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THESIS TITLE

Industrial valorization of garlic (*Allium sativum* L.) under the effect of endemic microbial bioinputs.

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Dedication

In order to express my gratitude to those who supported and encouraged me to undertake this research project, I dedicate this dissertation:

To the dearest person who has shown unwavering support and commitment to my success, particularly during the challenging years when I was striving to achieve my goals: my mother.

To my father, for your unconditional love, your sacrifices and your unwavering support throughout my career. I am truly grateful for your unwavering belief in me, even in the most challenging of times.

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Industrial valorization of garlic (*Allium sativum* L.) under the effect of endemic microbial bioinputs.

Abstract

Garlic (*Allium sativum* L.) is an aromatic herbaceous cultivated for culinary and medicinal purposes. The industrial sector requires a high level of consistency in the biomass, flavor and nutritional profile of garlic. Which can prove challenging to achieve across variable growing conditions due to the impact of soil quality, climate and crop management practices on garlic yield and quality.

In order to increase the value of this plant in the pharmaceutical and agricultural sectors, our study was based on the cultivation of garlic in pots and under greenhouse using microbial bio-inputs, including two isolates of arbuscular mycorrhizal fungi, three isolates of *Trichoderma asperellum* and one isolate of Plant growth–promoting rhizobacteria (PGPR), a comprehensive analysis was conducted on a number of parameters including, growth, yield, secondary metabolites, antioxidant, antimicrobial and herbicidal potential of aqueous extracts prepared from each of the garlic plant samples. The findings of this study indicate that the inoculation of garlic with endemic microorganisms exerts a range of effects on growth parameters, functional groups of chemical compounds and the antioxidant and antimicrobial properties of their extracts.

The results obtained highlight the significance of employing endemic microbial isolates (T1) *Trichoderma asperellum*, (T7) PGPR and (MO) arbuscular mycorrhizal fungi to enhance the majority of the growth parameters under investigation. Moreover, the application of the mycorrhizal fungus isolate MO and the *Trichoderma asperellum* isolate T3 resulted in an enhancement of the quality of the secondary metabolites of this crop, as well as an increase in the antioxidant and antimicrobial properties of their aqueous extracts. Furthermore, the aqueous extract prepared from garlic plants inoculated with isolate T3 indicated a potential inhibitory effect on seed germination and the growth of *Lolium perenne* seedlings. In a similar manner, the aqueous extract prepared from garlic plants inoculated with the isolate T3 exhibited most notable antimicrobial properties, with this potential being retained even in the freeze-dried form for a period of six months. In light of these findings, a series of pharmaceutical products were formulated, encompassing both antimicrobial capsules and a gel, for both oral and cutaneous administration.

Keywords: *Allium sativum* culture, antimicrobial gel, biological activities, microbial bio inputs, secondary metabolites.

Valorisation industrielle de l'ail (*Allium sativum* L.) sous l'influence des bio-intrants microbiens endémiques

Résumé

L'ail (Allium sativum L.) est une plante herbacée aromatique cultivée à des fins culinaires et médicinales. Le secteur industriel exige un niveau élevé de constance dans la biomasse, la saveur et le profil nutritionnel de l'ail. Ce qui peut s'avérer difficile à obtenir dans des conditions de culture variables en raison de l'impact de la qualité du sol, du climat et des pratiques de gestion des cultures sur le rendement et la qualité de l'ail.

En vue de valoriser cette plante dans le secteur pharmaceutique et agricole, notre étude s'est basée sur la culture d'ail en pots et sous serre sous l'effet d'application des biointrants microbiens dont, deux isolats de champignons mycorhiziens à arbuscules, trois isolats de *Trichoderma asperellum* et un isolat de PGPR. Les paramètres étudiés concernent la croissance, le rendement de la culture, leurs métabolites secondaires, ainsi que, les potentialités antioxydantes, antimicrobiennes et herbicides des extraits aqueux préparés à partir de chacun des échantillons de plantes cultivés d'ail. De plus, deux formulations pharmaceutiques naturelles dérivées de l'extrait d'ail ont été mises au point.

Les résultats de cette étude indiquent que l'inoculation de l'ail avec des micro-organismes endémiques exerce des effets variables sur les paramètres de croissance, les groupes fonctionnels les propriétés antioxydants et antimicrobiennes de leurs extraits. Les résultats obtenus ont prouvé l'importance d'utilisation des isolats microbiens endémiques *Trichoderma asperellum* (T1), PGPR (T7) et champignon mycorhizien (MO) dans l'amélioration de la majorité des paramètres de croissance étudiés. Par ailleurs, l'application du champignon mycorhizien (MO) et l'isolat de *Trichoderma asperellum* (T3) était responsable d'amélioration de la qualité des métabolites secondaires de cette culture ainsi que, les propriétés antioxydantes et antimicrobiennes de leurs extraits aqueux. . En outre, l'extrait aqueux préparé à base de plantes d'ail inoculées par l'isolat T3 a montré un potentiel effet inhibiteur sur la germination des graines et la croissance des plantules de *Lolium perenne.* De même, celui préparé à base de plantes d'ail inoculées par l'isolat T3 s'est avéré comme un puissant antimicrobien qui a pu même conserver ses potentialités sous forme lyophilisée pendant six mois. Sur la base de ce résultat, des capsules et un gel antimicrobiens ont été formulés pour une administration orale et cutanée dans le domaine pharmaceutique.

Mots-clés : Culture d'*Allium sativum,* bio-intrants microbiens, métabolites secondaires, activités biologiques, gel antimicrobien.

التثمين الصناعي (.Allium sativum L) تحت تاثير المخالت الحيوية الميكروبية المتوطنة

الملخص

الثوم (.*Allium sativum* L) هو نبات عشبي عطري زُرع لغراض الطهي االغراض الطبية.
حُتاج القطاع الصناعي إلى كل من الكتلة الحيوةُ االنكهة االمزاا الغذائية للثوم. اقد كُون من الصعب تحقيق ذلك في ظل ظراف الزراعة المتغيرة بسبب تأثير جودة التربة االمناخ اممارسات إدارة المحاصيل على محصول الثوم اجودته.
بهدف استغلل هذا النبات في قطاعي الداةُ االزراعة، استندت دراستنا على زراعة الثوم في أصص اتحت الدفيئة باستخدام مدالت حيوةُ ميكرابية بما في ذلك عزلتان من الفطرأت الميكورزُةُ الشجرةُ اثلث عزلت من التركُودرُما اسبرلوم اعزلة االحدة من بكيراُ PGPR. كانت البارامترات التي تمت دراستها هي النمو، إنتاجية المحصول، االمستقلبات الثانوةُ، امضادات الكسدة، امضادات الميكرابات، إمكانات مبيدات العشاب للمستخلصات المائية المحضرة من كل عينة من عينات نبات الثوم. بالضافة إلى ذلك، تم تطورُ تركيبين صيدلنيين طبيعيين مشتقين من مستخلص الثوم.
تشير نتائج هذه الدراسة إلى أن تلقيح الثوم بالكائنات الدقيقة المتوطنة له تأثيرات متفاتة على بارامترات النمو االمجموعات الوظيفية امضادات الكسدة االخصائص المضادة للميكرابات في مستخلصاتها.

أثبتت النتائج التي تم الحصول عليها أهمية استخدام العزلت الميكرابية المتوطنة تركُودرما (T1) االبكتيراُ PGPR (T7) االفطرأت الميكورزُةُ الشجرةُ (MO) في تحسين معظم بارامترات النمو التي تمت دراستها.
هذه المواد الحيوةُ هي محفزات للنمو ابدلُ للسمدة الكيميائية.ابالضافة إلى ذلك، كان استخدام الفطرأت الميكورزةُ (MO) اعزلتالركُودرُما (T3) مسوالن عن تحسين جودة المستقلبات الثانوةُ للمحصول، فضل عن الخصائص المضادة للكسدة االمضادة للميكرابات في مستخلصاتها المائية. من المحتمل أن تسهم هذه المستخلصات في تطورُ بدائل طبيعية للمضادات الحيوةُ التقليدةُ.ابالضافة إلى ذلك، أظهر المستخلص المائي المحضر من نباتات الثوم الملقحة بالعزلة T3 تأثيرا مثبطا محتمل على نمو تتلت .L. perenne.

ابالمثل، أثبت المستخلص المائي المحضر من نباتات الثوم الملقحة بالعزلة T3 تركُودرما اسبرلوم أنه مضاد قوي للميكرابات، حتى أنه احتفظ بإمكانياته في صورة مجففة بالتجميد لمدة ستة أتهر. استنادا إلى هذه النتيجة، تم تركيب كبسولت مضادة للميكرابات للعطاء عن طرقُ الفم اهلم للجلد في المجال الصيدلني.

الكلمات الخالة: أنشطة بيولوجية، مدالت حيوةُ ميكرابية، مستقلبات ثانوةُ، مستنبت *Allium sativum* L، هلم مضاد للميكرابات.

List of abbreviations

AlCl3: Aluminum chloride

CaCl2: Calcium chloride

CMD: Corn meal dextrose agar medium

CO: Control

G. invermaium*: Glomus invermaium*

IAA: (Indole-3-Acetic Acid)

KOH: Potassium hydroxide

MA: Arbuscular mycorrhizal isolates

MO: Arbuscular mycorrhizal isolates

P.vulgaris*: Proteus vulgaris*

PDA: Potato dextrose agar

PGPR: plant growth-promoting rhizobacteria

R.intraradices*: Rhizophagus irregularis* **SNA:**

Saltwater Nutrient Agar Medium

T1: Trichoderma asperellum TMSKOLDZ20

T2: Trichoderma asperellum TMS11DZ15

T3: Trichoderma asperellum TMS5DZ15 **T7:**

Plant growth-promoting rhizobacteria **VOC**:

volatile organic compounds

List of tables

List of figures

Table of contents

Introduction

The garlic "*Allium sativum"* is an aromatic herbaceous annual spice and one of the oldest and most widely consumed bulbs. It is believed to have originated in Central Asia and has been cultivated for culinary, medicinal, and religious purposes for several millennia [1]. According to the Food and Agriculture Organization of the United Nations (FAOSTAT), global garlic production in 2023 is estimated to be approximately 28 million tons, cultivated on approximately 1.6 million hectares with an average yield of 17.00 tons per hectare [2]. The leading garlic-producing countries are China, India, Bangladesh, and Egypt, whereas, China and India are the predominant contributors to global garlic production, accounting for approximately 80 % of the overall yield [2]. Garlic plays an integral role in various culinary practices due to its distinctive flavour profile and aromatic characteristics. In the field of medicine, garlic is highly regarded for its healing properties and is believed by experts to have the potential to treat a wide range of ailments [1]. According to the Food and Agriculture Organization of the United Nations (FAOSTAT 2022), garlic in Algeria is the second most widely consumed allium, after onions. Its primary function is as a seasoning agent, predominantly as a condiment, and it is extensively utilized in the eastern highlands [3].

Bulb crops, such as garlic, have high nutritional requirements, necessitating a substantial input of nitrogen (N), phosphorus (P) and potassium (K) in the form of either inorganic or organic fertilizers or a combination thereof [4]. However, the lack of these essential nutrients can have a considerable effect on the yield and quality of garlic, which can prove challenging to achieve the industrial sector's requirements. Consequently, farmers seek to achieve higher yields of garlic through the application of specific fertilizers, with the objective of preserving the quality of the crop [5]. However, the extensive use of nitrogen-based fertilizers worldwide has been identified as a significant environmental issue in agricultural production systems which could potentially lead to environmental pollution and poses risks to ecosystems and human health as well as reduce the nutritional quality of garlic. [4].

A substantial body of research has demonstrated the potential of various microorganisms, including arbuscular mycorrhizal fungi (AMF), plant growth-promoting

rhizobacteria (PGPR) and *Trichoderma*, to enhance plant health and growth. These microorganisms possess the capacity to facilitate restructuring and stimulation of plant growth in both optimal conditions and under diverse biotic and abiotic stressors. This is achieved by enhancing the nutritional quality of plants and activating their defense response [6,7,8].

However, the impact of these microorganisms on garlic, and its chemical compounds in particular, remains to be elucidated.

The present study aims to investigate the impact of the application of endemic-microorganisms on the yield and quality of garlic cultivation, with a view to enhancing its potential applications in a range of fields, including agriculture and pharmacology on an industrial scale, while promoting sustainable cultivation techniques. In order to achieve this objective, the methodology is comprised of four principal elements.

- Impact of application of endemic-microorganisms on the growth and chemical composition of garlic,

- Study of some biological activities of garlic cultivated under the effect of application of endemic microorganisms including antioxidant, antimicrobial and, herbicidal activity.

- Study of the stability of the antimicrobial properties of two preparations based on garlic grown under the effect of application of endemic microorganisms,

- Formulation of an antimicrobial based on the garlic preparations studied.

Chapter: 1
Literature Review

1.1 Comprehensive overview of garlic

Garlic (*Allium sativum*) is a widely used plant known for its culinary, medicinal, and agricultural benefits. Its diverse applications make it a subject of interest across various fields.

1.1.1 Historical background

Garlic originated in Central Asia [9], although its widespread use has resulted in its distribution across the globe. The Sumerians (2600-2100 BC) were among the first to recognize its medicinal properties and subsequently introduced it to China. Nevertheless, some historians still believe that garlic originated in this country [10], where it has been used since 2700 BC. In Egypt, from the time of the pharaohs, it was an indispensable food supplement, a source of nourishment and a remedy for the impoverished [9]. In Greece, the plant was known as 'the stinking rose' and was apparently employed by early Greek army commanders prior to major battles, as well as by athletes in order to guarantee a favorable outcome [11]. In the middle Ages, Arab medicine played a significant role in the proliferation of garlic, largely due to its perceived medicinal value. Conversely, during this same period, Western Europe remained largely unaware of the therapeutic properties of this vegetable [9;11].

1.1.2 Botanical description

Garlic "*Allium sativum"* is a perennial herbaceous bulbous plant that has the potential to reach a height of up to 90 cm. The plant comprises a bulb, leaves, roots, flowers and a stem [12]. The root system of garlic is of the adventitious type, comprising a thick, little-branched structure with an epidermis, multicellular cortex, and endoderm surrounding the central stele [13]. The root development of the plant is sensitive to soil moisture and temperature [14]. The stem or pseudo-stem is notably short, forming a receptacle at the base from which adventitious roots emerge (Figure 1). It is composed of a series of leaves that are joined together by their leaf sheaths [13] . Its bulb is composed of a number of cloves, these are wrapped in a protective tunic and have a small bud at their centre. It should be noted that the number of cloves in a bulb of garlic can vary depending on the specific variety (Figure 1), however, a bulb will typically contain between 10 and 15 cloves [15].

The leaves of garlic are characterized by their elongated, flat and smooth morphology, exhibiting a cylindrical, hollow and linear blade (Figure 1). The blade is flat and solid with an acute apex (acuminate apex) [16]. The number of individuals in a given population

varies from nine to twelve [17]. The maximum length and width of an individual is 40 cm and 2 cm, respectively [12].

The inflorescences are umbels comprising perfect flowers with six petals, six anthers and three locules, each containing two ovules [18]. They can be either large or small, and may contain a greater or lesser number of sterile flowers and bulbils (Figure 1). However, the capacity to produce inflorescences is not universal, being most prevalent in varieties originating from Central Asia and Spain [13].

