorrhiza (*F. mosseae* M), *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

3. Sweet potato yield:

The results depicted in Figure 28 provide insights into the variations in yield observed across various treatments administered to sweet potato plants, particularly when incorporating arbuscular mycorrhizal fungi (*F. mosseae)* with biological agents. Notably, the combined treatment (M + Ap) and (M + T34)alone significantly increased yield, and the application of mycorrhiza alone exhibited the most substantial and statistically significant enhancements in tuber yield. Furthermore, treatments involving combinations such as (M + T34), T34 alone, Ap alone, and (M + Sg) also demonstrated heightened tuber weight compared to the control group.

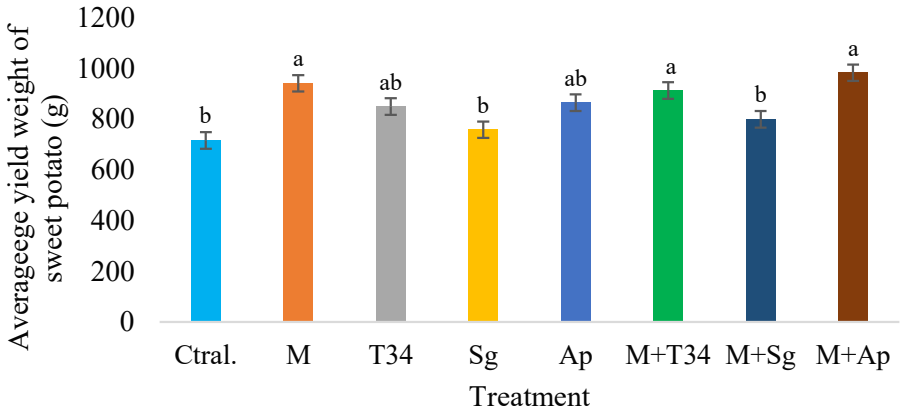


Figure (29) Tuber root weight measurement (g) Control: Ctral, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.4.5 Effect on dry weight

I- Upper part dry weight of okra plant

The effect of biological agents combined with mycorrhiza on the biomass of the upper part of okra plants with discernible dry weight outcomes is shown in Figure 30. In this study, the synergistic interplay between arbuscular mycorrhizal fungi (*F. mosseae*) (M) and host plants, coupled with three distinct microbial agents, evoked varied levels of dry-weight biomass augmentation in the upper portions of okra plants. Specifically, the synergistic combination treatments of arbuscular mycorrhiza with A. pullulans (M+Ap = 82%) and *T. asperellum* (M+T34 = 77%) showed significant differences in dry weight biomass compared to the control group. Similarly, individual treatments with mycorrhiza (M = 84%) and *T. asperellum* alone (T34 = 43%) resulted in an increase in the dry weight biomass. However, the combination treatment (M+Sg = 28%) slightly increased the dry weight compared to the control.

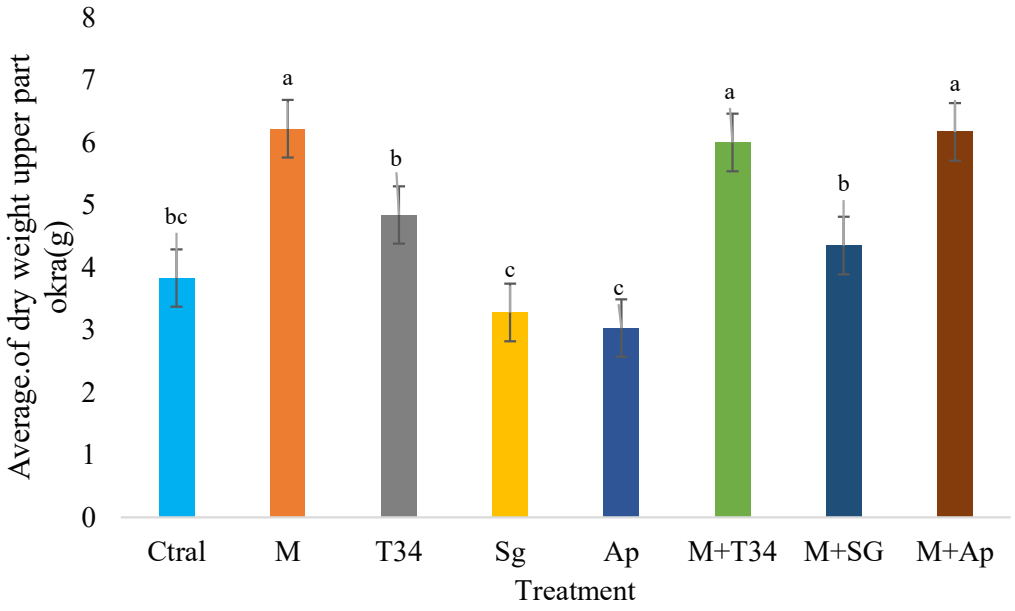


Figure (30) shows the average dry weight of the upper part of the okra plant: control: control, mycorrhiza (*F. mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

II - Upper part dry weight of tomato plant

The experimental trial results showed observable trends in the dry weight of the tomato plants. Quantitative measurements were conducted at predetermined intervals, allowing the characterization of temporal variations in plant biomass. Statistical analyses facilitated the identification of significant differences in dry weight among experimental conditions, providing insights into the factors influencing biomass accumulation. Figure (31) shows the difference

between the treatment of the vegetative dry weight of the tomato plants that arbuscular mycorrhiza (*F. mosseae)* combined with the biological agents and alone treatments (M), M+AP, M+T34, and M+Sg frequently showed significant differences compared to the control. The microbial agent treatments (T34, Sg, Ap) did not show an impact on tomato yield compared to the control.

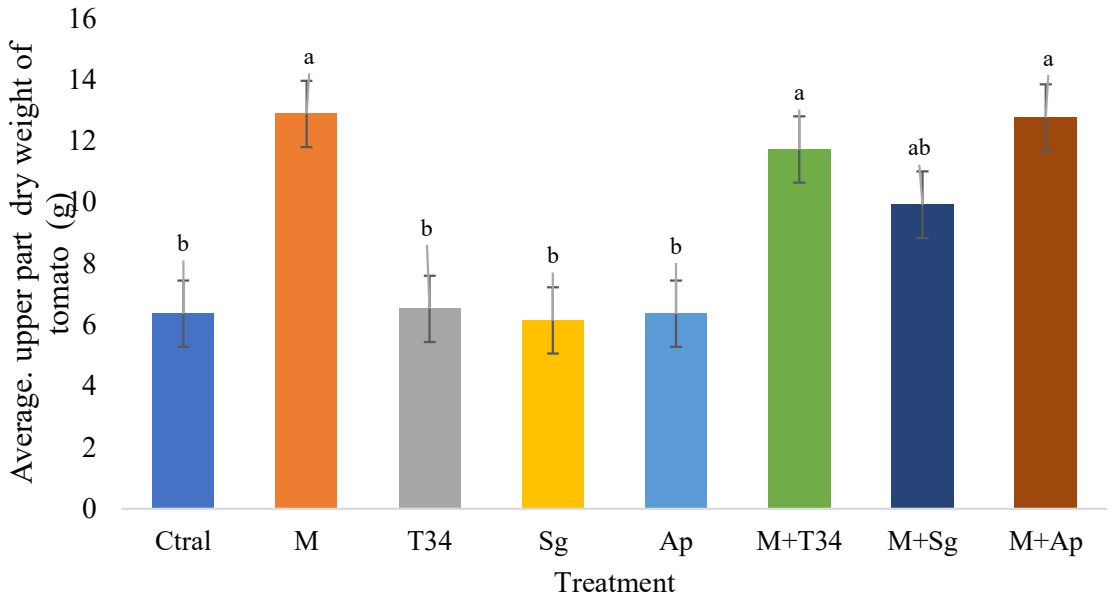


Figure (31) shows the average dry weight of the upper part of the tomato: treatment control: ctral, mycorrhiza (*F. mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

III- Root dry weight measurements of okra plant

The findings presented in Figure 32 illustrate significant differences in root dry mass among various treatment modalities applied to okra plants, particularly those involving combinations of arbuscular mycorrhizal fungi (AMF), *F. mosseae*, with biological agents. Treatment of mycorrhizal fungi with microbial agents, namely AMF +*A. pullulans* (M + AP), AMF + *T. asperellum* strain 34 (M + T34), AMF alone, and AMF + *S. griseoviridis* (M + Sg), resulted in increased root dry weight compared to treatments consisting solely of microbial agents or the control group. Notably, the inclusion of microbial agents in the T34 treatment slightly amplified the disparity in root dry mass, whereas no statistically significant differences in root dry weight were observed between the Ap and Sg treatments.

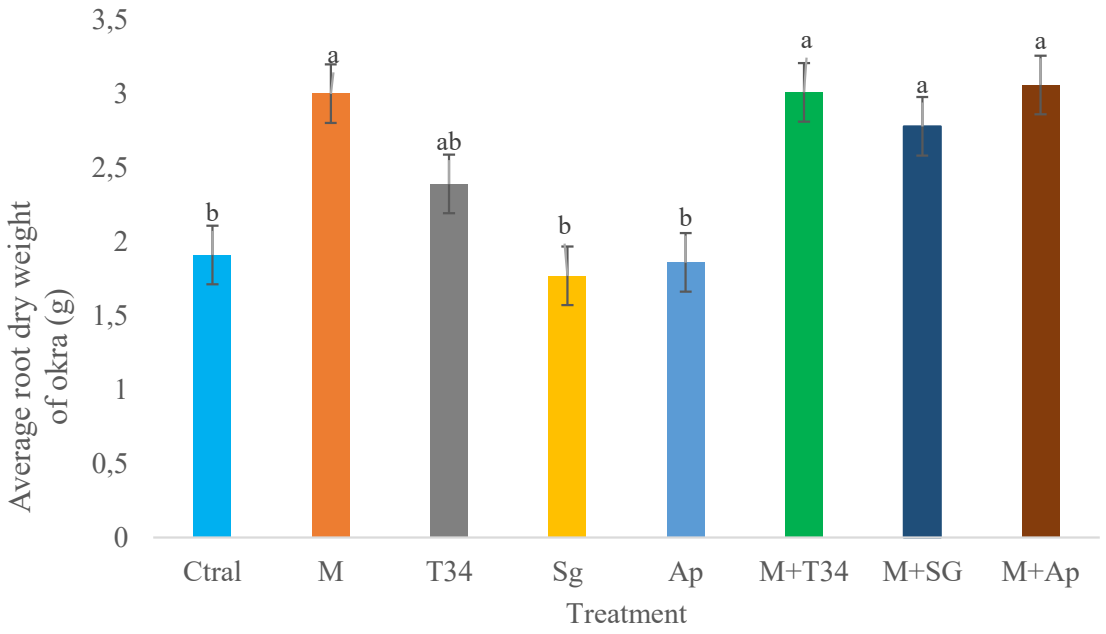


Figure (32) shows the average root dry weights of okra plants: Control: Ctral, mycorrhiza (*Funneliformis mosseae)* M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

IV- Root dry weight of tomato plant

The results presented in Figure 33 demonstrate the difference in root dry weight between various treatments of tomato plants in conjunction with arbuscular mycorrhiza (*F. mosseae)* and microbial agents. The combined treatments of arbuscular mycorrhiza with *A. pullulans* (M + AP), arbuscular mycorrhiza and *T. asperellum* strain 34 (M + T34), and arbuscular mycorrhiza and *S. griseoviridis* (M + Sg) all exhibited higher root dry weights than the control group. Treatment with arbuscular mycorrhiza alone (M) also resulted in a significant increase in root dry weight. Conversely, treatments of T34, Ap, and Sg with biological agents resulted in no significant disparities in root dry weight.