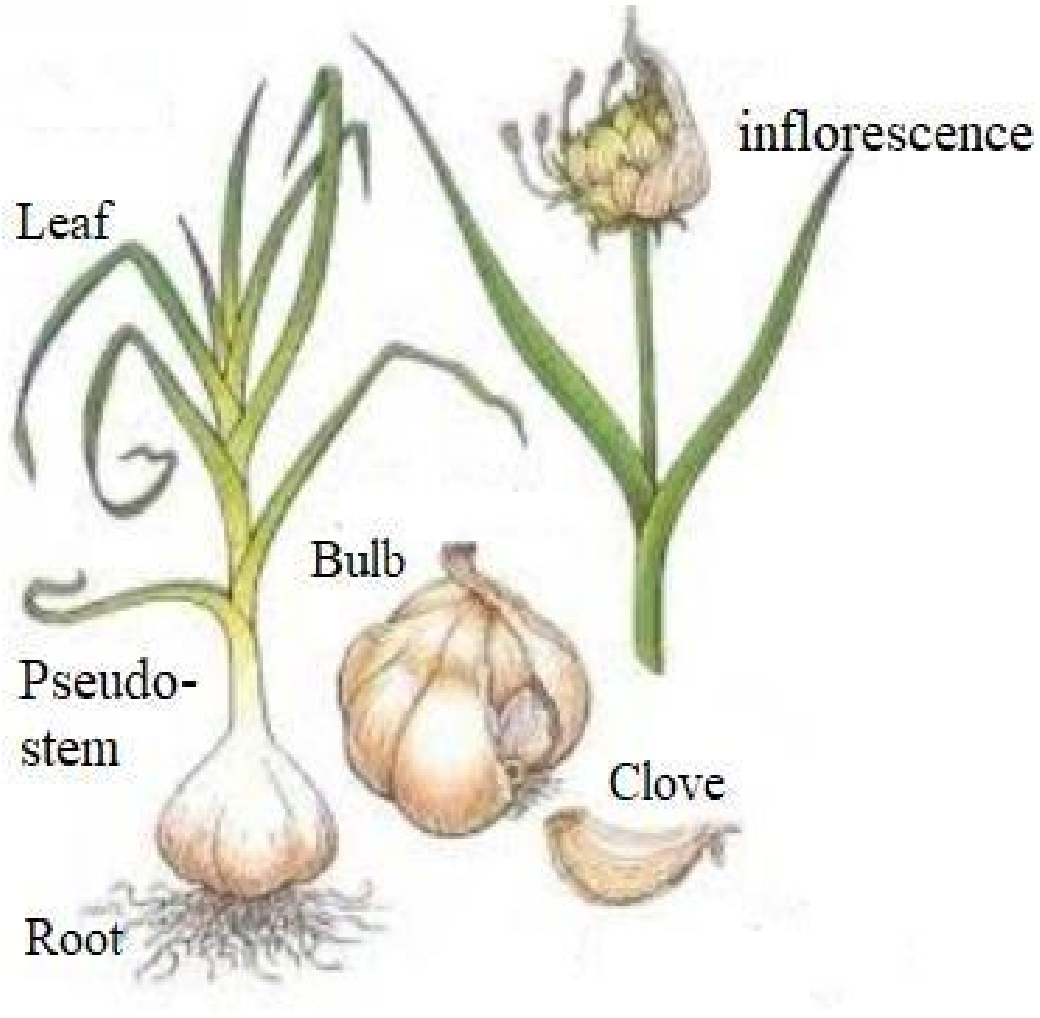


Figure 1: Garlic plant morphology [19]

1.1.3 Classification

In accordance with the taxonomic classification system outlined by So *et al*.[13], this plant has been categorized as follows :

Table 1: Garlic classification

Kingdom	**Plantae**
Subkingdom	Tracheobionte
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Equisetopsida
Subclass	Magnoliidae
Superorder	Lilianae
Order	Asparagales
Family	Amaryllidaceae
Genus	*Allium*
Species	*Allium sativum*

1.1.4 Varieties of garlic

The genus Allium includes a vast array of garlic varieties, distinguished by their varying sizes, Colors,, and flavors. As elucidated by Engeland et al. [20,21], these characteristics encompass a diverse range of garlic types (Table 2).

According to the ITCMI report [23], the varieties most widely cultivated in Algeria are as follows: Local Red, Kabylie Pink, Chinese Pink, Spanish Red, Thermidrome, Messidrome, Fructidor, Iranian Red, Germidour, Bulgarian Mocta and Simple California.

Table 2: Phenotypic types of garlic as set out by Engeland et al. (20, 21).

Varieties	Cloves	Clove wrapper tightness	Clove color	Bulb color	Flavor	Matu -rity	Stora -ge (Mon -th)	scapes	Climate prefered
Asiatic	4-7	Average	Brown Rosy purple	White	Hot	Early	4	Yes hardneck	Mild, softhern
Turban	6-8	Average	Pink brown	Striped purple	Hot	Very early	4	Weak bolting	Mild, softhern
Creole	8-12	Tight	Red	White	Spicy sweet	Mid	10-12	Weak bolting	Hot, humid, softern
Purple stripe	8-12	Average	Purpl, Pink	Striped purple	Full, great baked	Mid	4-6	Yes hardneck	Cold winters
Glazed	6-10	Average	Purple brown	Satin purple	Full, great baked	Mid	4-6	Yes hardneck	Cold winters
Marbled	6-10	Average	Purple brown	Mottled purple	Full, great baked	Early	4-6	Yes hardneck	Cold-cool winters
porcelain	4-6	Tight	Pink	White	Hot	Late	6	Yes hardneck	Cold winters
Rocambole	6-11	Loose	Brown	White	Rich, robust	Late	4-6	Yes hardneck	Cold winters
Silverskin	12-20	Tight	White pink	White	Hot	Late	10-12	No softneck	Cool-hot, versatile
Artichoke	12-20	Tight	White	White splotched	Mild	Early	10	No softneck	Cool-hot, versatile

1.1.5 Life cycle of garlic

Throughout garlic life cycle, the garlic plant *(Allium sativum*) experiences five sequential phenological stages: senescence, dormancy, dormancy break, vegetative growth, and bulb formation.

The dormancy period of mature cloves is initiated at temperatures ranging from 25 to 30°C, and the optimal temperature for its elimination is 6 to 7°C. Vegetative growth is observed to be more rapid at temperatures between 18 and 20°C [24]. The process of bulb swelling requires temperatures in excess of 20°C, and the photoperiod must exceed a threshold of 12-15 hours, depending on the cultivar, in order to satisfy the "need for cold" after dormancy has been eliminated. It is for this reason that bulb production in the tropics is more challenging for garlic than for onions [22]. However, it should be noted that there is considerable physiological variability among garlic cultivars. Those suitable for

cultivation in tropical conditions are not strongly dormant, their "need for cold" is low, and their photoperiodic threshold is barely 12 hours. The complete cycle varies from 4 months (in the tropics, or for highly dormant cultivars planted in spring in temperate climates) to 9 months (for less dormant cultivars planted in autumn in northern Mediterranean climates). In optimal conditions of altitude and latitude, some cultivars produce inflorescence regularly, while others produce well-developed flowers and seeds, provided the bulblets present among the flower buds have been removed early [22, 24]. Cultivars that normally produce inflorescences have flowers that remain sterile, while others do not produce inflorescences under normal conditions, but only at higher altitudes or latitudes.

1.1.6 Cultivation requirements

The cultivation of garlic is a relatively straightforward process, as the crop can be grown year-round in regions with a temperate climate and an average rainfall of 600 to 700 mm, followed by a sunny, dry summer. In cold climates, garlic cloves are planted for propagation during the autumn season before the time of freezing and cooling of the soil, about six weeks after that, the output is harvested for the period from before the end of spring until the beginning of summer [25]. In order to obtain optimal results, it is essential to plant these cloves at a specific depth in the soil to prevent climate-related fluctuations that could potentially infect the crop with fungal diseases [25]

Garlic (*Allium sativum*) is a plant that is cultivated in nutrient-rich, well-drained soils that can maintain sufficient moisture levels throughout its growth cycle [26]. It has been observed that a sandy clay soil environment is conducive to its optimal development [26]. The optimal soil pH range for garlic production is between 6 and 7, with some regions experiencing slightly higher values [27]. Sunlight exposure is a crucial factor for garlic growth, as it requires ample phototrophy [27].

Garlic is a very demanding plant in terms of fertilizer, requiring a sufficient quantity of nutrients in the soil throughout the growth period. Conducting a comprehensive soil analysis is paramount to formulate a fertilization strategy that aligns with available resources [28]. Nutrient elements such as phosphorus, potassium, and nitrogen are pivotal for optimal root and bulb development, as well as carbohydrate storage [28].

In its early stages of development, garlic requires a period of cool conditions to initiate growth. It demonstrates a preference for relatively mild climates with temperatures of 20-22°C, yet it requires temperatures of up to 30°C for optimal bulb development [29]. Garlic

is sensitive to water shortage, as drought can lead to a drop in yield, which is why it needs to be adequately irrigated during the vegetative period [29].

1.1.7 Garlic situation in the world

Since the 2000s, garlic has become an increasingly significant crop in global agricultural production. Between 2000 and 2018, the combined production of garlic for seed and consumption worldwide increased by a factor of two and a half, from 11 million tonnes to 28.5 million tonnes, according to the FAO 2020 (Figure 2) [3].
The highest production of garlic is in Asia, with 26 million tonnes in 2018, followed by Europe, the Americas, Africa and finally Oceania (Figure 3) [3].

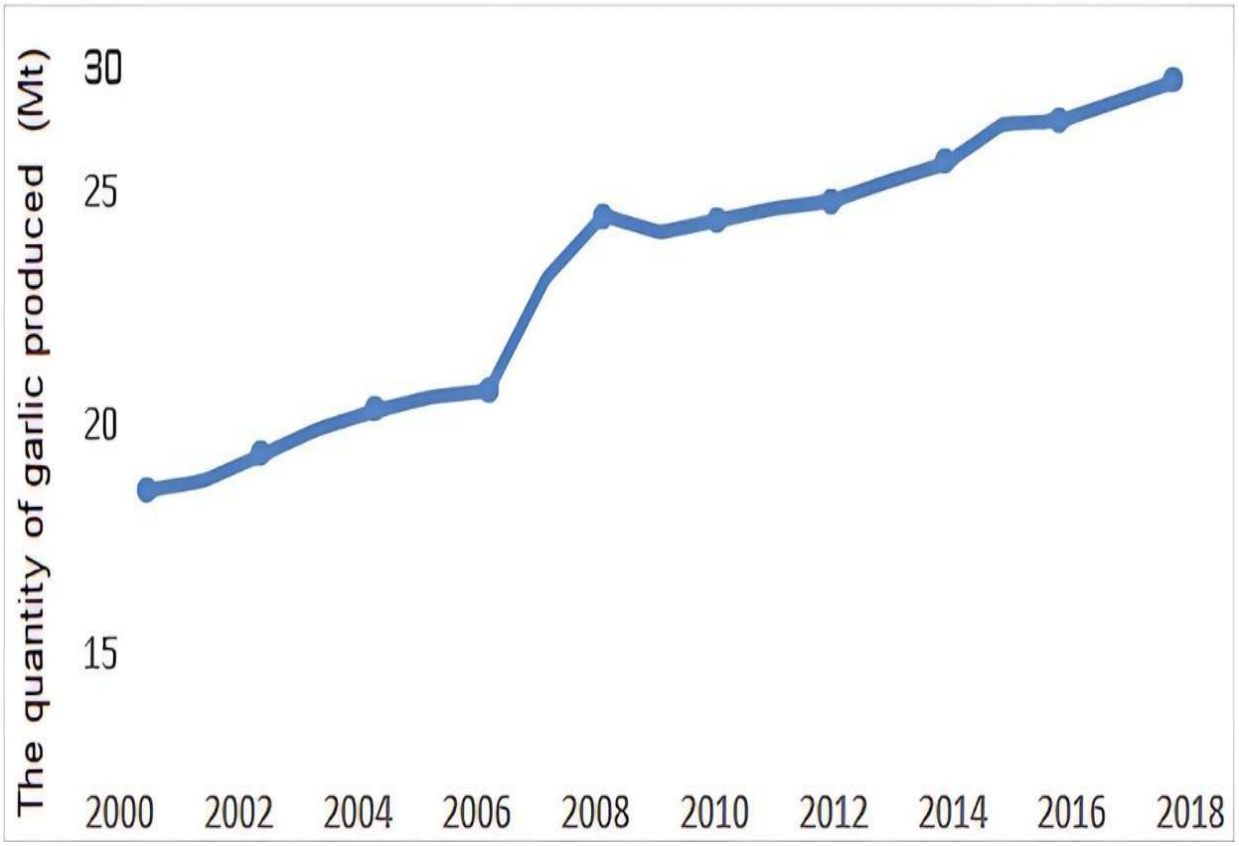


Figure 2 : World garlic production from 2000 to 2018 [3].

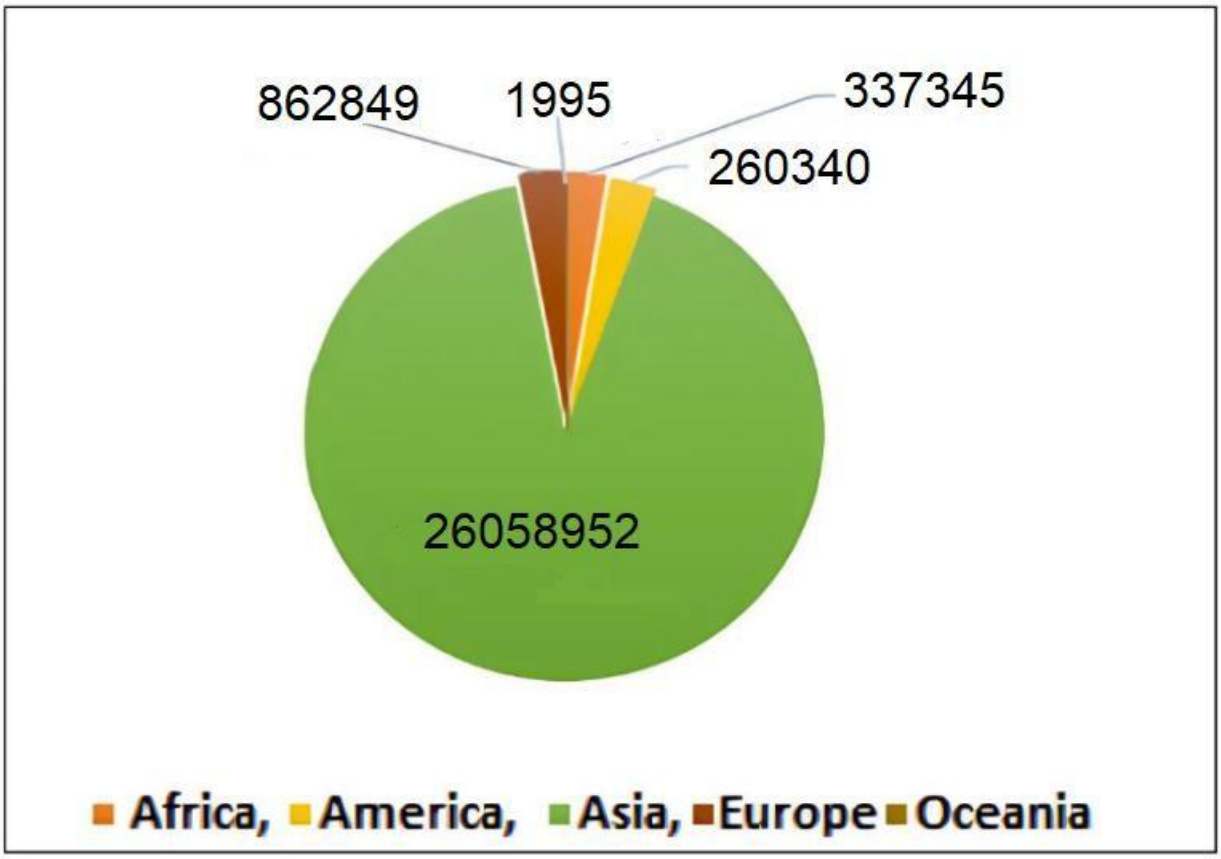


Figure 3 : World garlic production by continent in 2018 [3].

In 2020, China was the leading producer of garlic, with a yield of 20.7 million metric tons, as reported by the Food and Agriculture Organization of the United Nations (FAOSTAT 2020). India ranked second with a production of 2.9 million metric tons. The remaining three principal garlic-producing countries are South Korea, Egypt and Russia [3].

Table 3: The top 20 garlic-producing countries in the world 2018 [3]

N°	Pays	Quantité en tonnes
1	Chine, continentale	22273802
2	Inde	1721000
3	Bangladesh	461970
4	République de Corée	331741
5	Égypte	286213
6	Espagne	273476
7	États-Unis d'Amérique	260340
8	Ouzbékistan	254857
9	Fédération de Russie	211981
10	Myanmar	207094
11	Algérie	202201
12	Ukraine	187020
13	Argentine	148156
14	Turquie	143207
15	Éthiopie	124801
16	Brésil	118837
17	Pérou	104574
18	Mexique	94692
19	Pakistan	81167
20	Thaïlande	74288
totale	Monde	28 494 130

1.1.8 Garlic situation in Algeria

Algeria is the eleventh largest garlic producer in the world, and the second largest in Africa in 2018, with 202201 tonnes covering an area of 12,945 hectares and yielding 1562303 kg/h (figure 4)[3].

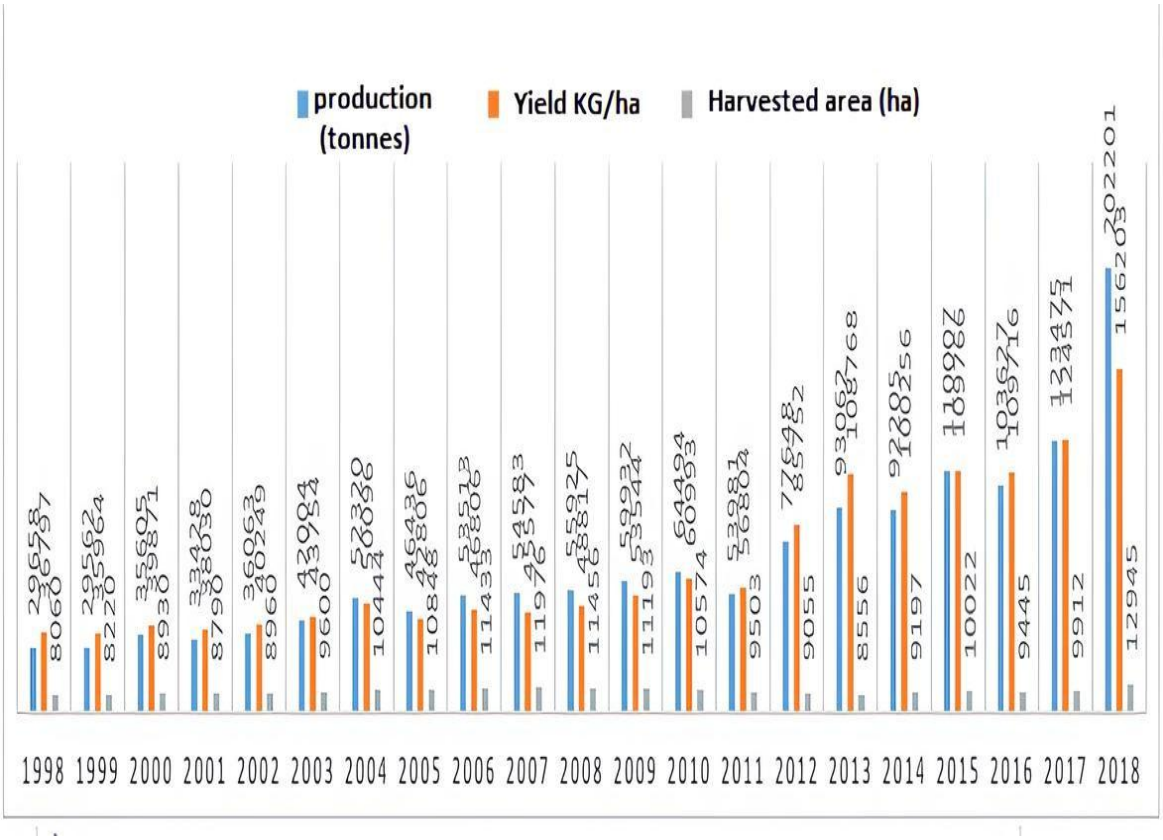


Figure 4 : Area, production and yield of garlic in Algeria during the 1998 /2018 seasons [3].

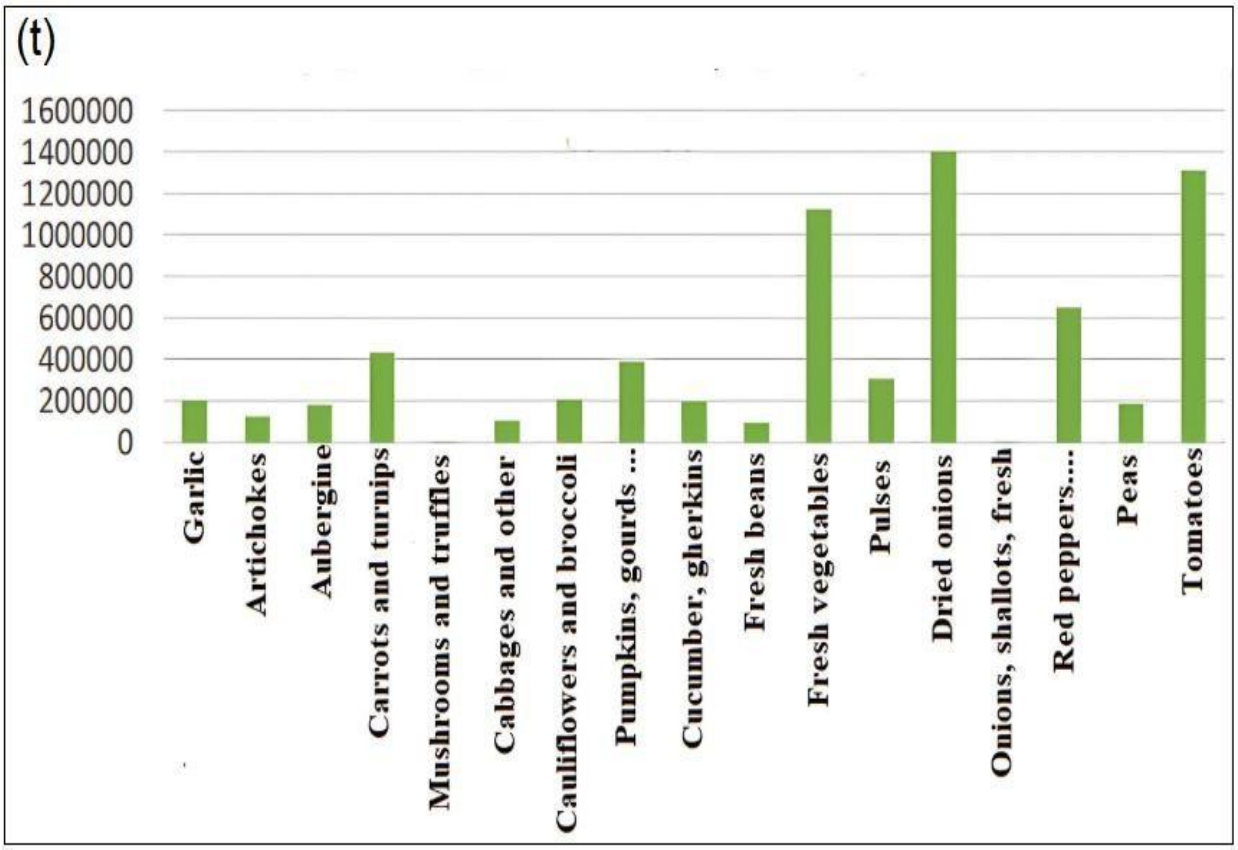


Figure 5: Vegetable production in Algeria 2018 [3]

Garlic production in Algeria remains very low in contrast to the production and consumption of other vegetables in 2018. This can be explained by the fact that garlic consumption is not identical to that of other vegetables such as onions and tomatoes. Garlic is used more for seasoning dishes, mainly as a condiment, and is widely used in the eastern highlands. Garlic can be grown in all Algerian regions, as it tolerates the cold well, although clay and sandy soils give a better yield. However, it is the second most widely consumed allium after onions. (Figure 5) [3].

According to DSA horticultural statistics estimates for the 2018/2019 season, the main garlic-producing areas in Algeria were: Mila (1,903 ha), Médéa (968 ha) and Batna (788 ha), Skikda (620 ha), M'sila (495 ha), Tizi Ouzou (322 ha), Guelma (290 ha), Setif (236 ha) and Bordj Bou arreridj (132 ha) (Table 4).

Table 4: **Potential garlic production areas in Algeria in 2019-2020 [3].**

Etats	**Area (ha)**	**Production(qx)**
Mila	1903	1Million
Batna	788	125300
M'sila	595	40968
Setif	236	45870
Bordj Bou Arreridj (BBA)	132	9228
Médéa	968	45870
Skikda8	620	38400

1.1.9 Nutrient Composition

Garlic is a rich source of non-sulphur compounds, including nutrients, antioxidants and other beneficial substances.

The average garlic bulb is composed of 65% water, a figure that contrasts with the 85% or more typically found in most fresh vegetables [19,30] Carbohydrates constitute approximately 27.5 to 30% of the composition, including simple carbohydrates such as

fructose, glucose and sucrose, and complex sugars such as fructosan [31]. It contains pigments, including chlorophyll, carotenoids and anthocyanins.

As demonstrated in the relevant literature, a low concentration of lipids has been documented, including the essential (polyunsaturated) fatty acids linolenic acid (omega-3 fatty acid) and linoleic acid (w6 fatty acid) [31]. Furthermore, the total composition consists of 2% proteins, which include essential amino acids such as methionine, cysteine, tryptophan and arginine [31]. The total composition is made up of approximately 4.7% dietary fibre. This encompasses both soluble fibers, such as pectin, and insoluble fibres, including cellulose and hemicellulose [31]. Additionally, the subject is a rich source of vitamins, including vitamins A, B1, B2, B6, C and E [32].

The total composition is made up of approximately 4.7% dietary fibre. This encompasses both soluble fibers, such as pectin, and insoluble fibers, including cellulose and hemicellulose (Senninger, 2009). Additionally, the subject is a rich source of vitamins, including vitamins A, B1, B2, B6, C and E [32]. It contains also a substantial quantity of minerals and trace elements, including calcium, phosphorus, magnesium, iron and selenium [30].

Garlic contains a variety of antioxidant compounds, particularly flavonoids and polyphenols, with apigenin and myricetin being the most prominent molecules [31].

In addition to the various phytochemicals present in garlic cloves, they also contain a multitude of sulphur-containing organic compounds. The majority of the health benefits, including its antibacterial activity, have been attributed to these organosulfur compounds [33, 34, 35] Similar compounds are found in other members of the Liliaceae family, which includes onions, leeks and chives. Intact garlic contains a multitude of organosulphur compounds derived from cysteine, with allin being a crucial component in its antibacterial activity. The process of crushing or cutting a garlic clove results in the release of the allinase enzyme, which converts allin into allicin and other compounds, Allicin is regarded as one of garlic's most potent and earliest identified antibacterial constituents [36].

Allicin is a volatile compound, and as a consequence, it undergoes degradation and rearrangement, resulting in the formation of a multitude of organosulfur compounds. Many of these compounds are organosulfides, which are composed of different lengths of alkene and alkane chains attached to one or multiple sulfur atoms [36]. The most prevalent organosulfur compounds include diallyldisulfide (DADS), diallyltrisulfide (DATS), allylpropenyldisulfide, methyl allyldisulfide (MADS), diallyltetrasulfide (DATTS), and a number of sulfur-containing cyclic compounds, including vinyl dithiins. Ajoene is a

compound formed by the rearrangement of allicin [37]. The majority of these compounds exhibit some degree of water solubility, yet they are predominantly oil-soluble and volatile organosulfur compounds present in garlic have been demonstrated to exhibit inhibitory activity against pathogens. These sulphides are present in highly concentrated form in garlic oil [38]. These organosulfur compounds are responsible for the various health benefits associated with garlic consumption, as well as the distinctive aroma and flavor of garlic. Aqueous extracts of garlic, in particular aged garlic extracts, are notable for their high concentration of s-allylmercaptocysteine (SAMC), S-allyl cysteine (SAC), and S-methyl cysteine [39, 40].

1.1.10 Garlic applications

The diverse range of biochemical compounds and active ingredients present in garlic may contribute to a variety of beneficial properties, making it a versatile ingredient with numerous potential applications.

1.1.10.1 Culinary use

Garlic is a versatile ingredient with a multitude of culinary applications. In the present era, the bulbs are employed in a variety of ways, including as a fresh ingredient, as well as in a dried, granulated, or powdered form, which are utilized as a condiment. The entire clove can be subjected to either steaming or baking. Garlic salt is a common ingredient used to enhance the flavor of a variety of foods. For several years, garlic flowers have been available for purchase. These are the flowering stems that are harvested as soon as they appear. They are consumed in a cooked or marinated state [41].

1.1.10.2 Agri-food industry use

Garlic is used as an antioxidant in oils to preserve them for a long time. Garlic powder is used instead of antibiotics in cattle, poultry and fish feed to avoid antibiotic residues in meat [42]. Garlic is added to smoked fish, sausages and fresh meat stored at 4°C to prevent spoilage and rancidity [43].

1.1.10.3 Agricultural use

It has been demonstrated that garlic exhibits robust inhibitory activity against a diverse array of plant pathogenic bacteria and fungi. Furthermore, it has been demonstrated that garlic-based extracts possess larvicidal properties against Anopheles larvae [44].

1.1.10.4 Medical use

Garlic products have a long history of use as medicinal agents in human medicine. Their efficacy is attributable to a range of properties including [45]:

Antimicrobial properties

The antimicrobial activity of garlic has been employed as a treatment for bacterial infections worldwide for a considerable period of time. Garlic displays a range of notable antibacterial properties, including bactericidal, antibiofilm and antitoxic efficacy, against a vast array of bacterial species, including strains resistant to numerous antibiotics. This is attributed to the presence of organic sulfur compounds [46].

Allicin represents a significant component of garlic, exhibiting potent antibacterial activity [47].

A study demonstrated that a fresh garlic extract (FGE) was capable of eradicating over 90% of both *Staphylococcus epidermidis* and *Salmonella typhi* within a three-hour period. The antibacterial activity of garlic paste has been demonstrated against *Escherichia coli* O157:H7, a food-borne pathogen. Moreover, the antibacterial activity of FGE has been documented against foodborne pathogens, including *S. aureus, S. typhi, E. coli and Listeria monocytogenes* [48]. Additionally, the antibacterial activity of garlic powder and FGE has been observed against enteric bacteria and vancomycin-resistant enterococci [49].

Antifungal activities

The application of garlic extracts has been demonstrated to exert a fungicidal effect against a diverse range of fungal species, including *Candida, Torulopsis, Trichophyton, Cryptococcus, Aspergillus, Trichosporon* and *Rhodotorula*. Allicin is the principal component responsible for the inhibition of fungal growth. Its antifungal activity is particularly marked against *Candida albicans* [49]. Diallyl disulphide (DADS) has been demonstrated to exhibit antifungal activity against a number of fungal species, including *Candida albicans*, *tropical Candida* and *Blastoschizomyces capitatus*. The combination of ajoene at a dose of less than 20 μg/ml with certain drugs has been demonstrated to inhibit the growth of *Candida albicans* and *Aspergillus niger*, the mechanism of action of garlic extract is through the disruption of the fungal cell wall, leading to irreversible structural changes in fungal cells. This results in a loss of structural integrity and affects the capacity for germination [50].