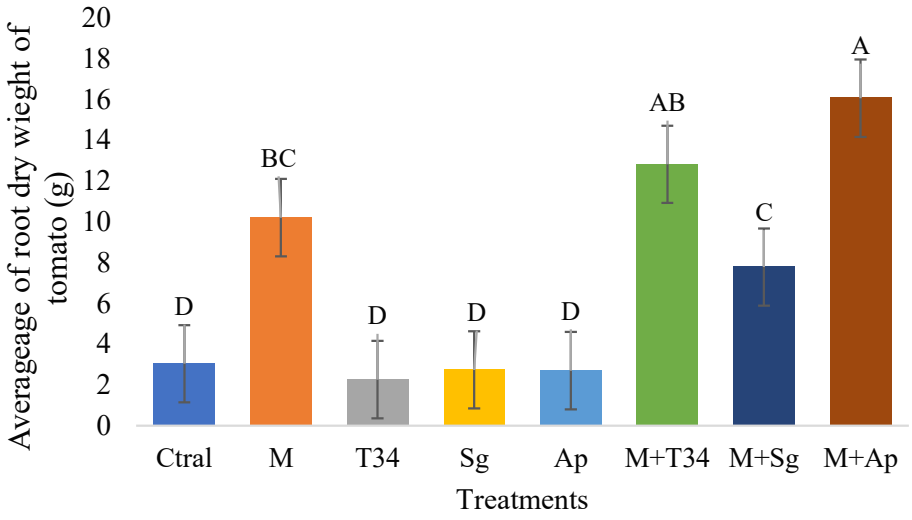
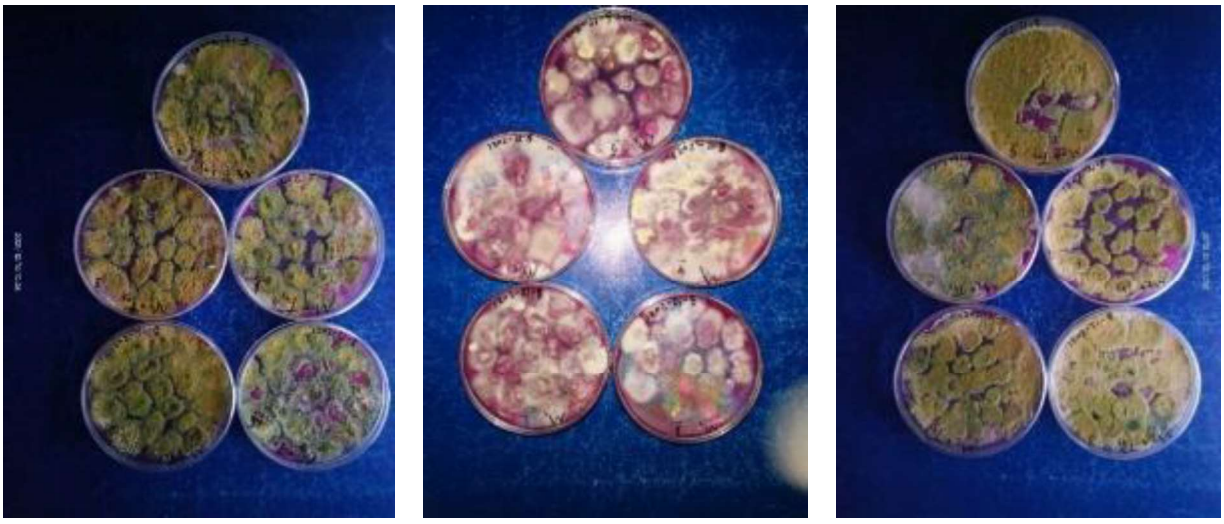


Figure (33) shows the average tomato plant root dry weight: Control: Ctral, Mycorrhiza (*F. mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.5 Root colonisation by Trichoderma

1. Trichoderma colonisation in okra root

The findings illustrated in Figures 34 and 35 show that the combination of *T. asperellum* strain 34 and arbuscular mycorrhiza (*F. mosseae*) did not exhibit deleterious effects on either organism. This finding suggests that the concurrent application of *T. asperellum* strain 34 and arbuscular mycorrhiza holds promise as a viable strategy for mitigating plant diseases and augmenting agricultural yields. In summary, the results of this investigation underscore the potential advantages of employing *T. asperellum* strain 34 and arbuscular mycorrhiza in tandem as a means of fostering plant growth and bolstering resistance against pathogens.



A B C

Figure 34: Shows the Trichoderma colonisation in the root of okra. A: *T. asperellum T34* treatment, B: arbuscular mycorrhiza (*F. mosseae*) treatment, C: Combination treatment *(T34*+M)

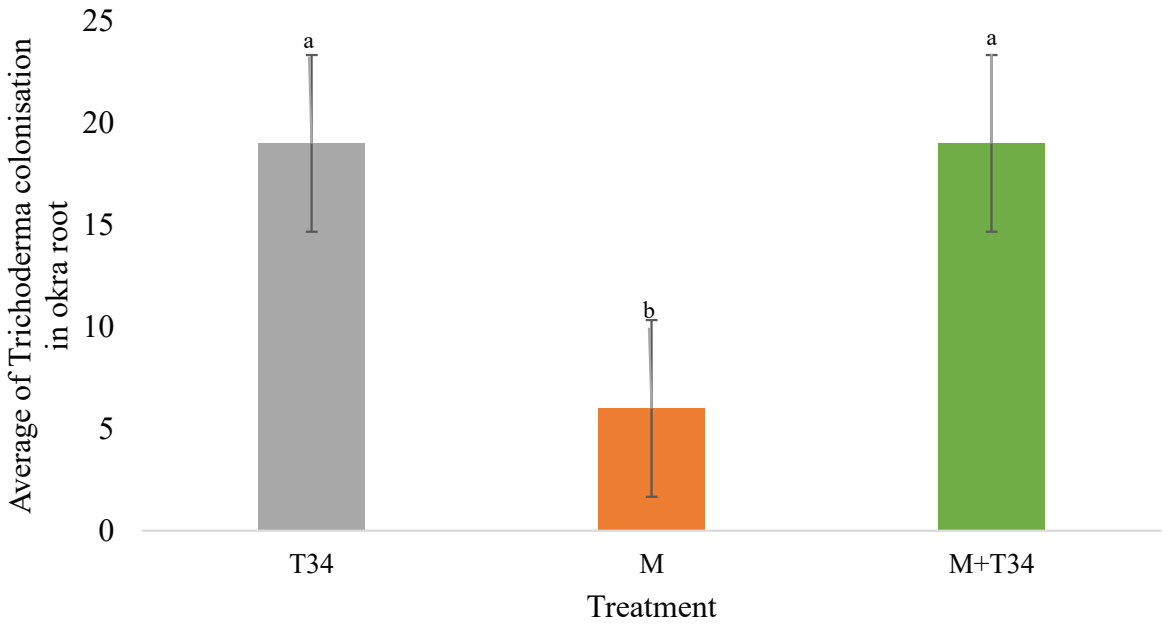


Figure (35): show colonization of *T. asperellum* in okra roots. T34: *T. asperellum*, M: arbuscular mycorrhiza (*F. mosseae*).

2. Trichoderma *asperellum* colonisation in tomato root

The data acquired from the root colonization evaluation elucidated the presence and spatial distribution of *T. asperellum* (T34) in tomato roots. Colonization density was meticulously quantified, thereby delineating the prevalence and colonization of the roots and the percentage of symbiotic associations between *T. asperellum* (T34) and the tomato root system. Observations of root colonization on PDA media have provided valuable insights into the spatial distribution of *T. asperellum* (T34) hyphae across distinct root zones of tomato plants. Figure 36 shows the robust cooperative behavior of *T. asperellum* strain 34 with arbuscular mycorrhizal fungi (*F. mosseae*)., underscoring their ability to effectively colonize the root system, thereby fortifying plant growth, bolstering plant defenses, and fostering mutualistic relationships conducive to enhanced plant health and productivity.

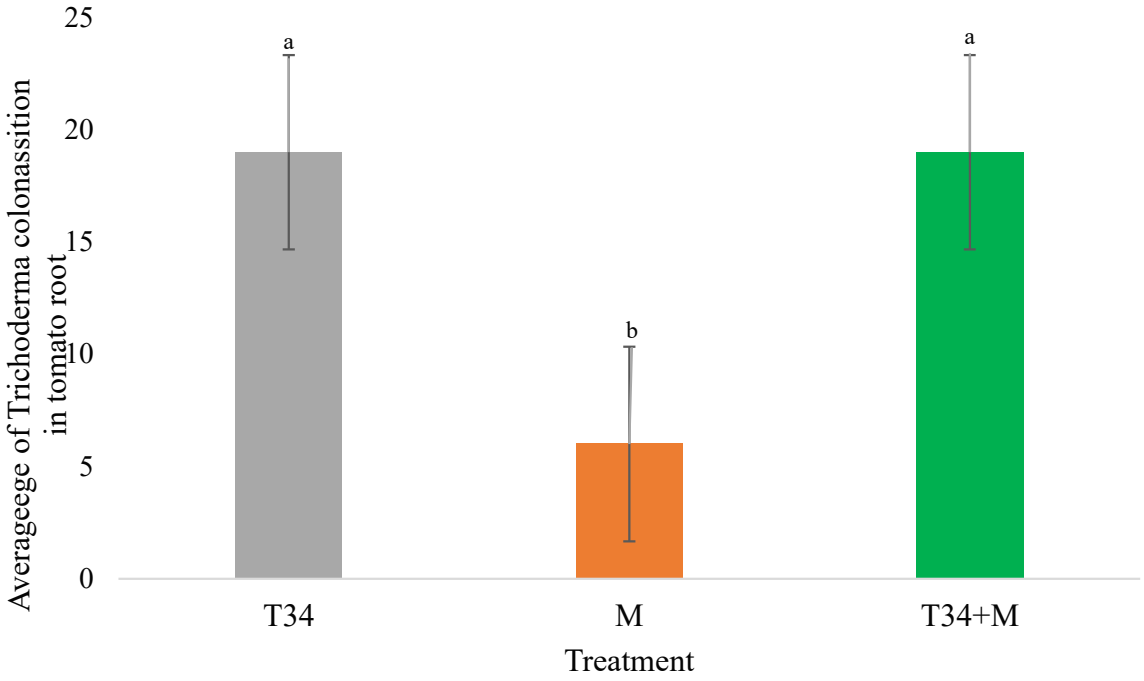


Figure 36: Average Trichoderma colonization in tomato roots. Plants treated with microbes alone or in combination with T34: *T. asperellum*; M: Mycorrhiza.

3. Evaluate *Trichoderma asperellum (T34)* colonisation in sweet potato root

Preliminary findings revealed a notable influence of co-inoculation on sweet potato root colonization by *T. asperellum* (T34), with observed enhancements in the root. Furthermore, the presence of T34 in the root system is anticipated to affect nutrient assimilation, thereby contributing to increased plant vigor. Figure 37 shows the treatment of the T34 cooperation with arbuscular mycorrhiza (*Funneliformis mosseae)* (M+T34), and T34 alone, the roots were 100% colonized with T34 and did not affect each other. Thus, they can work together to protect and enhance plant growth, and induce plant resistance.

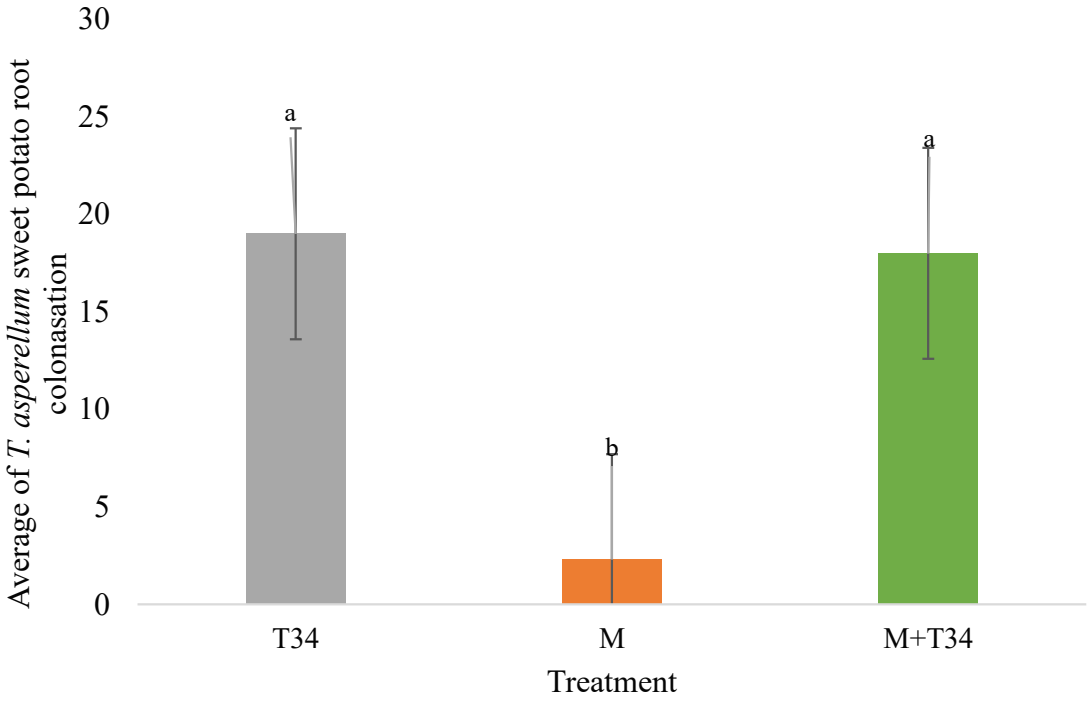


Figure (37) shows Colonization of *Trichoderma asperellum* T34 in Sweet Potato Plant Roots Treated with Arbuscular Mycorrhiza (*Funneliformis mosseae*), and a combination treatment T34 + M.