Anti-oxidant activities

A number of studies have yielded significant findings regarding the antioxidant properties of garlic. It seems that garlic may contain a variety of antioxidant compounds, including flavonoids and tocopherols, as well as sulphur compounds (allicin, diallyl sulphide, diallyl disulphide, diallyl trisulphide, etc.) and vitamins E and C. These compounds are thought to contribute to the antioxidant action of garlic [51]. A substantial body of research indicates that the anticancer properties of garlic may be associated with its potential to function as an antioxidant, neutralize free radicals, and impede tumor growth and DNA damage [52].

1.2 General information on mycorrhizal fungi

Mycorrhizal fungi are specialized soil fungi that establish symbiotic associations with the roots of most terrestrial plants.Understanding their biology, ecology, and agricultural applications is essential for optimizing crop productivity and promoting sustainable agricultural systems."

1.2.1 Historical background

In the early 1840s, the German forester Theodor Hartig employed the use of a microscope to observe and describe the intricate structure of the fine roots of various tree species. His observations revealed the presence of a compact interlacing of cells situated between the cells of the root cortex. However, he was unaware of the fungal nature of this cellular network. Hartig's work demonstrated the existence of a fundamental symbiotic relationship between plants and fungi [53].

Other biologists made comparable observations during the nineteenth century and identified the fungal nature of the organism colonizing tree roots. In 1883, Gibelli provided an accurate description and illustration of this fungal association [53].

In 1885, the German botanist Albert Bernhard Frank provided a comprehensive summary of this work and proposed the term 'mycorrhiza' (derived from the Greek words 'myco' and 'rhiza') to describe the mixed plant-fungus organ that represents the foundation of this recurring association. Moreover, Frank conducted experimental trials to demonstrate the beneficial role of the fungus in the growth of Scots pine [54]. In 1886, Robert and Hartig endorsed the novel theory and had the designation 'Hartig Network' formally adopted in tribute to his father, Theodor Hartig. Subsequently, throughout the 20th century, a number of researchers conducted studies on other types of mycorrhizae [53].

1.2.2 Arbuscular mycorrhizal fungi

AM fungi are obligate biotrophs that form mutualistic symbioses with more than 80% of terrestrial plants [55]. In this context, the term "obligate" denotes that AM fungi are unable to complete their life cycle in the absence of a host plant [5]. The fossil evidence indicates that AM fungi have interacted with plants since approximately 470 million years ago (57,58,59). Moreover, it has been postulated that the symbiosis between early plants and AM fungi played a pivotal role in the colonization of land by plants [58,59]. This hypothesis is supported by the discovery of structures that are morphologically similar to those of AM fungal hyphae in the root systems of early wetland plants [60, 61].

In this association, the plant provides the fungus with carbon, derived from photosynthesis, and a microhabitat in which the fungus can complete its life cycle. In return, the fungus provides the plant with nitrogen, phosphorus and other minerals [62].

Consequently, mycorrhizal symbiosis is currently being investigated for its favorable impact on plant growth and its prospective contribution to agricultural and forestry practices [63]. The presence of mycorrhizae has been observed to result in the formation of new biological compartments within the rhizosphere [64].

1.2.3 Taxonomic diversity of AMF

Morphological and phylogenetic studies have enabled the classification of all arbuscular mycorrhizal species within the phylum *Glomeromycetes* [65], with the identification of four orders (Figure 6): *Glomerales*, *Paraglomerales*, *Archaeosporales*, and *Diversisporales* [66].

The morphological diversity of *Glomeromycota* is considerable, with notable variation observed in spore characteristics. These spores exhibit a wide range of sizes, colours, and shapes, which can vary significantly even within a single species. At present, approximately 18 genera are recognized, encompassing 250 species [66].

This estimate may turn out to be well below reality, as current taxonomy is based on species isolated from spores, whereas new molecular techniques allow sequences to be obtained from roots or soil, providing access to species that are non-sporogenic or difficult to detect [67].

The phylogenetic tree of *Glomeromycetes* is divided into four orders: *Diversisporales*, of which the species *Gigaspora rosea* is a member; *Glomerales*, of which the model species *Rhizophagus irregularis* is a member; *Archaeosporales*; and *Paraglomerales* (Figure 6) [66].

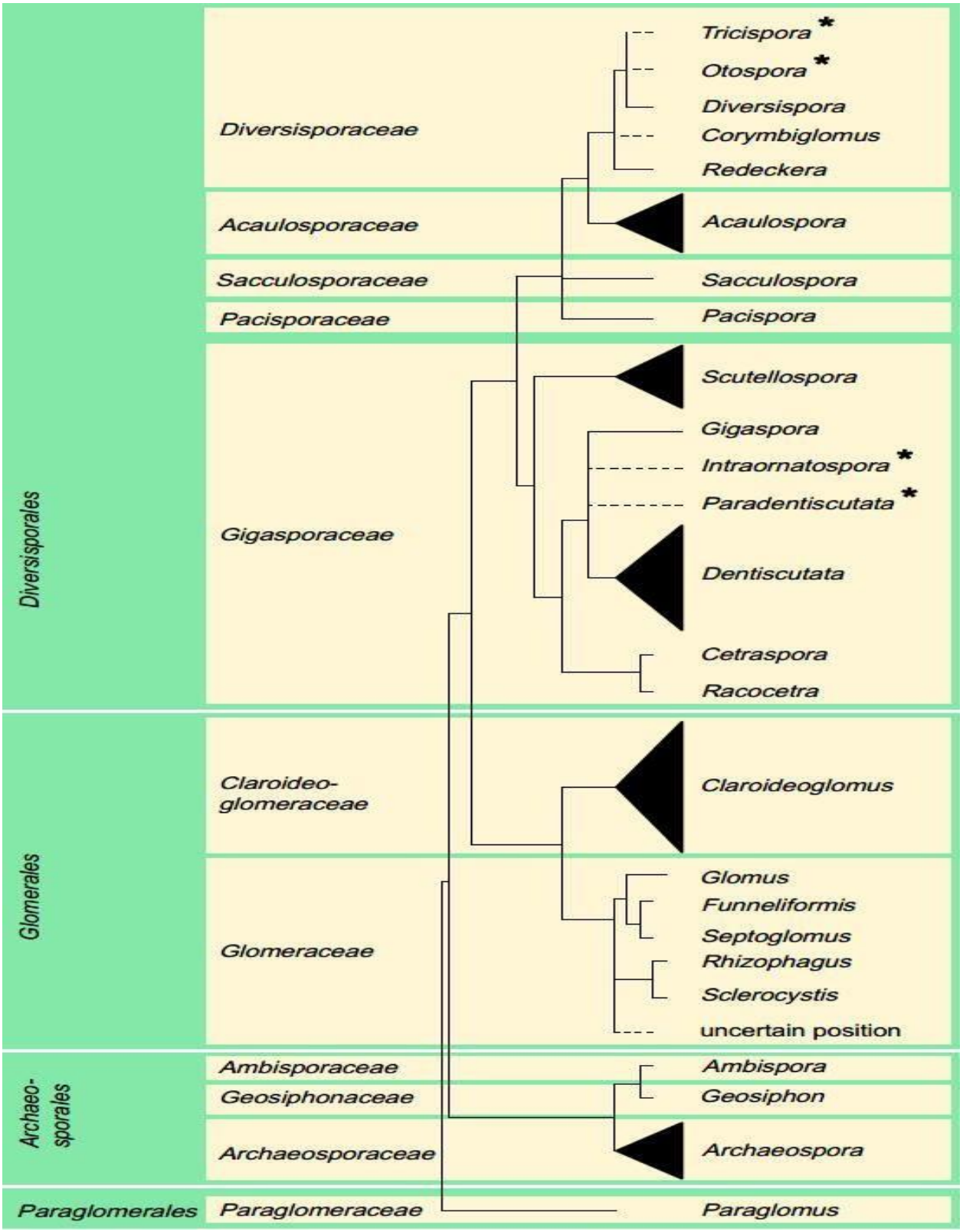


Figure 6: Phylogenetic tree of the *Glomeromycetes* [66]

1.2.4 Life cycle of the arbuscular mycorrhizal symbiosis

The life cycle of arbuscular mycorrhizal symbionts is completed only in the presence of the host plant during the formation processes of this symbiosis [68]. The plant accepts the colonization of the fungus without any rejection reaction and a series of root-fungus interactions leads to the integration of the two organisms (69).

According to [70], the establishment of an arbuscular mycorrhizal symbiosis begins with contact between a compatible root and germinating hyphae produced by AMF propagules (asexual spores or roots that have already been mycorrhized). This process goes through the following five stages (Figure 7).

1.2.4.1 Stage 01

The initial stage of the process is the germination of spores and the formation of a primary mycelium, also known as a promycelium.

1.2.4.2 Stage 02

The formation of a hyphopod, a structure comprising a fungus and root. Subsequently, the mycelium infiltrates the root system, enlarging into vesicles and forming arbuscules.

1.2.4.3 Stage 03

The mycelium then penetrates the root system, undergoing a swelling process that results in the formation of vesicles and arbuscules.

1.2.4.4 Stage 04

The mycorrhiza thus formed gives rise to an extra-root network from which new spores are differentiated.

1.2.4.5 Stage 05

Subsequent to maturation, these spores give rise to the initial promycelium (stage 1).

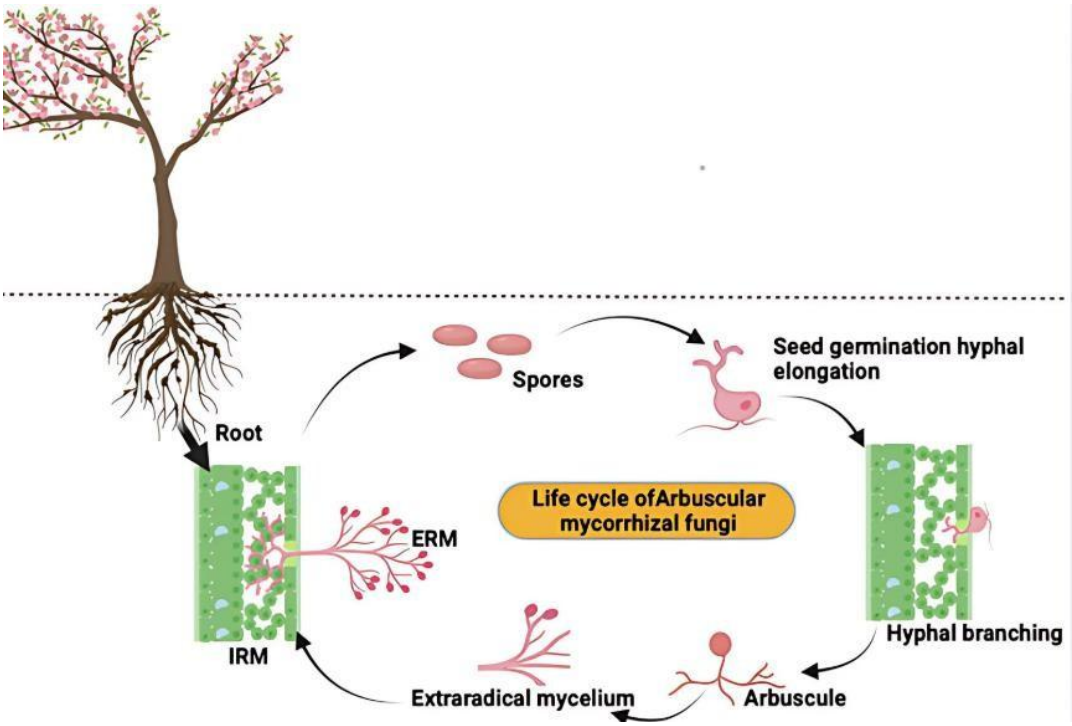


Figure 7: Life cycle of arbuscular mycorrhizal fungi [71]

1.2.5 Arbuscular mycorrhizal fungi structures

The AMFs form several structures within the roots

1.2.5.1 Spores

Arbuscular mycorrhizal fungal spores are produced in the extra-root hyphae of AMF by asexual reproduction [72, 73]. The diameter of these fungal spores has been observed to vary from 20 to 1000 µm [74]. They are typically spherical in shape, although some species may exhibit a basal ampulla or be accompanied by a sort of annexed sac (Figure 9) [75, 53].

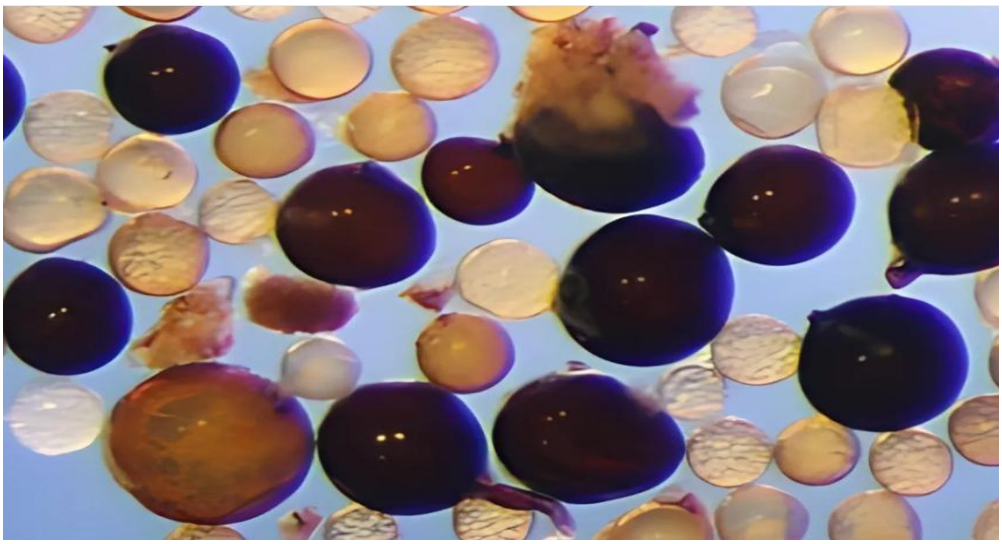
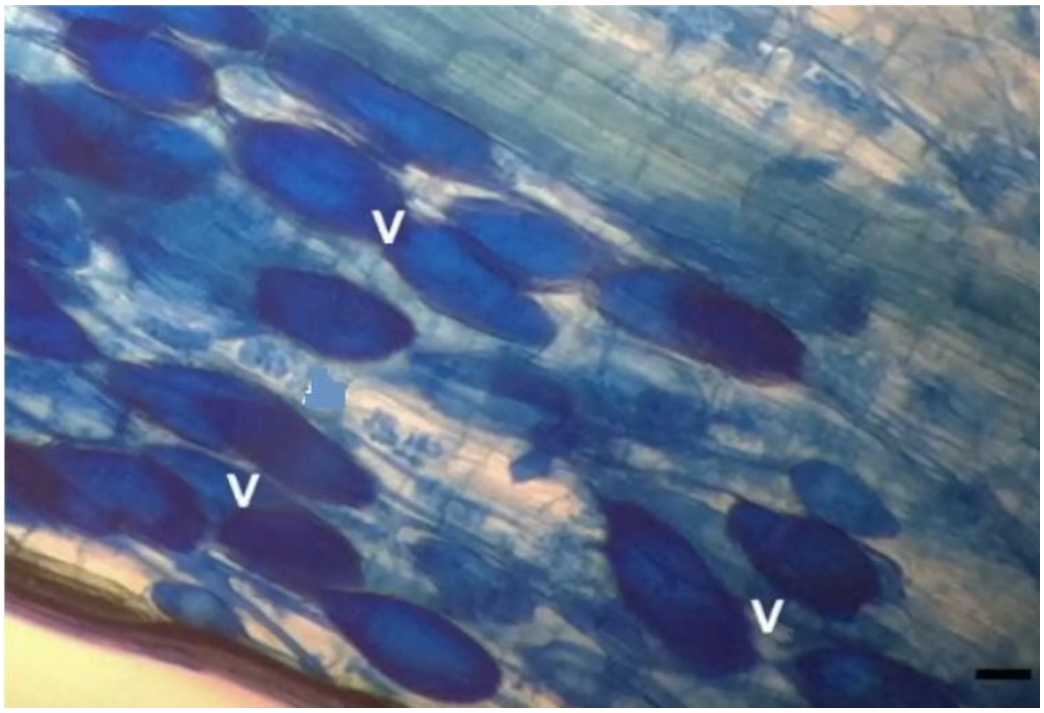


Figure 8 : Morphology of Spores of AMF (40x). [76]

1.2.5.2 Vesicles

These structures are formed within the root cortex. They are swellings of hyphae that are largely spherical, irregular or lobed in shape and vary greatly in size (from 10 to 100 µm in diameter) (Figure 9). They may be intracellular or intercellular [75]. The high concentrations of lipids and glycolipids present in these structures suggest that they play a role in nutrient storage [77].

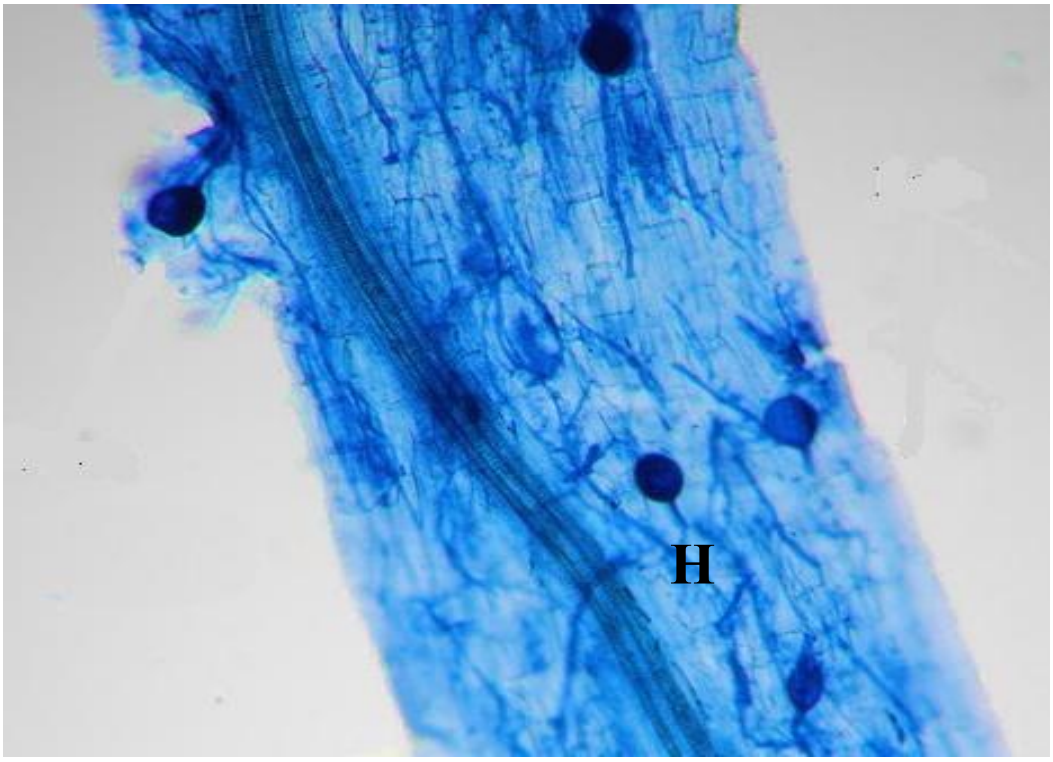


V: **Vesicle**

Figure 9 : Vesicle morphology of AMF (400x). [78].

1.2.5.3 Hyphae

The exploration of the soil by the hyphae enables the fungus to make contact with other host plants and ensure its survival. Mycorrhizal hyphae develop in the root cortex where they form intracellular arbuscules and vesicles (Figure 10) [79].

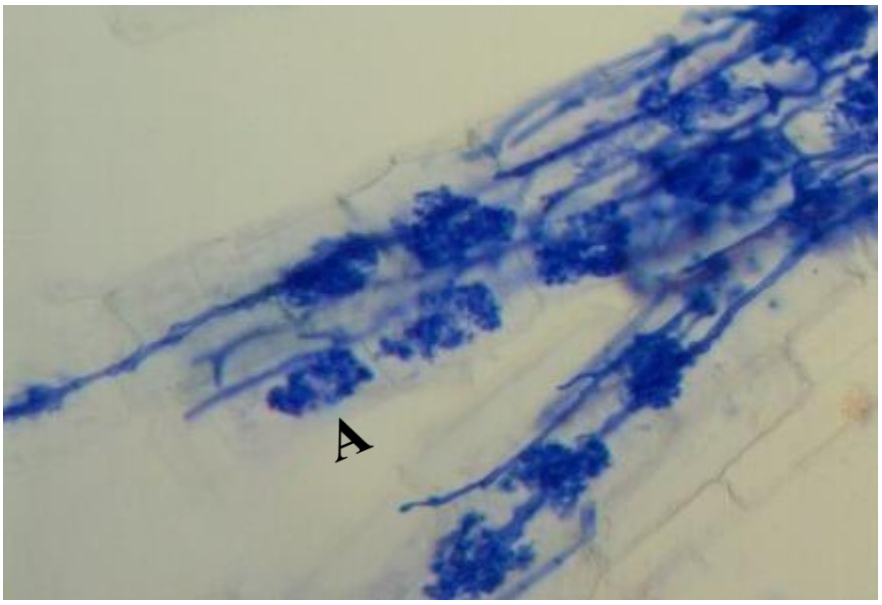


H:Hyphae

Figure 10: Morphology of hyphae of AMF (400x). [80]

1.2.5.3 Arbuscules

Arbuscules are defined as specialized hyphae representing the primary site of exchange of elements between the two partners (plant and fungus). It is a highly branched fungal structure that develops in the cortical cells of the host root (Figure 11) [81].



A : Arbuscule

Figure 11: Morphology of arbuscule of AMF(400x) [81]

1.2.6 Ecology of AMF

Arbuscular mycorrhizal fungi must evolve in an appropriate environment to establish a fully effective symbiosis [83]. The identity of the partners and the environmental conditions (biotic and abiotic) in which the mycorrhizal interaction takes place are of significant importance [84, 85]. These factors are:

1.2.6.1 The plant

AMF species are obligate biotrophs, requiring a host plant for nutrition and to complete their life cycle [86].

1.2.6.2 Soil

Arbuscular mycorrhizae represent a defining feature of soils where phosphorus represents the primary nutrient limiting plant growth. This observation has been made in tropical and subtropical regions [87]. Moreover, evidence suggests that the formation of mycorrhizae is inversely correlated with soil fertility. Mycorrhizae are typically found in soils that are deficient in one or more minerals [88].

1.2.6.3 pH

The mycorrhizal association is most likely to develop in acid soils. The optimal pH varies according to the different species of fungi, with abundance dependent on soil moisture. In a study conducted by [89], the relationship between rhizosphere soils and AMF in two mangrove plants (*Avicennia germinans* and C*onocarpus erectus*) was investigated. The findings revealed that matrix pH was one of the primary edaphic variables influencing changes in mangrove rhizosphere AMF. Additionally, pH was observed to have a direct negative impact on AMF species richness. Secondly, an increase in soil pH was found to result in a reduction in the rate of AMF colonization of plant roots and the number of spores produced.

1.2.6.4 Light

The plant can provide up to 20% of its photosynthetic product to the fungus in the form of carbohydrates [86]. A reduction in photosynthesis due to lack of light can indirectly affect mycorrhizal colonisation, spore production and hyphal network elongation [83].

1.2.7 Importance and modes of action

The prevalence of mycorrhizal symbiosis in the plant kingdom is indicative of its ecological significance, this symbiotic relationship confers numerous benefits upon its mycorrhizal partners:

1.2.7.1 growth and nutrition

The primary impact of mycorrhizal fungi in ecosystems is to enhance the growth of mycorrhized plants [90]. The increase in plant biomass is linked to the provision of limiting nutrients by the fungus (Figure 12). The mycelium of AM fungi enables the supplementation of plant nutrition with limiting elements, such as calcium (Ca), magnesium (Mg) and potassium (K), which are deficient. It has been demonstrated that the extraradicular hyphae of the fungus are capable of taking up and transporting these elements to the roots, particularly phosphorus [91, 92, 93, 94].

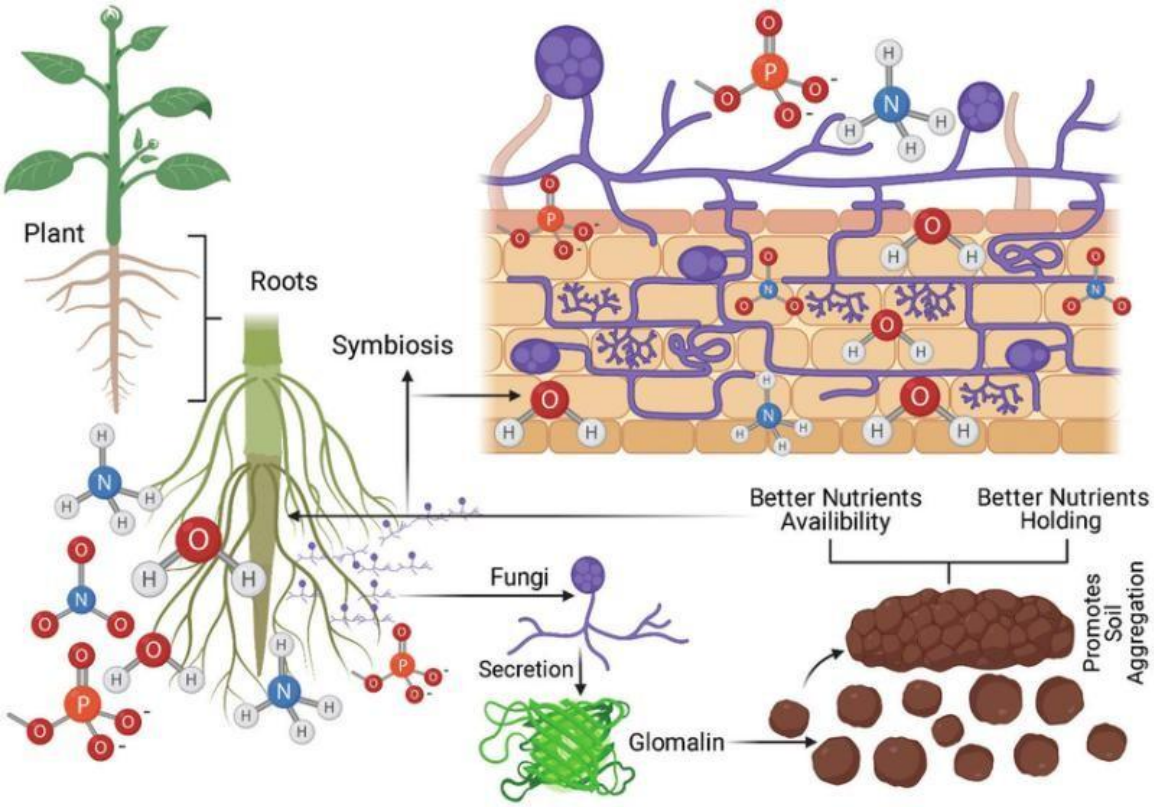


Figure 12 : Regulation of nutrients and water by arbuscular mycorrhizal fungi AMF [95]

1.2.7.2 soil structure effect

Fungal hyphae have been demonstrated to exert a beneficial influence on the macro-aggregation of soil constituents, thereby enhancing soil stability [96]. The maintenance of

soil stability is of great consequence in the context of combating erosion and the concomitant loss of nutrients and organic matter through leaching, which ultimately results in a decline in agricultural productivity [97]. Mycelia excrete a glycoprotein, designated glomalin. Glomalin functions as a glue, binding together the finest soil particles to form aggregates. These aggregates play a fundamental role in soil fertility, retaining water and mineral elements, promoting gas exchange and aeration [98].

1.2.7.3 Abiotic and biotic stress resistance

The presence of arbuscular mycorrhizal (AM) fungi in plants has been demonstrated to alleviate the effects of stress by enhancing the plant's intrinsic defensive capabilities [99]. The response of mycorrhized plants to drought conditions is reflected in higher rates of transpiration and photosynthesis, which in turn result in superior growth compared to non-mycorrhized plants [99]. The mechanisms by which mycorrhized plants are protected from water stress are thought to be linked to enhanced phosphate nutrition, which improves photosynthesis and increases biomass, as well as improved access to soil water and maintenance of water balance within the plant [100].

The experimental evidence indicates that plants inoculated with arbuscular mycorrhizal fungi demonstrate enhanced resistance to pathogenic fungal attack and exposure to soil toxins [94, 101]. At the scale of the mycorrhizosphere, microorganisms are subject to competition and antagonism. The propagules of pathogenic fungi are unable to proliferate and remain at relatively low numbers. The second mechanism allows mycorrhized plants to demonstrate elevated resistance to disease. In response to pathogen attack, plants produce antibiotic compounds that impede the growth of these organisms [98].

1.2.7.4 Soil remediation

AMF functions as a soil reclamation and biocontrol agent for contaminated or polluted soil [102]. It has been demonstrated to enhance plant growth in the presence of toxic trace elements (TEs), including Zn. The regulatory role of AMF in heavy metal transport is contingent upon the concentration of the elements in question. The direction of transport in plants is influenced by AMF in a manner that varies in response to TEs. AMF has been observed to increase the absorption of Zn through growth dilution and Cd through biological enrichment. Furthermore, the interaction between Cd and Zn is subject to modulation by AMF and other components [103].

1.3 General information on *Trichoderma* species

Trichoderma is a genus of filamentous fungi that are widely found in soil and plant root environments. they play a crucial role in agriculture.
Understanding the biology and ecological functions of *Trichoderma* is essential for developing bio-based solutions to improve crop yield and resilience.

1.3.1 Historical background

The genus *Trichoderma* (teleomorph: *Hypocrea*) is classified within the subphylum Ascomycetes, class Sordariomycetes, order Hypocreales, and family Hypocreaceae [104]. The term *Trichoderma* was first used by the South African mycologist Christiaan Hendrik Persoon in 1794. He was the first to describe the genus *Trichoderma* on the basis of samples collected in Germany. In 1825, Elias Fries initially delineated the ideal form, designated *Hypocrea*, as a species characterized by the presence of hyaline ascospores [105]. In 1865, the Tulasne brothers suggested that *Hypocrea rufa* is the teleomorph of *Trichoderma viride* Pers. Brefeld [106] was able to obtain a culture of *T. viride* from a single ascospore of *H. rufa* [107].
From 1939 to 1969, any fungal species with green spores belonging to the genus *Trichoderma* was considered to be the only species: *Trichoderma viride* [108]. This single-species system was very popular, and no special skills were needed to arrive at a new identification. The "single species" approach to systematics proposed by Bisby prompted John Webster and his student Mein Rifai to undertake a revision of the taxonomy of *Trichoderma* and *Hypocrea* [109, 110]. This effort reached its culmination with the publication of his thesis, a groundbreaking monograph on the *Trichoderma* species that employs the concept of "aggregated species," based on anamorphic microscopic characteristics. An aggregated species is defined as an entity comprising groups of species that are morphologically and genetically similar, and which are therefore difficult to distinguish from one another. Rifai established nine aggregated species of *Trichoderma*, including *T. aureoviride* Rifai, *T. hamatum* (Bonord.) Bain, *T. harzianum* Rifai, *T. koningii* Oudem, and *T. longibrachiatum* Rifai. Additionally, the following species were identified: *T. piluliferum* Rifai, *T. polysporum* (Link: Fr.) Rifai, *T. pseudokoningii* Rifai, and *T. viride* [111]. This system is arguably the most readily accessible to the scientific community.
Subsequent revisions of Rifai's aggregate species were conducted by Bisset [112, 113, 114, 115] and Doi et al. [116]. In 1991, Bissett highlighted the challenge of distinguishing between the aggregate species of Rifai, noting that only five of these species had been

narrowly defined (*T. harzianum, T. reesei, T. pseudokoningii, T. piluliferum*, and *T. polysporum*). As a solution to this problem and to cope with the growing number of new *Trichoderma* species, Bissett adopted the notion of a "section," which has no connection with aggregated species [117].