4.6 Root colonization by mycorrhiza

I- Okra Root colonization

Arbuscular mycorrhizal fungi *Funneliformis mosseae* (AMF) Funneliformis mosseae plays a crucial role in promoting biomass growth in food crops (Alam, Choudhury and Mridha, 2023), and the extent of root colonization by AMF is indicative of the strength of the symbiotic relationship between the fungi and the plant. In this study, we examined the colonization of okra plant roots treated with AMF alone and in combination with biological agents (*T. asperellum*, *A. pullulans*, and *S. griseoviridis*) (T34, Ap, and Sg) (Figure 38). Our laboratory investigations revealed varying levels of root colonization in different treatments. The M+Sg treatment showed the highest colonization rate (85.16 %), followed by the M treatment (84.68 %). Conversely, M+T34 and M+Ap treatments exhibited lower colonization rates of 61.18% and 60.92%, respectively. Significant increases in AMF root colonization were observed with M and M+Sg treatments compared with the other microorganisms. Additionally, simultaneous infection with *T. asperellum*, *A. pullulans,*and *S. griseoviridis* led to a dramatic decrease in AMF root colonization levels, potentially owing to the suppression of AM parasitism by T34 and Ap (Wang et al., 2023).

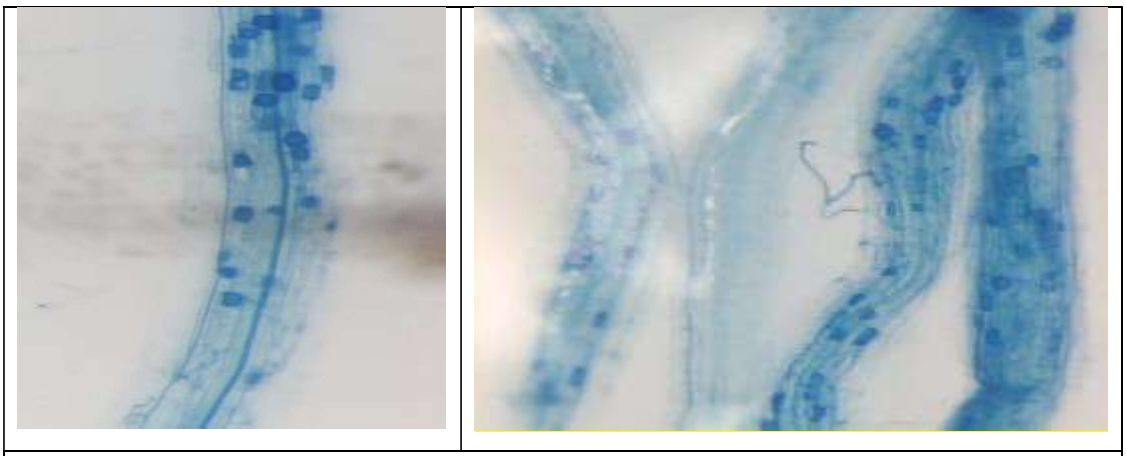


Figure 38: Mycorrhiza colonasation (*Funneliformis mosseae)* root of the okra plant

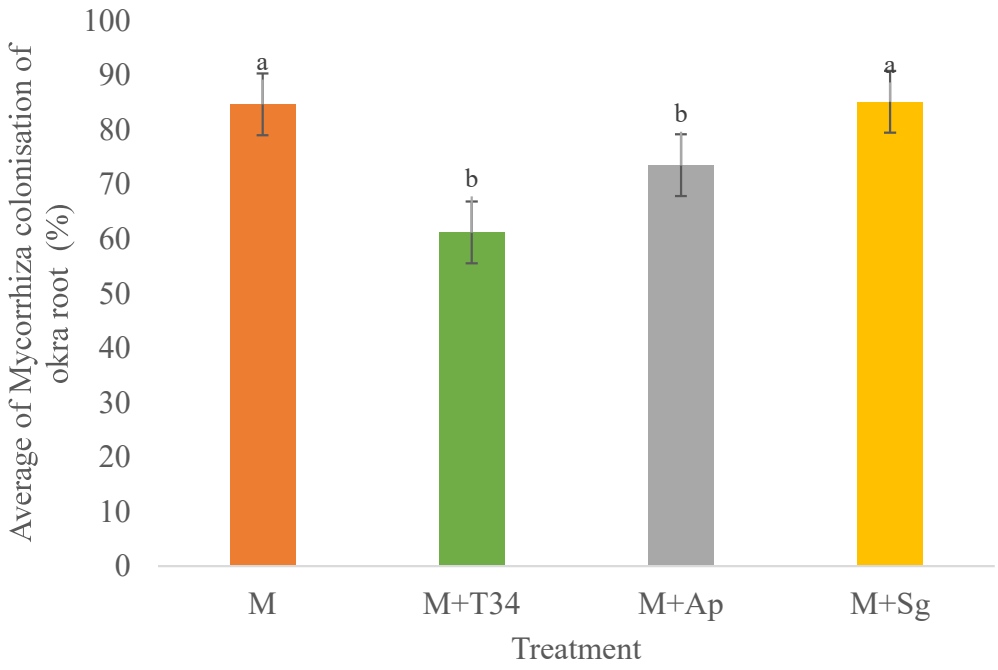


Figure (39) shows the percentage of arbusular mycorrhizal (*Funneliformis mosseae)* colonization in the roots of okra. M, mycorrhiza; *T, Trichoderma asperellum* strain 34; Ap, *Aureobasidium pullulans*; Sg, *Streptomyces griseoviridis.*

II- Root colonization of tomatoes by mycorrhiza (Funneliformis mosseae)

The results presented in Figure 40 demonstrate the difference in arbuscular mycorrhizal (*Funneliformis mosseae)* (AMF) colonization between various treatments of tomato plants in conjunction with microbial agents. The treatments that included arbuscular mycorrhiza alone (M) and arbuscular mycorrhiza combined with *T. asperellum* strain T34 (M + T34) did not show a negative effect on each other compared to the treatments that included arbuscular

mycorrhiza combined with *S. griseoviridis* (M + Sg) and arbuscular mycorrhiza combined with *A. pullulans* (M + Ap). These findings suggest that the combined use of arbuscular mycorrhiza and *T. asperellum* strain T34 may be particularly effective in promoting tomato plant growth, while the use of arbuscular mycorrhiza alone also exhibited positive effects on plant growth. The results of this evaluation highlight the potential benefits of utilizing these microorganisms in combination to enhance tomato plant growth and productivity.

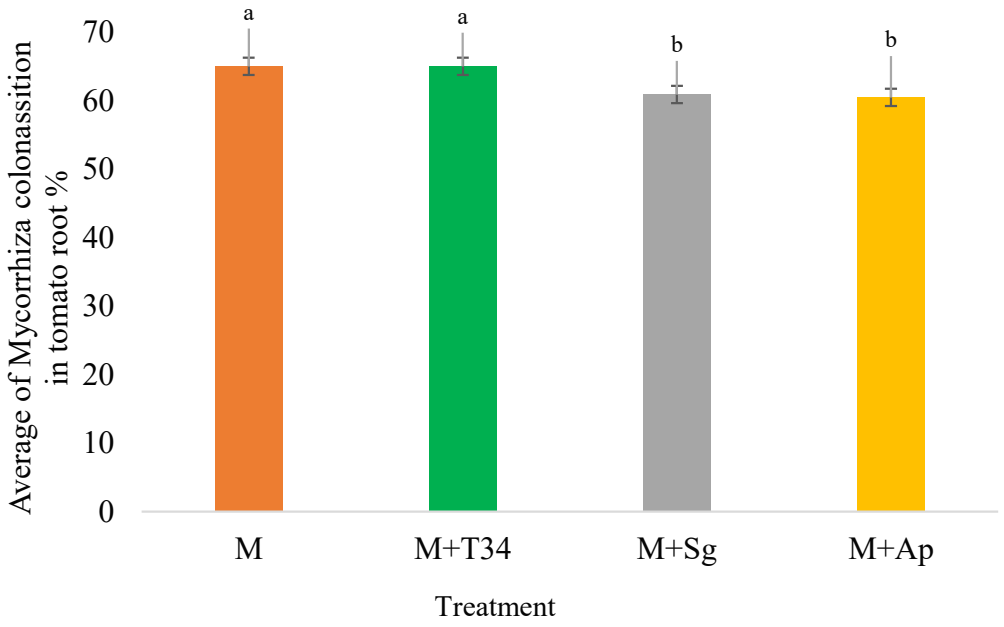


Figure (40) shows the percentage of mycorrhiza (*Funneliformis mosseae*) root colonisation of tomato. M, mycorrhiza with perlite; T34, *Trichoderma asperellum* strain 34; Ap, *Aureobasidium pullulans*; Sg, *Streptomyces griseoviridis*.

III- Root colonization of sweet potatoes by mycorrhiza (*Funneliformis mosseae)*

Figure 41 shows the rate of arbuscular mycorrhizal colonization in the sweet potato between the treatment of the sweet potato plant in which the companions of arbuscular mycorrhiza with the microorganism agents with the highest treatment of length M +A, M +*T. asperellum* (T34), M, M +*S. griseoviridis* were highly significant compared to the control.

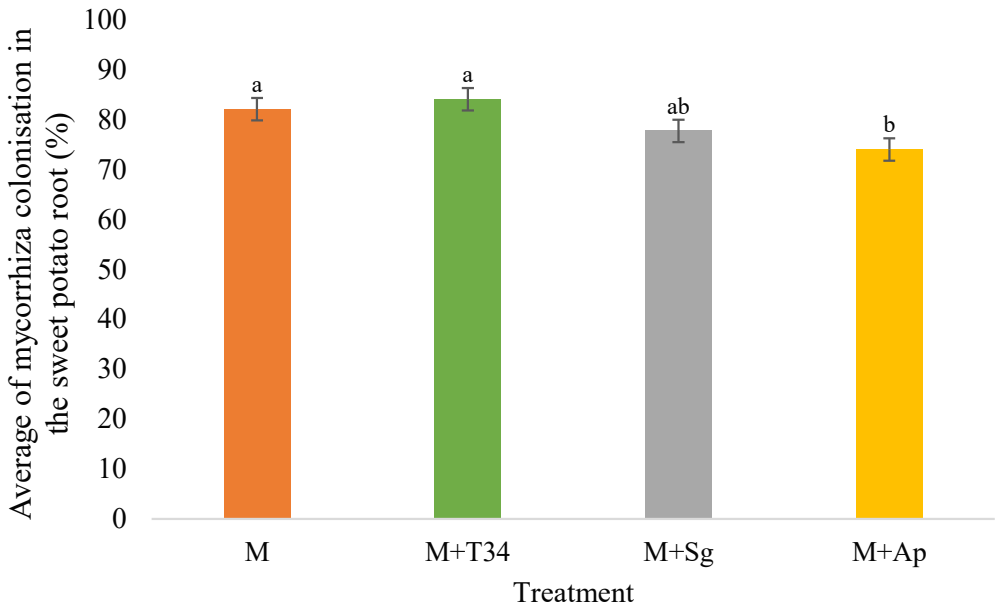


Figure (41) shows the percentage of mycorrhiza (*Funneliformis mosseae*) colonization (root)in sweet potato roots. M, mycorrhiza with perlite; T34, Trichoderma asperellum strain 34; Ap, Aureobasidium pullulans; Sg, Streptomyces griseoviridis.

4.7 Effect enzymes and antagonist treatment on the Glutathione-S-Transferase activity of okra plant

The observed variations in GST activity among the different microbial treatments suggest distinct influences on the antioxidative responses of okra plants. Mycorrhiza (*Funneliformis mosseae*) (M) treatment exhibits the highest GST activity, followed by *A. pullulans* (Ap) and *T. asperellum* (T34), indicating potential contributions to the plant's ability to counteract oxidative stress. *S. griseoviridis* (Sg) treatment shows a moderate increase in GST activity compared to the control.