Bissett enhanced the classification system for *Trichoderma* species through the implementation of more comprehensive morphological investigations of anamorphs. He replaced the nine aggregated *Trichoderma* species with five sections (*Trichoderma, Pachybasium, Hypocreanum, Longibrachiatum*, and *Saturnisporum*), comprising 27 species [118]. In addition to morphology, a variety of other taxonomic methods have been employed, with particular focus on the analysis of secondary metabolites. This approach has revealed a striking diversity within this genus [119]. Furthermore, physiological characteristics may also prove to be a useful system for identification purposes. Isoenzyme profiles have been demonstrated to be an effective taxonomic tool [120,121]. Molecular methods, based on DNA sequence polymorphism, have enabled the finest taxonomic entities to be resolved, and above all, the discovery of many new species of *Trichoderma*/*Hypocrea*. The molecular identification work conducted up to 2000, including the identification of *T. reesei* as the anamorph of *H. jecorina* (the most industrially important species) and the revision of the *Longibrachiatum* section, was primarily based on the sequencing of the internal transcribed spacer (*ITS*) region of ribosomal DNA [122, 123].

In 1998, Kindermann et al. undertook the initial attempt at constructing a phylogeny of the genus *Trichoderma*, utilizing sequences from the internal transcribed spacer (*ITS*) region. He divided the *Pachybasium* section into two distinct phylogenetic groups, designated as "A" and "B." This classification has been subsequently adopted and corroborated in other studies [124, 125].

Lieckfeldt et al. [126] were able to identify the new species *T. asperellum* through the application of morphological and phylogenetic (*ITS* region) identification methods. The development of new methods and techniques for the phylogenetic analysis of sequences has made it possible to identify numerous *Trichoderma* species on the basis of multiple genes. Kullnig-Gradinger et al. [124] developed the first "multi-gene" phylogenetic classification of *Trichoderma*, based on the *ITS1/ITS2* region, the *endochitinase 42* gene (*ECH42*), and the *translation elongation factor* gene (*tef1*). The study was conducted on 47 distinct species of *Trichoderma*. Samuels et al. [136] used the *ITS* region of the rDNA and the *tef1* gene to study the systematics of two closely related species, *T. harzianum* and *T. aggressivum* Samuels and W. Gams.

In 2005, *Hypocrea*/*Trichoderma* was estimated to comprise 88 distinct species, of which 14 were identified as holomorphs, 49 as teleomorphs, and 25 as anamorphs [127]. By 2006, the genus had already been shown to comprise more than 100 species that could be defined on the basis of their phylogenetic relationships [118].

In the same year, the International Subcommission on *Trichoderma*/*Hypocrea* Taxonomy published a list of 104 *Trichoderma*/*Hypocrea* species on its website (http://www.isth.info/) [128].

The number of newly described species has doubled following the development of molecular identification programs for *Trichoderma*/*Hypocrea* at http://www.isth.info/: TrichOKEY, TrichoBLAST, and TrichoCHIT, based on DNA oligonucleotide barcodes [127, 129, 130]. TrichOKEY is a program used to identify *Trichoderma*/*Hypocrea* on the basis of several genus-specific characteristics located in the *ITS1* and *ITS2* sequences [127].

TrichoBLAST is a database supported by sequence diagnostics and similarity search tools based on these frequently used phylogenetic markers, including *ITS1* and *ITS2*, and the introns *tef1-int4* and *tef1-int5* [130].

Jaklitsch [112] included 135 different species names in his phylogenetic tree based on the combined analysis of the *tef1* and *rpb2* genes. Bisset et al. [132] published a list of 256 accepted *Trichoderma* species names against which they should be protected.

1.3.2 Morphological characteristics

Since the revision of the genus *Trichoderma* by Rifai in 1969, researchers have employed morphological characteristics to distinguish and characterize various *Trichoderma* species [133].Furthermore, Samuels *et a*l. [134] have provided comprehensive observations on the morphological characters of defined *Trichoderma* species. The genus *Trichoderma Pers*. is distinguished by its rapid and extensive growth rate, accompanied by a profusion of sporulation on the culture medium [135].The majority of *Trichoderma* cultures exhibit rapid growth at temperatures between 25°C and 30°C, with no growth observed at 35°C [134]. Nevertheless, it has been observed that certain species exhibit optimal growth at temperatures of 35°C, thus constituting an essential criterion for distinguishing between morphologically similar species. For instance, *T. harzianum* can be distinguished from morphologically similar species, including *T. aggressivum* and *T. atroviride,* by subjecting them to growth conditions of 35°C. Following a 96-hour incubation period at 35°C, the diameter of the colonies of *T.*

aggressivum and *T. atroviride* did not exceed 5 mm, whereas that of *T. harzianum* demonstrated robust growth and sporulation [136].

The genus *Trichoderma* can be readily identified in culture due to the green coloration of its spores [137]. The conidia of *Trichoderma* give rise to a mycelium that is initially white in color. Two days later, the aerial parts of the mycelium exhibit a green coloration, which corresponds to the onset of conidiogenesis [137].

Conidia typically form within a week in compact or slightly flaky clumps of greenish, whitish, and occasionally yellowish coloration. The pigmentation of the phialides dictates the color of the colonies. Mycelial development and pigmentation characteristics can be more readily observed in a rich medium such as PDA (Potato Dextrose Agar) [134].

Some *Trichoderma* species exhibit yellow pigmentation on PDA medium, while others, such as *T. viride*, produce a distinctive odorant reminiscent of coconut [133, 138].

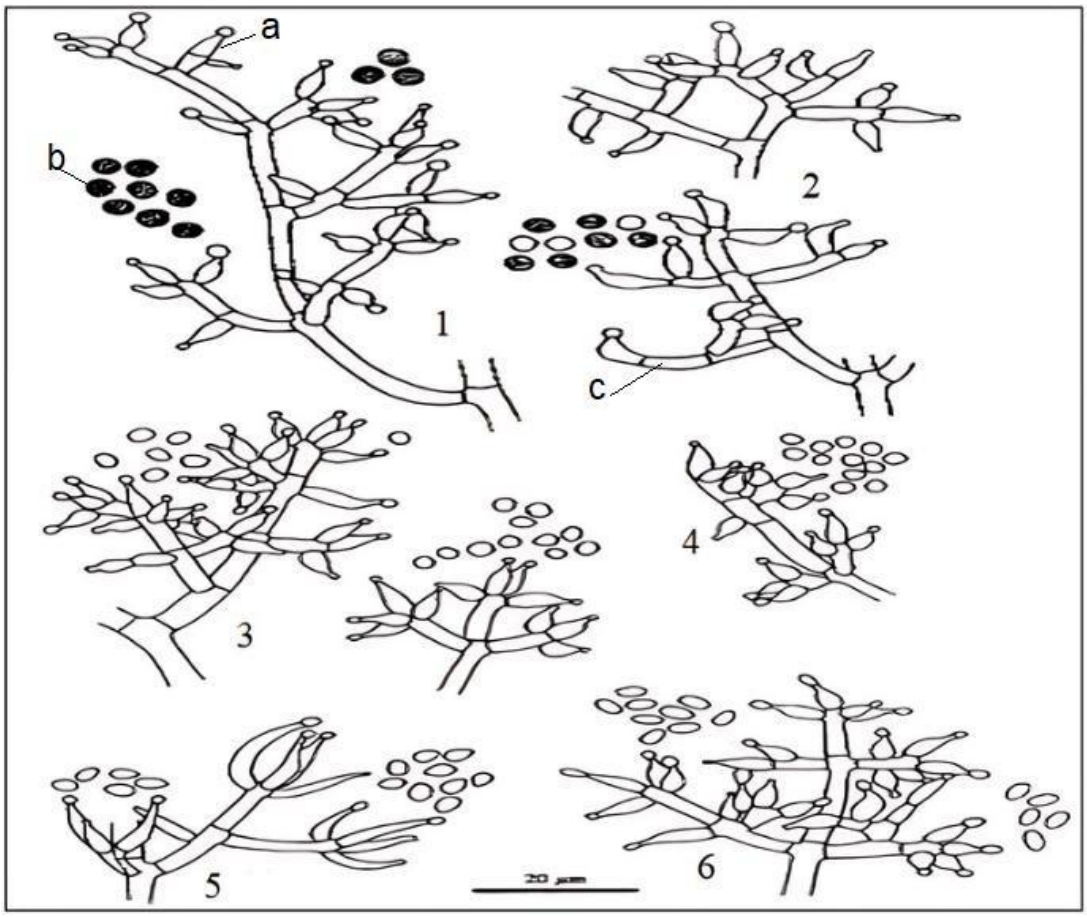
Microscopically, conidia are typically observed to be hyaline, ellipsoid, and smooth in most species. Globular conidia are less prevalent. These conidia frequently gather in clusters at the tip of the phialides, forming what are known as "false heads."The mycelium is composed of smooth-walled, branched, and septate hyphae.

The conidiophores are characterized by a strong branching pattern in a pyramidal structure, with one or more phialides at the terminal end.

These phialides are typically flask- or keel-shaped (Figure 13).

The phialides are attached at right angles to the conidiophores, which in turn bear conidia. Some species are capable of producing globose chlamydospores, which are either intercalary or terminal. These chlamydospores are typically unicellular, although they can be multicellular in certain species, such as *Trichoderma stromaticum* [107, 138, 139].

The optimal Hypocrea form is characterized by a fleshy, rounded stroma of varying hue (pale to highly colored), equipped with perithecia within which are cylindrical asci with a predominantly fine apex (Figure 13). Each ascus contains eight spherical, disarticulated spores.



1:Trichoderma viride, 2: *Trichoderma atroviride,* 3: *T harzianum,* 4: *T. inhamatum,* 5: *T. aureoviride,* 6: *T. koningii,* a: conidia, b: conidiophores c: phialides

Figure 13: Morphological description of a, b and c of *Trichoderma* spp. [140]

1.3.3 Ecology

The field of ecology is concerned with the study of the interactions between organisms and their environment. The genus *Trichoderma* is noteworthy for its remarkable capacity to adapt to diverse climatic conditions, which has facilitated its extensive distribution in both terrestrial and marine ecosystems [142]. *Trichoderma* species are notable for their capacity to utilize a range of substrates, which has resulted in their becoming a predominant component of terrestrial and marine microflora [142].

In marine environments, *Trichoderma* can be considered facultative marine organisms. In a study of marine microfungi from the depths of the North Sea and North Atlantic [143], *Trichoderma* were identified at all levels. Moreover, they have been isolated from marine algae. Rhodophyta sp. and *Phaeophyta* sp. have been collected from the Iberian Atlantic and Mediterranean coasts, as well as from mussels in Canada [144].

Trichoderma can grow at temperatures ranging from 15 to 35°C. However, the optimal temperature varies depending on the species under consideration. Furthermore, Dommergues and Mangenot classified *Trichoderma* as an indifferent microorganism,

exhibiting the capacity to proliferate across a broad pH range [140]. The growth of these organisms is dependent on the availability of carbon and nitrogen in the surrounding environment. *Trichoderma* are typical inhabitants of soil, with their prevalence contingent upon a number of factors, including the quantity of organic matter present. Furthermore, their cellulolytic activity enables them to penetrate dead wood [140].

1.3.4 Biotechmological potential

The genus *Trichoderma* has emerged as a key biotechnological resource in modern agriculture due to its diverse applications in plant growth promotion, biocontrol,

1.3.4.1 plant growth promoter agent

According to Hyakumachi and Kubota [145] fungi (PGPF) plant growth-promoting fungi as a microorganism that can stimulate plant growth. They affect crop growth, yield and productivity. Recent research shows that *Trichoderma spp*. can be an effective PGPF. It enhances plant health by establishing an optimal environment and producing secondary metabolites.Plant growth is affected by many factors, such as temperature, light, nutrients and microbes. The rhizosphere is the specific zone of soil surrounding plant roots that is characterised by an elevated concentration of nutrients, largely due to the substantial quantity of photosynthetic by-products released from the roots [146]

In a study published in 2004, Hyakumachi and Kubota proposed that *Trichoderma* could serve as an exemplary plant-promoting fungus. It is evident that plants are capable of discerning and responding to the microbial communities present in their rhizospheres, which encompass a multitude of chemical compounds. To date, unfortunately, the discovery of synergistic mechanisms as well as the secondary metabolites and plant signals is still an open question [145]

1.3.4.2 biocontrol agent of plant disease

The term 'biocontrol' is employed to describe the process of reducing the population of pests through the utilization of living organisms. This approach is environmentally friendly [146]. The most commonly employed biological control agent against pathogens is the fungus species *Trichoderma.* Siemering *et al*. [147] reported that roots provide the primary habitat for the fungus, particularly at root surfaces and beneath the outermost layer of root cells.

In order to facilitate the establishment of the fungus in and on plant roots, it is recommended that *Trichoderma* be used during the seeding process. The application of a

seed treatment has been shown to be an efficacious method for the establishment of *Trichoderma* within the roots of the plant, thereby conferring benefits to the plant itself. The genus *Trichoderma* has been observed to attack pathogens via a variety of mechanisms, including antibiosis, competition, parasitism and induced resistance [147].

In the present study, our focus was directed towards a specific species of *Trichoderma: T. asperellum Samuels, Liechf. & Nirenberg.*

1.3.5 *Background on T. asperellum Samuels , Liechf. & Nirenberg*

The diameter of the colonies increased radially from 7 to 64 mm over a period of 72 hours following the completion of mycelial growth in the dark on PDA medium at a temperature of 30°C. The mycelium was observed to be aerial, with no evidence of yellow pigment diffusing into the medium and no odor emanating from the culture. Sporulation was noted to be dense and organized in the form of five concentric rings, with dark green conidia observed towards the center and others that were just beginning to form towards the margin [141].

Pustule formation is prevalent throughout the colony on CMD and SNA media after five days at 20°C, with alternating periods of darkness and white light. The conidia are either discrete or confluent, initially exhibiting a yellow coloration that rapidly transitions to green. The diameter of these structures ranges from 0.5 to 2.0 mm [141]. Conidiophores are produced in pustules on CMD medium, though they are less frequently observed in the aerial mycelium. They are symmetrical in appearance and terminate in two or more phialidia. The primary branches that emerge from the base of the structure are frequently paired and oriented at an angle of approximately 90° relative to the main axis. The width of these structures ranges from 1.7 to 7.0 µm [142].

Phialides are typically produced at the apices of primary, secondary, and tertiary branches, though they may also emerge directly along the length of the branches on rare occasions. They are typically arranged in whorls of two to four, exhibiting a straight morphology, ampullate shape, and only slight enlargement at the center (4.6-27.5µm) x (2.0-6.8µm) in size. The conidia are dark green in color and globose to subglobose in shape, with a fine spinose ornamentation that may be difficult to discern with a light microscope. Their dimensions are (2.8-7.0) x (2.5-6.0) µm (141).