The combination of mycorrhiza with Trichoderma (M+T34) and *A. pullulans* (M+Ap) demonstrated noteworthy changes in GST activity, suggesting potential synergistic effects on the plant's antioxidant capacity. Conversely, the combination of Mycorrhiza with *S. griseoviridis* (M+Sg) exhibited lower GST activity than mycorrhiza alone.

Figures(42,43) show the difference between the enzyme activity of the okra and tomato plant treatments, showing that the combination and single treatments (M, Ap, T34, M+T34, Sg, and M+Ap) were highly significant compared with the Ctral and M+Sg treatments.

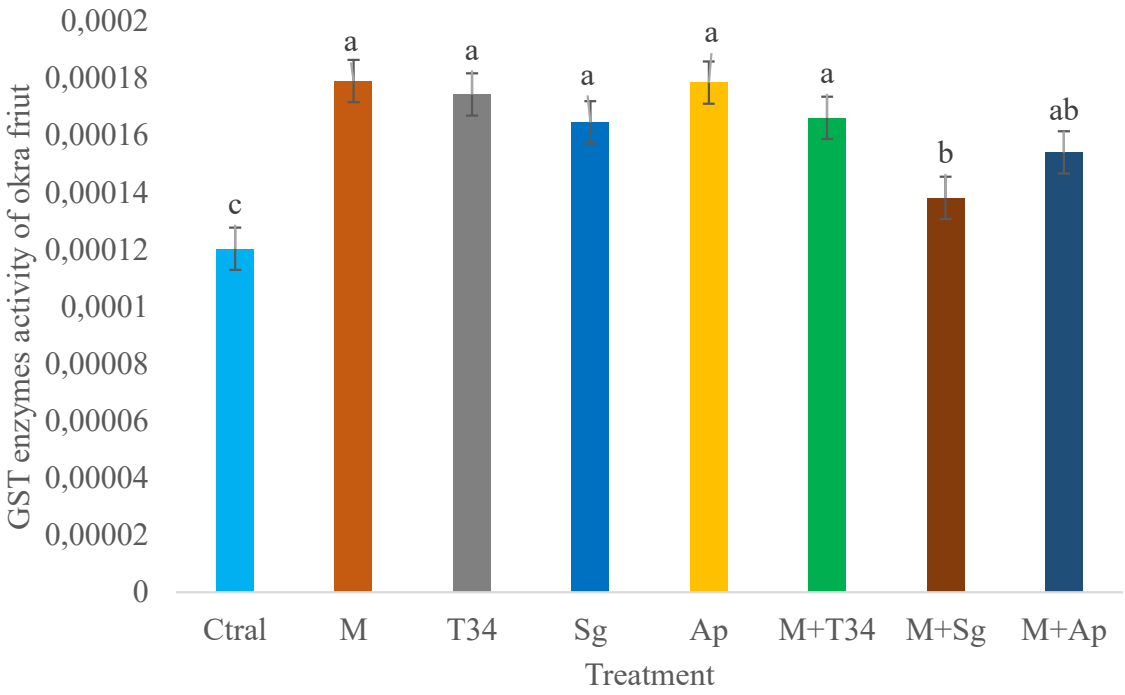


Figure (42) Enzyme activity of glutathione-S-transferase enzymes (GST) in okra fruits treated with microorganisms. Ctral, Control; M, *Funneliformis mosseae* with perlite; T34, *Trichoderma asperellum* strain 34; Ap, *Aureobasidium pullulans*; Sg, *Streptomyces griseoviridis.*

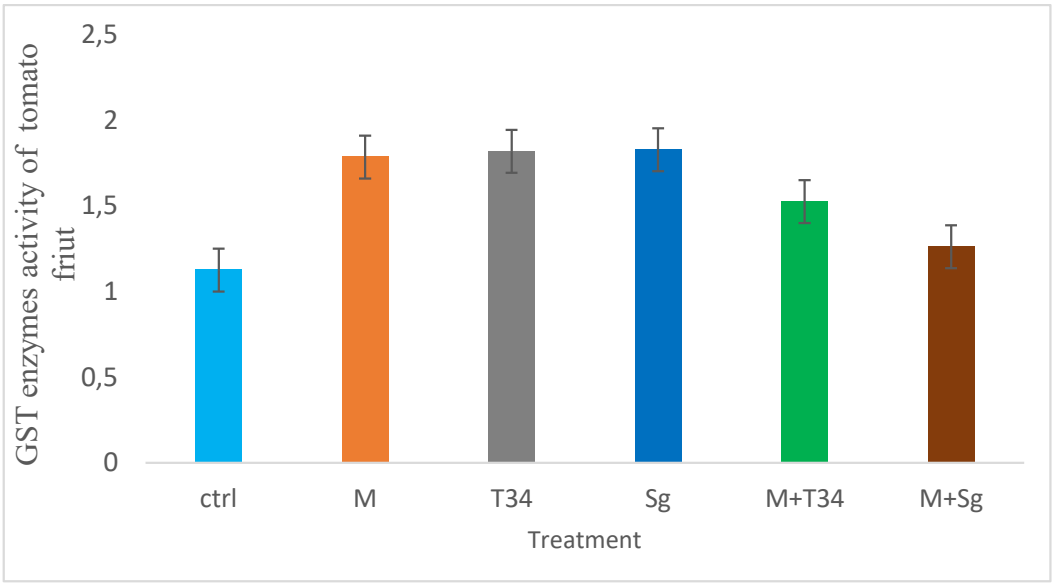


Figure (43) shows the activity of glutathione S-transferase enzymes (GST) in tomato plant leaves after combined treatment with biological agents alone. Ctrl: Control, M: *Funneliformis mosseae* with perlite, T34: *Trichoderma asperellum* strain 34, Sg: *Streptomyces griseoviridis*

4.8 Effect of mycorrhiza and antagonist treatment on the polyphenol content in okra plant:

The HPLC protocol applied to analyse poly phenols from the whole okra fruit allowed for excellent separation of the main compounds and their derivatives mainly dimers and glycosides (Figure 43). To make the discussion easier and meaningful, the obtained results were arranged in groups for the main compounds.

Almost all plants possess antioxidant defence systems including antioxidant enzymes and nonenzymatic antioxidants. Phenolic compounds or polyphenols, which consist of secondary metabolites, constitute a wide and complex array of phytochemicals that exhibit antioxidant actions. It is worthy to mention that most of the research on the antioxidant activity of phenolics from vegetables focused on the total phenol (TP) content). However, few articles dealt with the antioxidant activity of individual poly phenols (Hu et al., 2014).

4.8.1 **Kaempferol Derivatives**.

One of the important objectives of the present work is to investigate the effect of mycorrhiza alone and hyphenated with microbes on the content of polyphenols in okra fruits. Figure 44 shows the effect of mycorrhiza and antagonist agents on the content of kaempferol derivatives in the whole okra fruit.

The combination of arbuscular mycorrhiza with other microorganisms in different treatments (T34, M, Sg, M+Sg, M+T34, and M+Ap) caused a significant increase in the level of okra fruit phenols as compared to the control treatment. The results presented in Figure 44 demonstrated the effectiveness of various treatments on kaempferol content in Okra fruit, in conjunction with arbuscular mycorrhiza and microbial agents. Kaempferol and its derivatives one of the most important compounds stand out as prevalent dietary flavonoids with notable implications for health. Their therapeutic potential extends to various chronic diseases, such as diabetes and fibrosis. Recent scholarly reviews have delved into the pharmacological actions of KMF in diverse medical domains, including cancer, central nervous system diseases, and chronic inflammatory conditions (Mishra *et al.*, 2023). Figure (44) shows the impact of the combination of mycorrhiza and microbe agents on the okra to increase the percentage of fruit's phenols (Kaempferol derivatives). Inoculation of the plant with T. asperellum (T34), Mycorrhiza fungi (M), Sg, M+Sg, M+T34, M+Ap and Ap increased the content of kaempferol by 38%,29%, **27%**, 22%, 20**%**, 17% and14% respectively increased the rate of okra fruit kaempferol and was highly significant compared with the control. These findings suggest that the combined use of arbuscular mycorrhiza and these microbial agents may be particularly effective in promoting the metabolic pathways of some phenolic compounds in Okra fruit, which may have positive implications for plant health

and resistance to various stressors. The highest increase in the content of the kaempferol derivatives wase recorded for the T34 treatment. Shahrajabian, 2023 reported that microbes' infection increases some, but not all phenolic compounds in the plants.

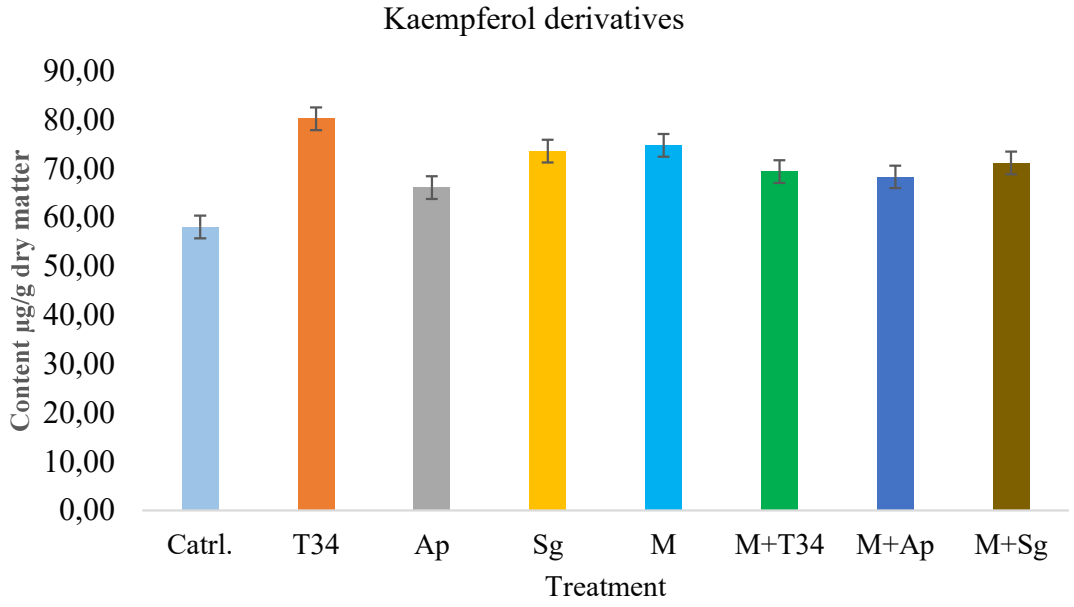


Figure 44 shows the Effect of Mycorrhiza and Antagonist Agents on Kaempferol Derivatives in Okra fruit: control: Catrl, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*

4.8.2 Coumaric acid and derivative.

The results presented in Figure 45 demonstrate the response of coumaric acid derivatives to various treatments. Such important polyphenolic compounds existed in Okra fruits in two free and coumaroyl-hexoside. The treatments of M + Sg, and M + AP showed an increase in the content of the two derivatives as compared to the control group. It is evident that a highly significant increase of coumaric acid and coumaroyl hexoside was recorded for treatments of M+AP and M+Sg respectively. It seems that the two treatments differ in their effect on glycosylation of coumaric acid in Okra fruits. As compared to the control, other treatments either decreased or had no significant effect on the level of coumaric acid derivatives. The results showed that the plants inoculation with microbes increased the level of coumaroyl derivatives by 43%, 22%, 12%, 9%, and 7% with M+Ap, M+Sg, M, Sg, and Ap respectively. Coumaric acid percentage enhancement has been found to be 29% with Mycorrhiza fungi (M), 27% with S. griseoviridis (Sg) according to (Ti *et al.*, 2006), who reported that microbes increased their concentration in the plants when infected. As for *T. asperellum* and *A. pullulans* no significant effect or enhancement on coumaric acid in the okra plant was noticed.

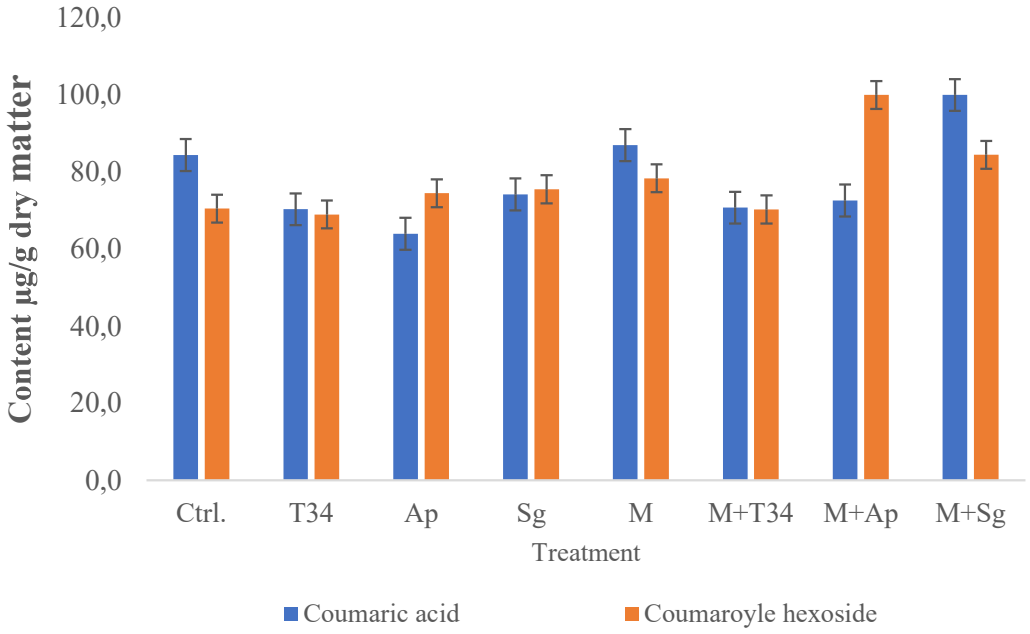


Figure (45) shows the Effect of Mycorrhiza and Antagonist Agents on Coumaric acid and coumaroyl hexoside in okra fruit: control: Ctrl, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.8.3 Chlorogenic acid-catechin-glucoside

The HPLC analysis of okra phenolics revealed that chlorogenic acid, the widely distributed phenolic compound ion plant kingdom, occurs in a form of dimer with catechin. Unfortunately, no available data on the antioxidant activity or biologically activity of such dimer. distinct patterns in the accumulation of chlorogenic acid-catechin-glucoside phenols in Okra fruit under the influence of Mycorrhiza and antagonist agents. Mycorrhiza (M), *T. asperellum strain* T34, *A. pullulans, S. griseoviridis* K61 treatment and combined mycorrhiza with microbe agents: Mycorrhiza sp+ *T. asperellum* strain T34 (M+T34), Mycorrhiza sp + A. pullulans (M+Ap)., Mycorrhiza sp+ Streptomyces griseoviridis (M+Sg). Quantitative measurements allowed for the characterization of phenolic content, with specific attention to variations induced by the microbial treatments Figure (46). It was found that Sg, M, M+T34, M+Ap, and M+Sg treatments significantly increased the content of chlorogenic-catechin dimer in okra fruits as compared to the control Figure 44. The significantly highest level of the dimer was recorded for mycorrhiza treatment. The biotic treatments increased by 163%, 116%, 86%,79%63%, 29% and 5% with M, M+T34, M+Sg, Sg, M+Ap, T34, and Ap the level of such polyphenol respectively. That means microbes have different abilities to induce plant immunity (M) and (M+T34) have significant roles in increasing concentration of chlorogenic acid compared to control but M+Sg, Sg, M+Ap and T34 exhibited no significant changes.

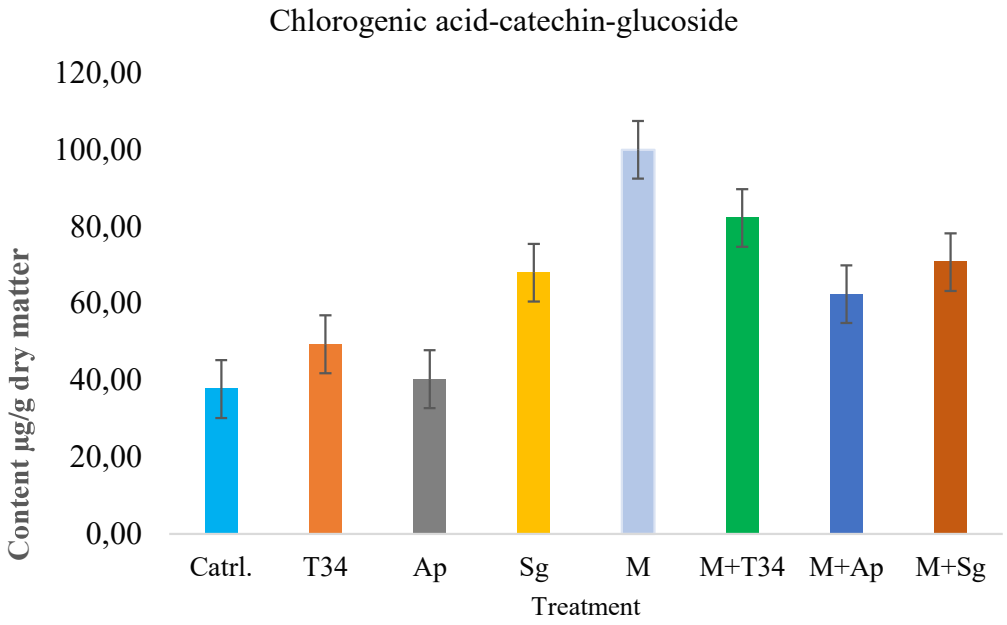


Figure (46) shows the Effect of Mycorrhiza and Antagonist Agents on Chlorogenic acid -catechin-glucoside in Okra fruit: control: Catrl, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.8.4 Sinapoyl feruloyl derivative

The results presented in Figure 47 Sinapoyl compounds in plants constitute a significant class of secondary metabolites with notable physiological roles. These compounds' presence in various plant species underscores their evolutionary significance. Sinapoyl malate is synthesized within the epidermal layer of leaves.(Kaling et al., 2015). Furthermore, the accumulation of sinapoyl malate in the epidermis suggests a specialized role in photoprotection. This antioxidative function is particularly crucial in the epidermal layer, where plants are exposed to environmental stressors such as ultraviolet (UV) radiation (Vink et al., 2023). It is evident from the results presented in Figure 45 that all treatments of mycorrhiza alone or combined with microbial agents promoted the biosynthetic pathways of sinapic acid derivative as compared to the control treatment., which was identified as a dimer of sinapoyl-feruloyl. The highest increase in the content of such dimer was found in fruits of okra treated with mycorrhiza combined with Ap. These results highlight the potential benefits of utilizing these microorganisms in combination with mycorrhiza as a means of enhancing Okra fruit phenolic content and productivity.

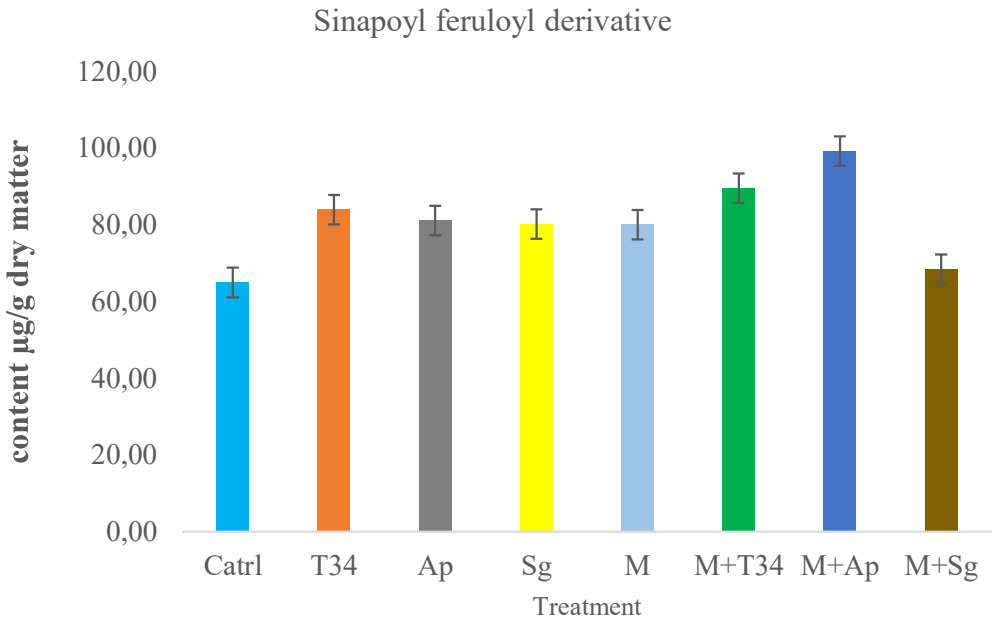


Figure (47) shows the Effect of Mycorrhiza and Antagonist Agents on Sinapoyl feruloyl derivative in Okra fruit: control: Catrl, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.8.5 Fumaric acid

The results presented in Figure 48 demonstrate the effectiveness of various treatments on the Fumaric acid content of Okra fruit, in conjunction with arbuscular mycorrhiza and microbial agents. The treatments included combining Arbuscular mycorrhiza +A. pullulans (M + Ap), Arbuscular mycorrhiza + *T. asperellum* strain T34 (M + T34) and individually of Arbuscular mycorrhiza and *T. asperellum* strain T34 respectively, to increase the Fumaric acid content of Okra fruit. On the other hand *A. pullulans*, *S. griseoviridis* and combining of Mycorrhiza + *S. griseoviridis* didn't show an effect or increase of Fomaric acid.

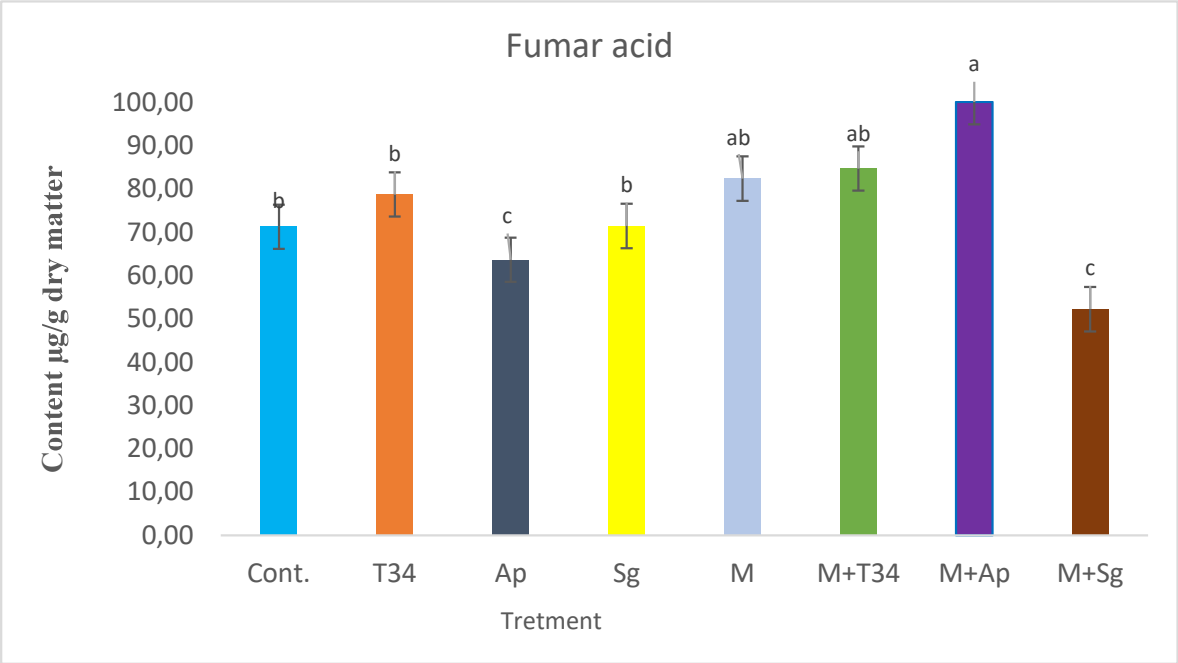


Figure (48) shows the Effect of Mycorrhiza and Antagonist Agents on Fumar acid in the Okra fruit: control: Cont., Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*: Sg, and *A. pullulans*: Ap.

4.8.6 Di- caffeolyl quinic acid

In Figure (49), the synergistic impact of the combined application of mycorrhiza and microbial agents on di-caffeoylquinic acid levels in okra fruits is depicted. Except Sg and M+Sg treatments all other treatments showed significantly higher content of di-caffeoylquinic acid in okra fruits. The highest increase in the concentration of such polyphenol glucoside was found in M, M+T34, and M+Ap with no significant variation between them in their impact on di-caffeoylquinic acid. The Sg type bacterial insulation particularly had a remarkable negative impact on the metabolic pathway of such polyphenol most probably by partial inhibition of the enzymes involved in the biosynthesis processes di-caffeoylquinic acid. Combination of Sg with mycorrhiza moderated slightly its negative effect on di-caffeoylquinic acid formation in okra fruit.