The presence of chlamydospores on CMD medium was observed over the course of seven days at 20°C and in the absence of light. They are terminal or, on rare occasions, intercalary, and are found on immersed 51 hyphae. They are subglobose to ovoid in shape,

smooth, pale green in color, and measure between 4.5 and 15 micrometers in diameter [141].

Trichoderma asperellum (Samuels, Liechfeldt, and Nirenberg) has been demonstrated to have the most significant biological control activity, both in vitro and in vivo, and has exhibited a substantial impact on plant development. Studies have demonstrated its efficacy in managing diseases such as *Fusarium* wilt in crops like bananas [148]

1.4 Plant growth-promoting rhizobacteria PGPR

Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance growth through multiple mechanisms,play a key role in sustainable agriculture, offering an eco-friendly alternative to chemical fertilizers and pesticides.

1.4.1 Historical backround

Plant growth-promoting rhizobacteria (PGPR) are defined as bacteria that inhabit the rhizosphere, a narrow region of soil that is influenced by root exudates. During the past century, numerous species and strains of PGPR have been identified which significantly contribute to enhanced plant growth [149]. However, despite belonging to the same species, different strains can demonstrate markedly different effects with regard to plant growth and their capacity to produce specific compounds may vary considerably [150]. These terms were initially defined by Klopper and Schrothwhile research on rhizobia dates to the 1890ties [151]. Due to their efficacy in stimulating plant growth and controlling plant disease, these strains are regarded as environmentally friendly alternatives to chemical fertilizers and pesticides. A diverse range of bacterial species, including *Bacillus*, *Burkholderia, Azospirillum*, *Azotobacter*, *Rhizobium*, and *Pseudomonas*, have been identified as plant-growth-promoting rhizobacteria PGPR. Among these, *Bacillus*, *Rhizobium*, and *Pseudomonas* are the most extensively studied and well-characterised species [149]. PGPR have been applied to a diverse range of plants, including chickpea, maize, pea, peanut, rice, soybean, sugarcane, wheat, and sugar beet [149,152].

1.4.2 Taxonomic diversity of PGPR

The number of PGPRs identified has increased significantly, primarily due to the growing recognition of the rhizosphere as an integral ecosystem within the broader functioning of the biosphere. Furthermore, the mechanisms of action of PGPRs have been

sufficiently studied, providing a deeper understanding of their role in the broader ecosystem. These cultivable microorganisms, which exhibit a high degree of diversity at the genus and species levels, can be classified into four main phyla, as follows: *Proteobacteria*, *Firmicutes*, and *Actinobacteria* [152;153].

1.4.2.1 *Proteobacteria*

The Proteobacteria phylum is comprised of three distinct classes.

Alphaproteobacteria

The *Alphaproteobacteria* can proliferate in low-nutrient conditions. PGPRs in this class include *Rhizobia*, which fix nitrogen and produce nodules in the root system. These bacteria form relationships with the roots of leguminous plants, and some of them are now classed as distinct genera. For instance, *Bradyrhizobium, Sinorhizobium,* and *Mesorhizobium*. The *Gluconacetobacter* genus, which belongs to the *Acetobacteraceae* family, comprises obligate endophytic bacteria that colonize the roots, stems, and leaves of sugar cane [154]. The *Azospirillum* genus, which is classified within the *Rhodospirillaceae* family, is regarded as a plant growth-promoting species. The strains belonging to this genus are found in the soil in the form of free cells or associated with the roots, stems, leaves, and seeds of cereals and grasses. The free cells are found in the soil or associated with the roots, stems, leaves, and seeds of mainly cereals and forage grasses [155].

Betaproteobacteria

This class, which includes the *Burkholderiaceae* family or the *Burkholderia* genus, constitutes a monophyletic group comprising diverse species with disparate physiological and ecological attributes. They are isolated from soils and plants. Some strains have the capacity to fix nitrogen asymbiotically. The genus *Ralstonia* is also included in the family *Burkholderiaceae.* The aforementioned genera are ubiquitous [156].

Gammaproteobacteria

This is the most numerous class of bacteria, comprising microorganisms with a wide range of physiological characteristics. The *Pseudomonaceae* family encompasses the genus *Azotobacter*, which comprises bacteria that facilitate plant growth due to their capacity to fix nitrogen and lack of production of nodules [157]. This family also includes the genus *Pseudomonas*. This is one of the Gram-negative microorganisms most present and abundant in the *rhizosphere.* The PGPR activity of certain species belonging to this

genus has been known for many years.This activity has been the subject of extensive research, and the resulting phenomenon can be attributed to a complex cascade of mechanisms. The bacterial genera that perform the PGPR function and are included in the *Enterobacteriaceae* family are *Citrobacter*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Kluyvera*, *Pantoea* and *Serratia* [158].

1.4.2.2. *Firmicutes*

The most prevalent genus within this phylum is Bacillus. Approximately 95% of isolated flora can be attributed to this genus. They are Gram-positive bacilli, isolated or in chains, capable of sporulation and generally mobile (some variants are immobile, e.g. *Bacillus anthracis*) [159].

These bacteria are facultative aerobic or aeroanerobic and are capable of forming endospores. Since its discovery in 1913, the presence of a spore has been employed as a principal criterion for classification. The Bacillus genus can be distinguished from other spore-forming bacilli by several characteristics. These include the nature of the respiratory type, whether it is strictly aerobic or facultative, the bacillary form and the production of catalase [159].

1.4.2.3 *Actinobacteria*

The Frankia genus, which belongs to the *Actinobacteria* phylum, is a symbiotic nitrogen-fixing microorganism. This characteristic ability is exclusive to the genus.These bacteria are associated with *actinorhizal* plants, which are responsible for the initial colonization of poor or degraded soils. Other *Actinobacteria* have been observed to promote plant growth, although they do not engage in the aforementioned symbiosis [160, 161, 162]

The aforementioned organisms are classified within the following genera: *Arthrobacter, Micrococcus* [160], Curtobacterium [161] and Streptomyces [162].

1.4.3. Biotechnological potential of PGPR

Plant Growth-Promoting Rhizobacteria (PGPR) hold significant biotechnological potential in sustainable agriculture, environmental and remediation applications. Their benefits stem from their ability to enhance plant growth, improve soil fertility, and protect against pathogens through multiple mechanisms [163,164]:

1.4.3.1 Mechanisms of action

PGPR exert their beneficial effects through multiple mechanisms, including [165,166]:

Nutrient acquisition

PGPR has been demonstrated to enhance the uptake of plant nutrients directly through modifications to root architecture and the solubilization of nutritionally unavailable forms Phosphorus (P) is essential element for growing plants. Although P is highly abundant in the soil, the available form, i.e. soluble P is limited [163,167].

Some PGPR strains, including *Azotobacter*, *Bacillus*, *Burkholderia*, and *Pseudomonas*, can produce organic acids and enzymes, including phosphatase and phytase, as demonstrated by Kumar *et al* [164168]. They render organic and inorganic forms of phosphorus available to plants [169, 170, 171]

Nitrogen (N) is essential for plant growth. Despite making up 78% of the air, N2 is not directly available for plants. Biological nitrogen fixation is essential for converting nitrogen dioxide into a form that plants can use. Nitrogenases, which are found in N2-fixing microbes, are the catalysts for this process. The most well-known nitrogen-fixing bacteria are *Rhizobium*, *Bradyrhizobium*, and *Azosprillum* [172]. They form nodules with legume plants such as soybean, pea, peanut, and alfalfa, and are defined as symbiotic nitrogen-fixing bacteria [172]. Another type of nitrogen-fixing bacteria, such as *Klebsiella pneumoniae*, *Paenibacillus polymyxa, Paenibacillus* massiliensis, *Bacillus megaterium*, and *Bacillus marisflavi*, are free-living diazotrophs that convert N2 into ammonia.

Phytohormone production

Phytohormones are compounds that regulate plant physiological processes. They are produced endogenously within plants to serve various important functions [173]. It is a well-established fact that PGPR can produce phytohormones. One of the most important phytohormones produced by microorganisms is the hormone auxin (indole-3acetic acid/indole acetic acid/IAA) [173].

It is thought to play a role in regulating important cellular processes, including plant cell proliferation, root development and photosynthesis. It has been suggested that IAA production may occur in PGPR, including *Rhizobium*, *Pseudomonas* and *Bacillus spp.* There is a possibility that its production is linked to the amino acid tryptophan, which is often found in root exudates [175].

It has also been suggested that other phytohormones, such as gibberellins and cytokinins, may be produced by PGPR and play an important role in regulating plant growth. There has been considerable research into the microbial production of IAA (an auxin compound) with a view to understanding its potential to modulate plant physiological processes and influence plant-microbe interactions [176]. It appears that IAA may play a role in modifying plant cell metabolism, which could potentially promote growth, regulate root morphology and lateral root formation, and be involved in various other processes, including responses to environmental stress and vascular development [177].

Pathogen resistance

In the context of plant-microbe interactions, the introduction of biological or chemical compounds can result in the development of resistance in non-exposed plant parts against pathogenic microorganisms. This phenomenon is referred to as induced systemic resistance (ISR). It has been observed that certain PGPR indirectly promote plant growth by enhancing plant survival when threatened by deleterious pathogenic microbes [170]. PGPR achieve this indirectly by priming the plant for pathogen attack and stimulating plant defence pathways.

The species *Pseudomonas* and *Bacillus* have been extensively documented as inducers of induced systemic resistance (ISR). It has been demonstrated that the ISR can be induced by cell envelope components, iron-regulated metabolites and antibiotics. Moreover, it has been demonstrated that some volatile organic compounds (VOCs) produced by microorganisms can also elicit induced systemic resistance (ISR). The signal transduction of induced systemic resistance (ISR) is contingent upon the presence of the hormone ethylene [177].

Chapter 2 :

Material Method

2. Material and method

The present study aims to investigate the impact of the application of endemic-microorganisms on the yield and quality of garlic cultivation, with a view to enhancing its potential applications in a range of fields, including agriculture and pharmacology on an industrial scale, while promoting sustainable cultivation techniques. In order to achieve this objective, the methodology is comprised of four principal elements.

- **Impact of application of endemic microorganisms on the growth and chemical composition of garlic**

The aim of this study is to gain a deeper understanding of the interaction between endemic microorganisms and garlic plants, with the aim of selecting the most efficacious isolate for the promotion of plant growth and the promotion of chemical compounds. which could be a useful alternative to the excessive and unselective utilization of chemical fertilizers in agricultural contexts. To achieve this objective, two isolates of arbuscular mycorhizal fungi, three isolates of *Trichoderma asperellum* and one isolate of PGPR were studied separately on garlic cultivation. The cultivation experiments were carried out separately in the greenhouse of the Department of Biotechnology and Agroecology of the University of Blida1.

- **Study of some biological activities of garlic cultivated under the effect of application of endemic microorganisms including, antioxidant, antimicrobial and herbicidal activity**

- **Study the stability of the antimicrobial activity of garlic cultivated under the influence of endemic microorganisms**

- **formulation of an antimicrobial from the garlic preparations previously investigated**

The laboratory work was conducted at three different locations. Laboratory of Research on Aromatic and Medicinal Plants at the University of Blida 1, Algeria, Laboratory of Micro-propagation at the Department of Soil, Plant and Food Science at the University of Bari Aldo Moro, Italy. And Laboratory of Crop Nutrition at the Department of Sustainable Agriculture at Rakuno Gakuen University, Japan. The study was conducted between November 2021 and August 2024.

2.1 Biological material

The study required the use of plant and endemic microbial materials.

2.1.1 Plant material

The plant material was represented by garlic cloves (*Allium sativum* L.) of the pink variety, obtained from a local market in Algeria.

2.1.2 Endemic microbial material

The endemic microbial material is represented by fungal and bacterial materials all sourced from the mycothèque and bactériothèque obtained from Dr Mesgo-Moumene S. Laboratory of Research on Medicinal and Aromatic Plants at Blida 1 University in Algeria. "These microorganisms were the subject of a university's research and training project (D00L05UN09012210001). The fungal material consisted of two isolates of endemic arbuscular mycorrhizal fungi and three strains of *Trichoderma asperellum*.

2.1.2.1 Arbuscular mycorrhizal fungi isolate

The isolate MO was obtained from *Allium triquetrum* plants, while the isolate MA was obtained from Mentha plants.

2.1.2.2 *Trichoderma asperllum*

This particular inoculant has been the subject of numerous research studies conducted by Dr. Mesgo-Moumene S. The strains TMSKOLDZ20 (T1), TMS11DZ15 (T2) and TMS5DZ15 (T3) were reactivated and developed on potato dextrose agar (PDA) medium (appendix 1) and incubated at 28°C.

2.1.2.3 Plant Growth promoting Rhizobacteria

The PGPR isolate is derived from the collection of rhizobacteria that have been shown to be highly effective in the context of the MSc thesis.

2.2 Installation of garlic cultivation

The installation of the crop is comprised of three distinct stages.

2.2.1. Setting up the culture

The experiment was conducted in the following manner:

2.2.1.1 Substrate preparation

The substrate for the cultivation trial was prepared from a mixture of two-thirds soil and one-third commercial moss peat. The soil was obtained from an uncultivated and pesticide-free fallow plot located within the Biotechnology Department at USDB1. Subsequently, the soil was sieved through a 3 mm diameter pore sieve in order to eliminate any large particles. For AMF inoculation experiment conducted in sterilized soil, the sterilization process was conducted at 100°C, with the operation repeated three times for one hour, with a 24-hour interval between each operation.

2.2.1.2 Preparation of conidial suspensions from *Trichoderma* spp. isolates

Conidial suspensions were prepared separately from each of the three *Trichoderma asperellum* strain culture by scraping the surface of the immersed cultures with sterile distilled water. Subsequently, the suspensions were transferred to sterile test tubes and vortexed in order to facilitate better homogenization. The sporulation rate was determined for each conidial suspension prepared using the Malassez cell under an optical microscope at 400x magnification. All concentrations were subsequently adjusted to 3x107[7] conidia/mL using sterile distilled water according to Caron *et al.* [178].

2.2.1.3 Preparation of bacterial suspensions from PGPR isolate

The preserved PGPR strains were revived by subculturing on agar media for 24 hours (King's B medium for *Pseudomonas* at 30°C). Bacterial suspensions were then prepared by culturing the PGPR strains in liquid Mueller-Hinton (MH) medium for 24 hours at 30°C. The cultures were subsequently standardized to a microbial concentration of approximately 1×10^8 CFU/mL (OD 0.45 at 610 nm) using a spectrophotometer, following the method described by Govindappa *et al*. [179]."

2.2.1.4 Preparation of arbuscular mycorrhizal inoculum

The inoculants were prepared by rinsing the mycorrhizal roots with water, drying them, and pulverizing them to create a powdered material.

2.2.1.5 Garlic cloves rinsing

A sufficient quantity of garlic cloves was peeled and soaked for 10 minutes in a 12° aqueous sodium hypochlorite solution, prepared at a concentration of 2%. This was followed by three rinses with abundant running water and a subsequent overnight drying period [180].

2.2.1.6 Garlic cloves germination

The garlic cloves were germinated in trays that had been previously filled with sterile peat moss. Each seed was planted in a separate tray. The trays were regularly irrigated with tap water.

2.2.2 Growing garlic in pots and greenhouses

The process of planting garlic seedlings in pots and greenhouses comprises two distinct stages

2.2.2.1 Preparation of the pots

The substrate, which had been previously prepared, was transferred into 250 g plastic pots. The young garlic clove sprouts were then carefully transferred directly into the substrate.