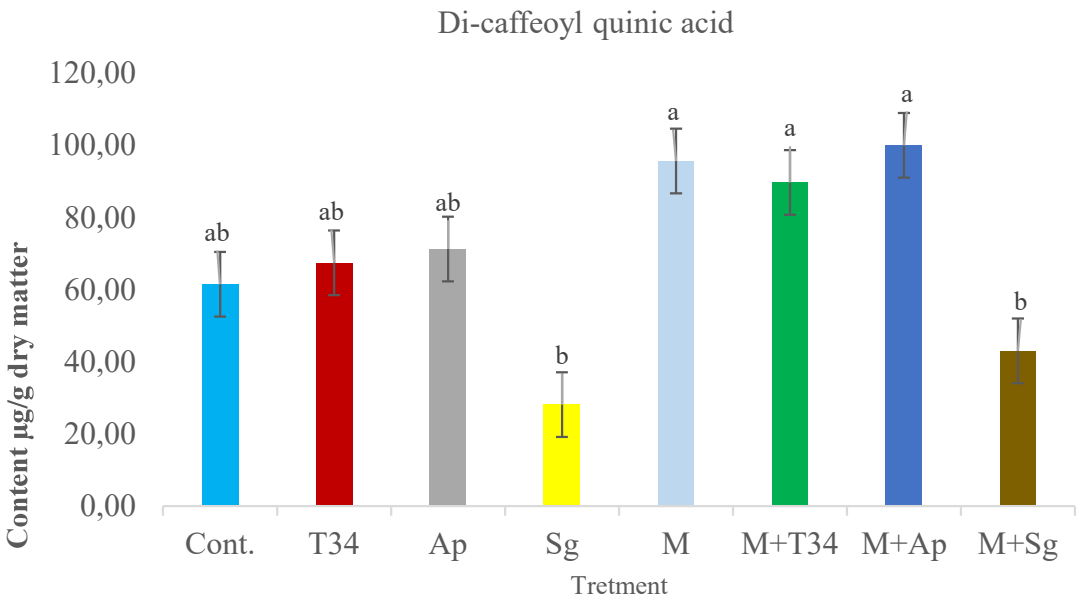
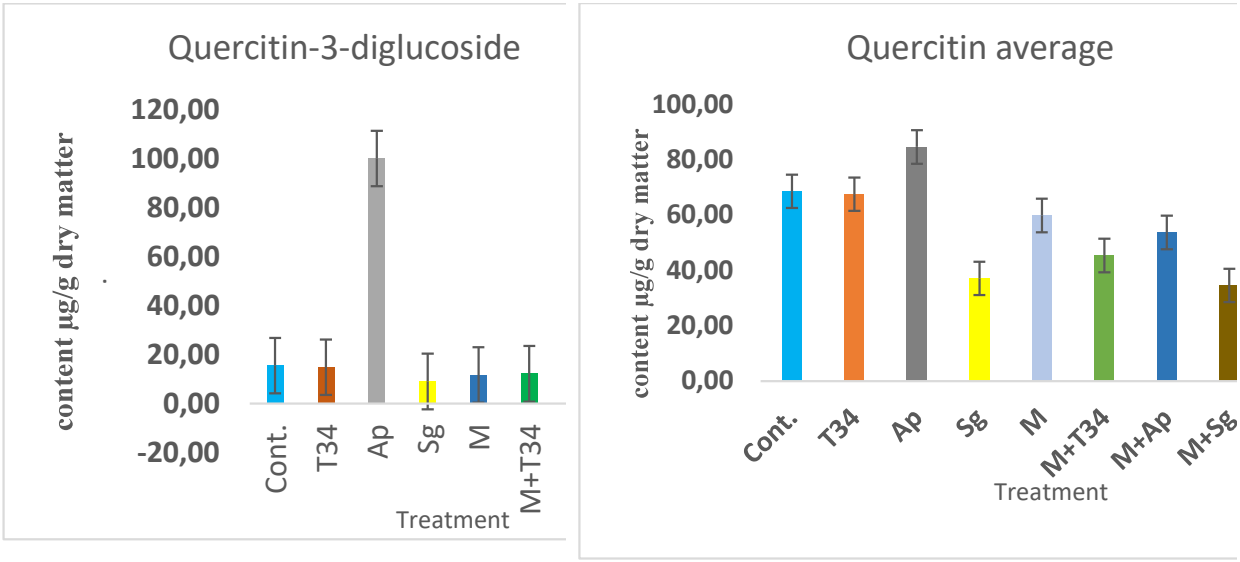


Figure (49) shows the Effect of Mycorrhiza and Antagonist Agents on di-caffeoyl quinic acid in the Okra fruit: Cont, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*:Sg, and *A. pullulans*: Ap.

4.8.7 Quercitin-3-diglucoside

In Figure (50), Quercetin was found in the extract of the whole Okra fruit to exist in quercetin-3-o- glucoside, quercetin- di-glucoside, and Quercitin-3-O-(melanoyl) glucoside. The impact of the different treatments on the quantity of quercetin derivatives is shown in Figure 4. It is of interest that Ap treatment resulted in a highly significant increase in the average content of quercetin which was found to be due to higher activation of quercetin-3-diglucoside biosynthesis. It is of higher interest that the combination of mycorrhiza with Ap (M + Ap) yielded fruits with a significantly higher level of quercetin 3-diglucoside as compared to other treatments, but not as much as Ap did alone.



A B

Figure (50) A: Shows the Effect of Mycorrhiza and Antagonist Agents on Quercitin-3-diglucoside, B: Quercitin-Average di-caffeoyl quinic acid in the Okra fruit: Cont, Mycorrhiza (*Funneliformis mosseae*): M, *T. asperellum* strain 34: T34, *S. griseoviridis*:Sg ,and *A. pullulans*:Ap.

5. DISCUSSION

The in vitro Okra tolerance assay for the assessment of Sclerotinia sclerotiorum disease infections was performed using Okra seeds cultivated in a petri dish positioned on moist filter paper. Following a ten-day incubation period for seed germination, inoculation with the plant pathogen occurred. Initial manifestations of white mold appeared on the plant stems approximately one week after the inoculation process. Consequently, both the root and shoot regions exhibited inhibited growth, wilting, and formation of moist lesions on the stalks. Progressively, this pathological progression culminated, and all the plants died, accompanied by the presence of white mycelium and sclerotia in the stalks during seed germination. It is noteworthy that an escalation in symptom severity was observed around 12 days post-inoculation, markedly distinct from the control plants, which remained devoid of symptoms. However, post-germination infection of okra plants by S. sclerotia reached 100%. Furthermore, studies have indicated that S. sclerotia, being the predominant pathogen, can also invade okra bud buds (Prova *et al.*, 2017), signifying reduced resistance or heightened susceptibility of okra plants to this pathogen. This study provides valuable insights into the susceptibility of okra plants to *Sclerotinia sclerotiorum*, thereby facilitating the formulation of strategies aimed at mitigating the adverse impact of this pathogen on okra cultivation.

The primary strategy for interacting microbes with arbuscular mycorrhizal fungi (*Funneliformis mosseae)* on okra plants involves plant growth promotion and increased plant immunity using microbes registered in EU regulations as environmental friends and suitable for human health instead of synthetic fungicides (Posta and Duc, 2020).Arbuscular mycorrhizal *Funneliformis mosseae* (AMF) fungi, one of the most commonly used microorganisms, effectively colonize most agricultural plants and enhance plant nutrition and performance (Posta and Duc, 2020). This study highlights the advantages of AMF inoculation on crop yield, particularly under conditions of water scarcity.

Several beneficial microorganisms, such as *Trichoderma asperellum, Aureobasidium pullulans* (fungi), and *Streptomyces griseoviridis* (bacteria), share similar environmental requirements and are widely utilized in biological and integrated crop protection (Posta and Duc, 2020). When combined separately with arbuscular mycorrhizal fungi, these microorganisms show promising results in enhancing plant growth. This experiment was designed to investigate the interaction of three plants with three different microbes possessing distinct characteristics when interacting with AMF.

In assessing the virulence of these bioagents against plant fungal diseases, including *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*, on PDA Petri dishes, Trichoderma asperellum effectively inhibited the growth of these pathogens when in direct contact (Posta and Duc, 2020). This finding is consistent with the existing evidence (A, D and Y, 2017; Stracquadanio *et al.*, 2020).

Similarly, *Streptomyces spp.* have been recognized for their potential as biofertilizers and biocontrol agents against plant diseases (Pereira, Santana and Van Der Sand, 2022; Soltani Nejad *et al.*, 2022). Therefore, it is crucial to identify natural alternatives. (Salla *et al.*, 2014; Pacios-michelena, González and Alvarez-perez, 2021). Bioactive compounds are environmentally safe and biodegradable (Dziggel, Schaefer and Wink, 2017).

Streptomyces griseoviridis was found to inhibit the growth of *M. phaseolina and S. sclerotia* in the initial days of the test, further supporting its potential as a biocontrol agent (Posta and Duc, 2020; Vurukonda, Giovanadri and Stefani, 2021; Díaz-Díaz *et al.*, 2022). However, its effectiveness against *R. solani* varies, highlighting the need for comprehensive treatments that target multiple fungi simultaneously.

Furthermore, *A. pullulans* demonstrated promising results as a biological control agent against *S. sclerotia,* although its efficacy against *M. phaseolina* and *R. solani* is limited (Posta and Duc, 2020). While some studies have reported *the ability of A. pullulans* to control *R. solani*, discrepancies in results may stem from variations in the experimental conditions (Di Francesco and Baraldi, 2021).

These results highlight the potential of various beneficial microorganisms as alternatives to synthetic fungicides for enhancing plant growth and combating plant pathogens. However, the effectiveness of these biocontrol agents may vary depending on the specific pathogen and the environmental conditions. Further research is warranted to optimize their application in agricultural practices.

Numerous investigations have highlighted the multifaceted biocontrol mechanisms employed by *Trichoderma asperellum* 34, including mycoparasitism, antibiosis, competition for nutrients and space, stress tolerance through enhanced root and plant development, systemic acquired resistance, and enzyme inactivation (Manzar *et al.*, 2022; Tyśkiewicz *et al.*, 2022; Martinez *et al.*, 2023; Podgórska-Kryszczuk, 2023; Lorenzetti *et al.*, 2024; Miriam *et al.*, 2024)

Regarding our results evaluating the potential of *T. asperellum* strain (T34) as a biocontrol agent against various plant pathogens, a test was conducted exposing *T. asperellum* (T34) to three

distinct pathogens: *S. sclerotiorum*, *M. phaseolina*, and *R. solani*. The results indicated that *T. asperellum* (T34) effectively inhibited the growth of all three pathogens. This suggests that *T. asperellum* (T34) holds promise as a biocontrol agent for managing infections caused by *S. sclerotiorum, M. Phaseolina,* and *R. solani*. The results of this activity test are shown in Figure (18,19). *T. asperellum* has been shown to effectively inhibit the growth of various plant pathogens when in direct contact, which is consistent with previous studies that have demonstrated the biocontrol capabilities of Trichoderma species (A, D, and Y, 2017; Stracquadanio et al., 2020).

The objective of this study was to evaluate the efficacy of utilizing MBCA (*S. griseoviridis*, *T. asperellum*, and *A. pullulans*), which are biological agents, to mitigate the occurrence of *Botrytis cinerea* infections in okra plants. Volatiles produced by *S. griseoviridis* have been observed to induce vacuolation and degradation of the mycelial surface of *B. cinerea*, consequently impeding conidial germination (Bello *et al.*, 2022). Similarly, A. pullulans emits volatiles that induce rough cell surfaces with numerous outgrowths on *B. cinerea* (Yalage Don *et al.*, 2021).

The results of the experimental investigation (Figure 20) underscore the impact of microbial inoculants on enhancing the resistance of okra plants against *B. cinerea* infection. Specifically, the combined treatments of biological agents with arbuscular mycorrhiza (*Funneliformis mosseae*) (M+T34, M+Ap, and M+Sg), as well as the synergistic pairing of arbuscular mycorrhiza + *S. griseoviridis* (M+Sg), demonstrated a significant increase in plant tolerance to *B. cinerea* infection when compared with the control group. The enhanced plant tolerance observed can be attributed to several mechanisms that promote systemic plant resistance and suppress pathogen growth. These mechanisms include an increase in phenolic compounds and GST enzyme activity, which are known to enhance plant tolerance to biotic and abiotic stresses. These beneficial microorganisms induce systemic resistance in plants and produce antimicrobial compounds such as peptaibols, which inhibit the growth of *B. cinerea.*

Fruit number

The diverse outcomes observed regarding mycorrhizal symbiosis with biological agents and their effect on okra fruit production (specifically, fruit number) can be attributed to several factors elucidated in the literature. Albrechtova et al. (2012), Abdul-Alhussein, Alawi, and Abood (2019) and Sarkar and Sadhukhan (2023) have highlighted the importance of specific microbial agents, their synergistic interactions with mycorrhizal fungi, and the overall health status of plants. The variability in the significance of increasing fruit number percentage for the combination treatments, such as Mycorrhiza + *T. asperellum* (M+T34) = 130%, Mycorrhiza + *A. pullulans* (M+Ap) = 96%, mycorrhiza (M) = 94%, and Mycorrhiza + *S. griseoviridis* (M+Sg) = 85%,

underscores the influence of these factors. Conversely, inoculation of plants with individual microbes, such as Ap = 41%, Sg = 37%, and T34 = 27%, yielded less significant effects on fruit number than the control. These findings align with those reported by Boyno et al. (2023), who documented fruit-enhancing effects of T34 and Ap.

The observed variations in fruit number can be attributed to the unique ability of specific biological agents to enhance nutrient uptake, promote fruit production, and suppress pathogens. Furthermore, synergistic interactions between mycorrhiza and certain microbes have been shown to enhance nutrient uptake, improve plant growth, and increase resistance to pathogens, thereby leading to higher fruit production. This underscores the importance of understanding the intricacies of plant-microbe interactions and their implications for crop productivity in agricultural systems.

Discussion of plant growth okra tomato and sweet potato

This investigation delves into the effects of individual and combined microbial treatments on the growth parameters of okra, tomato, and sweet potato plants. A symbiotic relationship between arbuscular mycorrhizal fungi and *T. asperellum* was observed to enhance nutrient uptake and provide protection against microbial agents, consistent with previous studies (Duc, 2017; Posta and Duc, 2020; Sain et al., 2023). Similarly, combined treatments of Mycorrhiza with S. griseoviridis (M+Sg) and A. pullulans (M+Ap) significantly increased okra plant height, indicating a synergistic effect on growth attributed to the secondary metabolism of pullulans and nutrient uptake enhancement by mycorrhiza, in line with previous studies (Wachowska et al., 2016; Di Francesco et al., 2021). Furthermore, this study revealed the efficacy of novel combinations, such as Mycorrhiza with A. pullulans and S. griseoviridis, in promoting okra plant height, underscoring the potential synergistic effects of these combinations under field conditions.

Individual microbial treatments, including T34, arbuscular mycorrhizal fungi (*Funneliformis mosseae)*, and *A. pullulans*, exhibited modest increases in okra plant height compared to the control, albeit not statistically significant, consistent with previous findings (Guzmán-Guzmán et al., 2023; Wahab et al., 2023). However, further investigation into the effect of microbes on mycorrhizal colonization in okra roots is warranted to elucidate the mechanisms underlying the observed growth enhancement.

In the context of environmentally sustainable farming, the integration of beneficial microorganisms, such as *T. asperellum*, Mycorrhiza, *A. pullulans*, and *S. griseoviridis*, has garnered attention. Studies focusing on tomato plants have indicated the variable effects of these microbial treatments on plant growth, biomass, and yield (Kiss, 2020; Ibrahim *et al.*, 2023). The dynamics of these interactions are crucial for understanding how beneficial microbes contribute to

plant resilience and growth (Srivastava *et al.*, 2010; Journal, 2011; Mwangi, Meinzen-Dick and Sun, 2011; Hong, Csintalan and Posta, 2018). Notably, the application of antagonist agents alongside arbuscular mycorrhiza leads to diverse effects on tomato plant height, with certain treatments exhibiting significant enhancements compared to the controls. Additionally, the initial growth of mycorrhiza-treated plants, either alone or in combination with other beneficial microbes, had a positive effect on plant height, suggesting their utility in agricultural practices without inhibiting plant growth.

Moreover, examination of sweet potato plants revealed significant improvements in yield and shoot length with the application of microbial treatments, particularly when combined with arbuscular mycorrhizal fungi (*F. mosseae*). Various microbial combinations exhibit diverse effects on sweet potato growth parameters, indicating the importance of strain selection and microbial interactions. Differences in yield among identical treatments may stem from optimal microbial temperatures or resistance to mycorrhiza among other factors. Despite these variations, treatments involving microbial mixtures consistently outperformed untreated controls, underscoring their potential for enhancing crop productivity.

In summary, this study demonstrated the multifaceted effects of individual and combined microbial treatments on plant growth parameters in okra, tomato, and sweet potato plants. These findings highlight the importance of microbial interactions and strain selection in influencing plant resilience and productivity, and offer valuable insights for sustainable agricultural practices. Further research is warranted to elucidate the underlying mechanisms and optimize microbial treatments to enhance crop yield.

Dry weight

This study explored the impact of combining arbuscular mycorrhizal fungi AMF (*Funneliformis mosseae):* (M) with various biological agents on the dry-weight biomass of okra and tomato plants. In the case of okra, distinct outcomes were observed, with varying levels of dry weight biomass enhancement resulting from the symbiotic interaction between AMF and microbial agents. In particular, combinations such as M+*T. asperellum* (T34) and M+*A. pullulans* (Ap) exhibited significant enhancements in dry-weight biomass (77% and 82% increase, respectively), whereas M alone also led to a notable increase (84%). However, treatment with T34 (43%) or M+*S. griseoviridis* (Sg) (28%) showed slight improvement in dry weight. Previous studies have reported similar results, suggesting that these biological agents possess unique capabilities to enhance nutrient uptake, promote plant growth, and improve resistance to environmental stress.

Studies by (Yang *et al.*, 2014; Quiroga *et al.*, 2020) demonstrated the positive impact of arbuscular mycorrhizal (AM) fungi on plant biomass and root dry weight, respectively, under various conditions. Additionally, the stability of root hyphae and expansion of the mycorrhizal network contributed to alterations in root biomass, as indicated by (F. Zhang *et al.*, 2020; Liang *et al.*, 2021) Field experiments conducted in this study confirmed that the inoculation of M and biological agents significantly increased nutrient availability, stimulated plant development, and enhanced biomass accumulation, ultimately leading to increased plant height, basal diameter, and fibrous root extension in both okra and tomato plants.

Similarly, in the case of tomato plants, the integration of beneficial microorganisms, including *T. asperellum, A. pullulans*, and *S. griseoviridis*, has gained attention for sustainable farming practices. Extensive research has been dedicated to understanding the interactions and repercussions of these microorganisms on tomato plants. Figures indicate that combinations of Mycorrhiza with T. *asperellum, A. pullulans*, and *S. griseoviridis* have variable effects on promoting plant growth and dry weight. Moreover, the use of different microbial strains combined with mycorrhiza may result in varying effects on tomato plant dry weight, with the initial biomass of mycorrhiza-treated plants being significantly higher, regardless of the presence of other beneficial microbes. Importantly, none of the treatments inhibited plant growth, suggesting that the integration of mycorrhiza and selected biological agents into crop production systems is safe and beneficial.

Overall, these findings underscore the importance of exploring the synergistic interactions between mycorrhizal fungi and biological agents in enhancing plant growth and biomass accumulation, contributing to the development of sustainable agricultural practices.

Trichoderma colonasation

This text is best suited to the discussion section of an academic paper. It discusses the collaborative effects of two biocontrol agents, AMF (M) and *T. asperellum* (T34), on the T34 root colonization dynamics of various plant species, namely Okra (*Abelmoschus esculentus*), Tomato (*Solanum lycopersicum*), and Sweet potato (*Ipomoea batatas* (L.) Lam). This study aimed to elucidate the interactive influence of these beneficial symbionts on root systems, focusing on quantifiable measures, such as colonization density, distribution patterns, and the synergistic impact on host plants.

We conducted an in vitro assay to test T34 colonization in the roots of arbuscular mycorrhizal fungi AMF (*Funneliformis mosseae)* (M)-treated okra, tomato, and sweet potato plants, along with assessing the mycoparasitic ability of T34. The results demonstrated active colonization of all the

roots tested, whether treated with AM alone or in combination with T34. This finding corroborates that reported by (Sain, Dewasi and Singh, 2023). Additionally, the study found that the combination of M with T34 did not significantly affect each other's efficacy in inducing and protecting the host plant, consistent with the results observed by (Alam, Choudhury and Mridha, 2023; TANUI, 2023), who highlighted the role of Trichoderma spp. as an opportunistic fungus symbiont that aids in disease suppression and benefits the host plant. M facilitates the delivery of minerals to plant roots and provides photosynthetic products to the fungi.

These findings underscore the potential of optimizing biocontrol strategies by harnessing the synergistic benefits of Mycorrhiza and Trichoderma in root colonization. Future research endeavors should delve into the molecular mechanisms underlying this collaboration, emphasizing the development of tailored approaches to enhance plant growth, resilience, and overall productivity across diverse agricultural settings.

Mycorrhiza colonasation

Based on our results, we examined the effects of the combination of biological agents with arbuscular mycorrhiza fungi (*Funneliformis mosseae):* AMF on AMF root colonization in okra, tomato, and sweet potato plants. The root colonization of okra plants combined with arbuscular mycorrhizal fungi (AMF), biological agents (T34, Ap, Sg), and AMF alone was assessed in this study. The investigation revealed varying levels of root colonization across different treatments. Notably, the combination treatment of AMF with *Streptomyces griseoviridis* (M+Sg) resulted in the highest colonization rate of 85.16%, followed closely by AMF treatment alone at 84.68%. Conversely, colonization rates decreased notably in treatments involving simultaneous infection with *T. asperellum* (T34) and A. pullulans (Ap), with rates of 61.18% for M+T34 and 60.92% for M+Ap, suggesting that AMF parasitism could be suppressed by these microorganisms. Previous studies have documented the biocontrol mechanisms of T34 and Ap strains, which could account for the observed reduction in colonization(Wang *et al.*, 2023).

A. pullulans has been extensively studied for its biological control properties, with proposed mechanisms including augmentation of host defenses, resource competition, and production of antifungal volatile organic compounds(S M Yalage Don *et al.*, 2020; Iqbal *et al.*, 2023; Podgórska-Kryszczuk, 2023). Similarly, Trichoderma spp. exhibit antagonistic processes such as secondary metabolite production, parasitism, and pathogen inhibition, potentially influencing mycorrhizal colonization levels (Khuong *et al.*, 2023). Streptomyces griseoviridis K61, which is known for its antifungal metabolites and enzymatic activities, can effectively manage various phytopathogens.

In the case of tomato and sweet potato plants, the effectiveness of symbiotic associations in enhancing resistance or tolerance may vary depending on AMF symbionts and other microorganisms involved. AMF colonization levels were not significantly affected by Trichoderma or Streptomyces treatments, although minor differences were observed. Notably, the mycorrhiza-only treatments reduced the presence of Trichoderma fungi, suggesting potential interactions between these microorganisms in the rhizosphere.

GST

We present findings regarding the modulation of glutathione-S-transferase (GST) activity in okra plants by various microbial treatments. (GST) are a crucial enzyme involved in conferring antimicrobial resistance to host plants and mitigating oxidative stress by synergistically removing lipid hydroperoxides as antioxidants (Gullner *et al.*, 2018; Zhang *et al.*, 2023). Our investigation revealed that the combination of arbuscular mycorrhizal fungi (*Funneliformis mosseae)* (AMF) with biological agents significantly increased GST activity in okra plants compared to the control treatments. These effects align with previous reports on the impacts of AMF symbiosis and microbial treatments on plant physiology antioxidants by (Mayer *et al.*, 2017; Shijun Xing *et al.*, 2023)

Distinct GST activity patterns were observed for the individual microbial treatments. Notably, treatment with arbuscular mycorrhiza (*Funneliformis mosseae)* (M) and Mycorrhiza+Aureobasidium (M+Ap) demonstrated potential effects on GST activity, suggesting a role in enhancing the antioxidative response in okra fruits. Conversely, treatments with *T. asperellum* (T34), *S. griseoverdis* (Sg), and *A. pullulans* (Ap) individually may exert varied effects on GST activity, as depicted in Figure 41, warranting further investigation into their specific contributions to the plant's detoxification processes.

Understanding the modulation of GST activity by microbial treatment has significant implications for enhancing the ability of plants to counteract oxidative stress and environmental challenges. In conclusion, this study provides valuable insights into the modulation of GST activity in okra plants using various microbial treatments. The observed variations underscore the potential role of arbuscular mycorrhizal fungi (*Funneliformis mosseae)* and Mycorrhiza+Aureobasidium combinations in influencing the plant's antioxidative response, thereby enriching our understanding of the interplay between microbial treatments and biochemical pathways in plants.

Effect of treatments on phenolic compounds

Plants employ a diverse array of strategies to defend against pests, with one notable mechanism being the de novo synthesis of specific compounds such as phenolics. High-performance liquid chromatography (HPLC) analysis of okra fruit revealed that the crop was rich in bioactive polyphenols. These phenolics play a crucial role in maintaining plant health, with heightened production observed in response to various stimuli, including insect feeding, pathogen infection, and colonization by beneficial microorganisms (Wallis and Galarneau, 2020). Moreover, phenolic compounds especially flavonoids have emerged as potential substitutes for bioactive agents in the pharmaceutical and medicinal sectors, offering avenues for promoting human health and combating various diseases. (Sun and Shahrajabian, 2023). Recent scholarly reviews have extensively explored the pharmacological actions of kaempferol derivatives across diverse medical domains, including cancer, central nervous system diseases, and chronic inflammatory conditions (Mishra *et al.*, 2023)

It is repeatedly evident that the individual polyphenolic compounds responded in different ways to the microbial treatments alone or in combination with mycorrhiza. For instance, the levels of kaempferol and its derivatives were significantly increased only by T34 treatment, while most of the treatments resulted in a significant increase in the content of chlorogenic acid. Another interesting response to microbial treatments was observed for quercetin and its glucoside. Its biosynthesis was highly significantly influenced by the treatments, and the highest level was recorded for quercetin-3-glucoside with AP treatment. The impact of Ap treatment is highly appreciated because quercetin-3-glucoside has higher antioxidant activity than other polyphenols in okra fruit. The glucoside derivatives of quercetin have been reported to exhibit higher antioxidant activity than quercetin itself (Hu et al., 2014). The potential of microbes to increase the concentration of phenolic compounds in plants has been previously reported (Shahrajabian, 2023; Ti *et al.*, 2006). In addition, HPLC analysis of okra fruit revealed the presence of important phenolic components that play a vital role in inducing plant defenses against pathogens and predators (Sung and Lee, 2010). Furthermore, inoculation of plants with microbes significantly increases the concentration of some phenolic compounds, highlighting the role of microbes in enhancing plant immunity (Zhou *et al.*, 2022).

During pre-harvest, harvest, and postharvest steps, plants and their products are exposed to several biotic factors, including microorganisms such as bacteria, fungi, and viruses, which are the most important biotic factors that can influence secondary metabolic pathways of plants as well as the production of bioactive compounds (Zeng et al., 2013). Microorganisms can act in selected ways during plant developmental stages and in different anatomical parts (Vivanco et al., 2005).

Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are among the most widely studied microbial groups (Alfonso and Galán 2006). In general, the increased concentration of secondary metabolites in plant roots, leaves, and fruits is related to the defense response of plants to microorganism colonization (Suzuki et al., 2014; Toussaint, 2007).

It is also evident that the different microorganisms differ in the mechanism, by which they induce or promote the biosynthesis of phenolic compounds in plants Some reports indicate that some microorganisms promote the absorption of phosphorous by plants, which activates the methylerythritol phosphate pathway that significantly affects secondary metabolite production, (Carretero-Paulet *et al.*, 2006). On the other hand, studies indicated that some microorganisms could be used as antagonists or to induce some secondary metabolites to defend fruits and vegetables (fresh or processed) against deteriorative microorganisms ((Terry and Joyce, 2004), 2004) It is important to mention that biotic factors include a more sophisticated interaction of plant biochemistry and physiology (Briskin, 2000). It can be assumed that biotic effects are related to plant interactions with microorganisms or plant physiological aspects such as phenology and ontogeny (Petinatti *et al.*, 2012; Ochoa-Velasco *et al.*, 2017). The strong induction of selected phenolic metabolites has been attributed to the presence of nitrogen and subsequent nitrogen deficit in phenolic metabolites and physiology. Flavones increase, but flavonols decrease in response to a lack of nitrogen (Kovác, 2014).

Our research revealed that okra, particularly its fruits, are rich in phenolic compounds, including coumaric acid and coumaroyl acid, thus enhancing its nutritional value in the human diet (H). Additionally, our findings underscore the potential of using microbes to increase the concentration of phenolic compounds in plants, as shown in Figures 45-46. (Zhou *et al.*, 2022) (Shahrajabian, 2023 Ti *et al.*, 2006);). as well as, the analysis of okra fruit via HPLC revealed the presence of important components such as chlorogenic acid, which plays a vital role inducing plant defenses against pathogens and predators(Sung and Lee, 2010). Furthermore, the inoculation of plants with microbes significantly increased the concentration of phenolic compounds, including chlorogenic acid, highlighting the role of microbes in enhancing plant immunity (Figs. 45-46) . (Zhou *et al.*, 2022).

However, our study explored the use of microbes to enhance plant health by promoting the release of phenolic compounds such as sinapoyl compounds, as depicted in Figure 47. Our findings showed that microbes increased the concentration of sinapoyl compounds in okra fruit, which are another class of secondary metabolites with significant physiological roles in plants, particularly in photoprotection against environmental stressors such as ultraviolet (UV) radiation (Kaling et al., 2015; Vink et al., 2023).

Fumaric acid and coumaric acid, known for their antioxidant properties, serve as defence mechanisms against reactive oxygen species (ROS) in plants. Our research highlights the ability of microbes to stimulate the release of fumaric acid, coumaric acid in plants, suggesting their potential role in enhancing plant resilience (Goldberg, Rokem and Pines, 2006; Ti *et al.*, 2006)

Caffeoylquinic acid derivatives, a group of natural products commonly found in plants, exhibit bioactive properties including antimicrobial, antioxidant, and analgesic activities (Goldberg, Rokem and Pines, 2006; Mijangos-ramos *et al.*, 2018). We found that microbial treatment significantly increased the concentration of dicaffeoylquinic acid fractions, suggesting a potential avenue for enhancing plant bioactivity (Fig. 49).

Quercetin derivatives identified in the extract of okra fruit demonstrated potent antioxidant activity. Our study indicated that treatment with microbes, particularly A. pullulans, significantly increased the content of quercetin derivatives, underscoring the role of microbes in enhancing plant bioactivity (Xu *et al.*, 2020).

Microorganisms can act in selected ways during plant developmental stages and in different anatomical parts (Vivanco and Cosio, 2005). Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are the most widely studied microbial groups (Alfonso and Galán, 2006).

represent significant dietary flavonols with implications for health, particularly in the management of chronic diseases such as diabetes and fibrosis. In our research, we used beneficial microbes to increase the concentration of polyphenols, including kaempferol and its derivatives, in okra fruit.
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6. CONCLUSIONS

Recognizing the economic importance of okra, tomatoes, and sweet potatoes in healthy nutritional meals for humans, this study investigates the use of environmentally friendly microorganisms to reduce dependence on traditional pesticides and fertilizers. The goal is to align agricultural practices with sustainability, to promote ecological preservation, and to safeguard public health.

In conclusion, co-cultivation experiments involving *T. asperellum*, *S. griseoviridis K61*, and *A. pullulans* with arbuscular mycorrhiza did not have a detrimental effect on the reciprocal characteristics between the microbial agents. The absence of adverse interactions suggests the compatibility of these combinations and underscores their potential utility in fostering plant growth. Therefore, they can be safely combined with mycorrhizal treatments.

Inoculation of plants with a combination of antagonist agents and mycorrhiza is instrumental in enhancing plant growth and yield in the cultivation of tomato, okra, and sweet potato. This symbiotic association not only exerts influence on physiological processes, but also underscores its importance in sustainable agriculture and plant biology, contributing significant insights into the environment within modern agricultural systems.

Furthermore, investigation of the interaction between antagonist agents and mycorrhiza revealed a significant improvement in the resistance of okra plants. This strengthening underscores the effectiveness of their symbiotic relationship, providing protection against various diseases and contributing positively to the overall plant health.

Additionally, the assessment of GST enzyme activity in okra leaves revealed a pronounced elevation, particularly in glutathione S-transferase (GST), which increases the resistance of the plant to biotic and abiotic stresses. The significant increase in enzyme activity (GST) as a result of the symbiotic relationship between antifungal agents and mycorrhizae has a significant impact on biochemical processes within plants.

HPLC analysis of okra fruits indicated a noticeable increase in the concentration of phenolic compounds, which was attributed to the interaction between antagonist agents and mycorrhiza. Based on the significance of phenolic compounds in several human disease treatments, this investigation has favorable implications for human health, nutritional quality, and environmental sustainability, emphasizing the diverse benefits of this symbiotic association.

7. NEW SCIENTIFIC RESULTS

1- *Trichoderma* treatment exhibited no inhibitory effect on the mycorrhiza root colonisation. However, *Streptomyces* and *Aureobasidium* treatment slightly reduced the mycorrhiza colonisation
2- The combined application of biocontrol microbes and mycorrhiza increased okra resistance to grey mould infection as compared with treatments with antagonists or mycorrhiza alone. Also, these latter treatments reduced the grey mould infection.
3- The combined treatments increased the growth of the vegetative parts of okra and resulted higher yield both alone and in combinations
4- Glutathion-s-transferase activity was increased by each treatment, alone or combination. It was shown that ncreased GST level is favorable for protection, and probably favoring against pathogens. At the same time, this did not increase with multi-inoculation, the presence of more microbes probably did not appear as additional biotic stress.
5- The polyphenolic changes occurring in the okra plant due to the individual inoculation of mycorrhiza and the joint application of Trichodrema and Streptomyces and Aureobasidum pullulans highlighted the most important and most prominent components. The concentration of some phenolic compounds in okra fruits were increased in some combinations

8. SUMMARY

Sustainable plant production requires the limited use of synthetic chemicals for plant nutrition and plant protection. This summary provides an overview of the recent developments in the interaction of microbial consortia with mycorrhizal symbiosis to enhance plant growth, induce plant resistance, and increase productivity.

The synergy between microbial agents *Trichoderma asperellum* strain 34, *Aureobasidium pullulans*, and *Streptomyces griseoviridis* K61 with arbuscular mycorrhizal fungi represents a promising avenue for sustainable agriculture. The integration of microbial agents, such as bacteria and fungi, with mycorrhiza has demonstrated multifaceted benefits, including improved phenolic compound concentration, plant growth promotion, enhanced plant resilience and resistance, improved overall plant performance, and increased yield.

This synthesis of microbial interactions with mycorrhiza presents a novel approach for optimizing plant-microbe associations, contributing to the development of innovative strategies for fostering robust and resilient plant ecosystems. Further research is warranted to elucidate the intricate mechanisms governing these synergistic interactions to provide a foundation for the implementation of effective and eco-friendly agricultural practices. Mycorrhizal root colonization was not influenced by the antagonists. Mycorrhiza treatment slightly reduced the natural occurrence of *T. asperellum, A. pullulans,* and *S. griseoviridis* on the plants used in the experiment, but did not inhibit the colonization of roots by the applied Trichoderma strain.

These findings underscore the potential for natural resource synergies between beneficial arbuscular mycorrhizal associations and microorganism agents to optimize reproductive parameters, providing valuable insights into sustainable agricultural practices aimed at improving crop yield and environmental sustainability. This study is key to the scientific understanding of the interactive relationships between mycorrhiza and antagonistic agents in plant enhancement.

9. APPENDIXES

A1: **References**

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A2: Publications related to the dissertation

Yousif, A. I. A., **Almuslimawi**, A., Turóczi, G., Kiss, J., Kovács, A., & Körösi, K. (2023). Efficacy of some plant resistance inducers against several sunflower downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) isolates. *Acta Biologica Szegediensis*, *67*(1), 75-86.

Alaa Abdulkadhim A. Almuslimawi 1,2, Borbála Kuchár 1, Susana Estefania Araujo Navas 1, György Turóczi 2, Katalin Posta 1Combined application of biocontrol microorganisms and arbuscular mycorrhizal fungus on plant growth and yield of tomato (Solanum lycopersicum L.)

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Godollo, February 2024

Alaa Almuslimawi

Declaration

I at this moment declare that the work presented in this thesis has not been submitted for any other degree or professional qualification, and is the result of my independent work.

Alaa Abdulkadhim A. Almuslimawi (PhD - Candidate)

Date: 2024