With regard to the AMF inoculation experiment in sterilized and unsterilized soil, 1 g of each inoculum (MO and MA) was incorporated. The mycorrhization process was conducted in close proximity to the roots of the seedlings, specifically at a depth of 5 cm and a distance of 5 cm to the side, in order to facilitate optimal results. While the inoculation experiments involving *Trichoderma* and PGPR, the clove sprouts were irrigated separately with 20 mL of conidial suspension of *Trichoderma* and 20 mL of bacterial suspension of PGPR separately with three repetitions occurring at 15-day intervals

2.2.2.2 Experimental set-up and installation of garlic plants under greenhouse

Accordingly, 12 replicates were considered for each inoculum. Similarly, 12 pots were designated as the control group, comprising young seedlings whose substrate was not inoculated. The seedlings were irrigated on a daily basis and as required, in accordance with standard practice. Following a three-month growth period, the young plants cultivated in pots were transferred to plastic pots with a 3 kg capacity and irrigated with regular applications of tap water.

Subsequently, the pots were placed in a plastic greenhouse under conditions of total randomization.

The cultivation plants were monitored for a period of six months in order to assess the selected study parameters.

2.3 Studied parameters

A variety of parameters have been evaluated. The efficacy of the treatments was assessed based on the specific treatment modality, and the following elements were taken into consideration:

2.3.1 Growth parameters

- **Plant height**

The height of the plants was determined by utilizing a ruler for measurement.

- **Fresh weight**

Subsequent to the harvesting process, the fresh weight of the bulbs and roots was quantified utilizing a balance.

2.3.2 Leaf pigment content

Chlorophyll a, chlorophyll b and carotenoid contents were determined by the method described by Lichtenthaler [181].

0.25g fresh leaf sample was ground in a porcelain mortar with 10 ml of 80 % acetone. The homogenized sample mixture was transferred to a 25 mL volumetric flask and filtered through filter paper. The resulting solution was analyzed for chlorophyll a, chlorophyll b and carotenoids by spectrophotometer at wavelengths of 470, 646 and 663 nm, respectively. The concentrations of chlorophyll a and b and carotenoids were calculated using the equations described by Lichtenthaler [81].

Chl a=12.25 A663-2.79 A646.

Chl b= 21.5 A646-5.1 A663.

Cc= (1000 A470-1.82 Ch-a-85.02 Ch-b) /198

The variables are as follows:

A: Absorbance

Chl a: Chlorophyll a

Chl b: Chlorophyll b

2.3.3 Study of arbuscular mycorrhizal colonization

To gain a deeper understanding of the response of garlic cultivated in sterilized soil to arbuscular mycorrhizal isolates inoculation, both the root colonization frequency and the extraradical hyphal length of mycorrhizae were determined in the sterilized soil, while in the unsterilized soil only the root colonization frequency was determined.

2.3.3.1 Root colonization frequency

According to the method of Phillips and Hayman [182], the roots were meticulously rinsed with tap water in order to eliminate all residual soil matter. The finest roots were cut into fragments of approximately 1 cm in length and then immersed in a 10% KOH solution for 20 minutes at 90°C in a water bath, with the objective of emptying the cells of their cytoplasmic inclusions. Subsequently, the roots were rinsed with tap water and immersed in the trypan blue staining solution (0.05%) at 50°C for 10 to 12 minutes in a water bath. Subsequently, the roots were rinsed once more with distilled water and decoloured with lactoglycerol, a solution comprising 50% glycerol and 50% lactic acid.

Subsequently, ten root fragments were positioned on a glass with a drop of glycerol, which was then covered with a cover glass. Subsequently, the excess liquid was permitted to dry, after which microscopic observation was conducted using an optical microscope at the following magnifications (X125 and X500).

The frequency of mycorrhization (F) was defined as the percentage of mycorrhized root fragments in relation to the total number of fragments observed.

F (%) = Number of mycorrhizal fragments/number of total fragments observed

2.3.3.2 Extraradical hyphae length measurement

According to Jakobsen *et al* [183], the process of measuring extraradical hyphae is comprised of two distinct stages:

- **Mycelia staining.**

A quantity of 2.0 g of soil was weighed and then transferred to a 1 L beaker. A small amount of tap water was added and the soil clods were crushed with the fingers. Approximately 300 mL of water was added and passed through a 250 µm stainless steel sieve over a 500 mL beaker. The filtrate was passed through a 53 µm sieve and the residue remaining on the sieve (containing the mycelium) was transferred to a 500 mL beaker using approximately 300 mL of water. This procedure was repeated five times.

The residue remaining on the sieve was transferred to a 100 mL beaker, which was filled with water from the wash bottle or nozzle until the 100 mL mark was reached.

The beakers were then placed in the sonicator and filled with water to the specified level. The soil particles were then suspended for five minutes, followed by a further 30 second interval. The resulting supernatant is then transferred to another 50 mL beaker, which allows rapid dosing of 50 mL, and sieved through a 53 µm sieve. The residue from the sieve is then transferred to a 100 mL tall beaker, with water from the wash bottle or nozzle added to the corners and mixed to 50 mL.

The solution in the beaker is mixed thoroughly and allowed to stand for 15 seconds. Then 2 ml (equivalent to 1 ml x 2) of the portion 1 cm below the water surface was transferred with a pipette and placed on a 0.45 µm membrane filter attached to the aspirator. The pump was then activated for the purpose of suction filtration. At the end of the aspiration process, the pump was deactivated. Next, 1 mL of a trypan blue solution (containing 0.05% trypan blue in lactic acid) was added to the filter, which was then allowed to stand for 10 minutes to allow the staining process to take place. Upon completion of the staining process, the pump was activated and 5mL of water was transferred from the wash bottle down the wall in three consecutive cycles. The aforementioned procedure was conducted with the pump in the deactivated state. Subsequently, the membrane filter was transferred to a Petri dish with the aid of tweezers and placed in a thermostatic bath set at 40°C for the purpose of drying. Once the membrane filter has been sufficiently desiccated, it is then transferred to a glass slide. Subsequently, 100 µL of 50% glycerol is added, and the slide is covered with 18 mm round cover glass. Subsequently, the cover glass is gently pressed with the back of a pair of tweezers or a similar tool to displace any air bubbles and ensure that the wrinkles on the membrane filter are elongated and flattened.

The glass slide is then placed under a microscope and five field of view images are taken with a x500 objective lens [183].

- **Measurement of the length of mycelium**

The measurement of this parameter is based on the utilization of the Image J software, the methodology of which was outlined by Baláz and Vosátka, [184]. The length of the mycelium spread over the entire filter paper was determined by measuring the image area and the effective filtration area of the membrane filter. Mycelia that were stained with trypan blue and exhibited neither septa nor a linear outline were classified as AM mycelia. Conversely, other unstained brown mycelia were designated as other mycelia. In rare cases, unstained mycelia with a thickness of around 5 to 10 µm without septa are considered as AM mycelia. Conversely, mycelia with septa were not identified as AM mycelia, but rather as belonging to a different category. The average value was calculated

as the result, based on the observation of eight filters per sample. The soil moisture were measured and calculated as mycelium length per gram of dry soil.

2.3.4 Chemical analysis of garlic powder by FTIR

The chemical groups and functions of all the compounds were identified by Fourier transform infrared [FTIR] analysis. This chemical analysis method was conducted on the garlic powder samples using the potassium bromide (KBr) pellet technique, as described by Gorgulu *et al* [185]. One milligram of garlic powder, which had previously been dried at 40°C and ground to a fine consistency, was mixed with 75 milligrams of KBr, and then subjected to high pressure in a hydraulic press at a pressure of 1100 kilograms per centimeter for eight minutes to obtain the mixture in pellet form. The sample holder, containing the KBr sample pellet, was positioned within the measurement compartment. All spectra were recorded in the infrared region (4000-400 cm-1) using the spectrometer's Opus 6.5 software (BrukerTensor 27 FT-IR). The functional groups were assigned in accordance with the infrared spectroscopy correlation table reported in the scientific literature, as referenced in the following sources: [186, 187, and 188].

2.3.5 Extraction and determination of phenolic and flavonoid compounds

The polyphenol and flavonoids present in the aqueous extract of garlic were evaluated. This extract was selected on the grounds of its potential to be safe for humans and the environment

2.3.5.1 Extract preparation

The garlic bulb sample was peeled, cleaned and ground to extract the phytochemicals. The extraction process was conducted according to the method described by Akullo et al. [189].

25 g of fresh garlic was mixed with 100 mL of distilled water; the mixture was then shaken at 300 rpm on a mechanical shaker in the dark for 24 hours. Following this, the solution was filtered using Whatman filter paper No. 1.

2.3.5.2 Determination of total phenol content

The total phenol content was determined using Folin–Ciocalteu's phenol, in accordance with the methodology proposed by Jang *et al*.[190]. 0.5 mL of diluted extract to 2g/mL was combined with 2.5 mL of 10% Folin Ciocalteu reagent, to which 2.5 mL of 7.5% sodium carbonate solution was added. The samples were incubated for a period of

30 minutes at a temperature of 25°C. The optical density was determined using a spectrophotometer at a wavelength of 760 nm, employing a blank consisting of 0.5 mL ethanol, 25 mL Folin-Ciocalteu reagent prepared at 10%, and 2.5 mL sodium carbonate prepared at 7.5%.

The concentrations of polyphenols present in the garlic bulb were calculated using the calibration curve obtained at varying concentrations of Gallic acid. The results are expressed as milligram of Gallic acid equivalent per gram (GAE/g), (appendix 2) .

2.3.5.3 Determination of flavonoids content

The total flavonoid content was determined using the method adapted by Djeridane *et al.* [191]. 0.5 mL of diluted extract to 2mL/g was combined with 1 mL of AlCl3. Subsequently, the mixture was incubated at room temperature for a period of 10 minutes. The absorbance was determined at 510 nm, with a control sample included as a reference point for comparison. The flavonoid levels were calculated using quercetin standard at concentrations spanning from 37.5 to 2.3µg/mL. The flavonoid contents are expressed in milligram of quercetin equivalent per gram (mg EQ/g), (Appendix 2).

2.3.6 Evaluation of antioxidant antimicrobial and herbicide activities

Subsequent to the extraction process, the antioxidant, antimicrobial, and herbicide activities of garlic aqueous extract were also evaluated.

2.3.6.1 DPPH free radical scavenging activity

The free radical scavenging activity of each sample was quantified using the method of Blois [192], with certain modifications. In brief, 5 ml of 0.1 mM DPPH in 95 % ethanol was added to 1 mL of diluted extract to 2mL/g of each sample. The mixture was then vigorously shaken and incubated at room temperature for 30 minutes in the dark. Following this, the absorbance was measured at 517 nanometres. Ascorbic acid was used as standard antioxidant.

The result was expressed as the percentage of DPPH radical inhibition (I%). The percentage of DPPH radical inhibition (I%) is calculated using the following formula:

I (%) = (blank OD - sample OD) × 100/blank OD

Where I (%): DPPH radical inhibition (%).

Blank OD: optical density of the blank;

OD sample: Optical density of the sample.

The antioxidant activity was evaluated in relation to ascorbic acid, used as a standard, under the same conditions as the samples. The values of the concentration that inhibited 50 % of the DPPH radicals (IC50) were expressed in (mg/mL).

2.3.6.2 Evaluation of antimicrobial activity

The antimicrobial potential of garlic extract was evaluated against seven microbial strains (five bacteria and two yeasts), provided by DCQ-SAIDAL Medea (Table 5). The tests were conducted using the disc diffusion method, whereby the inhibition diameter around a disc impregnated with the extract was measured [193].

Sterile paper discs with a diameter of 6 mm were impregnated with garlic extract at varying concentrations (100%, 75%, 50%, and 25%) and positioned in the center of each Petri plate that had been inoculated with the microbial suspension at a concentration of 10^7-10^8 ufc/ml.

The Petri dishes were incubated at 30°C for 48 hours to facilitate the growth of the yeast. positive controls were prepared using discs with a diameter of 6 mm impregnated with distilled water.

Tableau 5: detailed information on the microbial strains

Microorganisms	**Strain**	**Family**	**N° ATC***	**Gram**
Bacteria	*Escherichia coli*	Enterobacteriaceae	8739	_
	Staphylococcus aureus	Micrococcaceae	6538	+
	Staphylococcus epidermidis	Micrococcaceae	12228	+
	Bacillus subtilis	Bacillaceae	6633	+
	Salmonella typhimyrium	Enterobacteriacea	14028	_
Yeast	*Candida albicans*	Cryptococcaceae	10231	
	Saccharoms cerevisiae	Saccharomycetaceae	9763	

* ATCC = American Type Culture Collection.

The sensitivity of the various samples of garlic extract was classified according to the diameter of the zones of inhibition of the strains (Table 6), described by *Ponce et al.* [194].

Table 6: Scale for rating the sensitivity and resistance of microbial strains based on the size of inhibition zones [194].

Sensitivity	Code	Diameter of the inhibition zone (mm)
Resistant	(-)	8
Sensitive	(+)	9 to 14
Very sensitive	(++)	15 to19
Extremely sensitive	(+++)	> 20

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++).

2.3.6.3 In *vitro* evaluation of the herbicidal activity

The *in vitro* herbicidal efficacy of each aqueous extract of garlic bulb inoculated with each specific endemic microorganism (arbuscular mycorrhizal fungi MO, *Trichoderma asperellum* T3 and PGPR T7) was evaluated on two species: monocot (*Lolium perenne* L.) and dicot (*Amaranthus retroflexus*)

Lolium perenne L.

Is an important cultivated grass species which becomes a very competitive weed when growing in cultivated crops

Amaranthus retroflexus

can compete with the summer crops and reduce yield substantially

A series of tests were conducted to evaluate the potential impact on:

- Seed germination.

- Seedling growth (root elongation for *Amaranthus retroflexus*, root and shoot elongation *Lolium perenne* L.).

- **Preparation of the treatments**

The extraction process was conducted in accordance with the methodology described by Akullo *et al.* [189]. Following the extraction process, the garlic extract was diluted in order to create four distinct concentrations, 100%, 50%, 25% and 10%.

- **Phase 1: Pre-emergence application or inhibition of seed germination**

The aim of this test was to evaluate the potential of the extracts to impede or delay seed germination. This could prove an invaluable tool for controlling weeds, removing weed seeds from the soil, or avoiding weed competition during the early stages of crop

development. In this phase, ten disinfected seeds of each species were placed in sterile plastic Petri dishes with two layers of Whatman filter paper soaked in 2mL of the aqueous extracts with different concentrations (100%, 50%, 25%, 10%), which were added at the time of sowing. The study was conducted using a completely randomized design with three replicates for each and foutreatment r concentrations in each treatment. The negative

control group was treated with distilled water while the positive control group was treated with two commercial herbicides. The number of germinated seeds was counted at 24-hour intervals for seven days as described by Batish *et al* [195].

- **Phase 2: post-emergent application**

The objective of this test was to ascertain whether the extracts could inhibit or reduce plant growth at the early stages of development. In this phase of the experiment, seeds of the *Amaranth* and *Lolium* species were germinated on two layers of Whatman filter paper in Petri dishes, which were moistened with distilled water in order to promote germination. Subsequently, the germinated seeds were transferred to Petri dishes containing two layers of Whatman filter paper, soaked in 2 mL of aqueous extract. The study was conducted using a completely randomized design, with three replicates for each treatment and four concentrations in each treatment. The negative control group was treated with distilled water, while the positive control group was treated with two commercial herbicide. Following a seven-day period, the length of the seedlings' roots and shoots were measured as described by Batish *et al* [195].

- **Roots Plasma membrane integrity**

The objective of this experiment is to gain insight into the impact of treatments on cellular health and viability, as well as to elucidate the underlying toxicity mechanisms. Three roots from each of each old seedling will be stained in order to study the membrane integrity.

The root plasma membrane integrity was determined by incubating the roots in an Evans blue solution (0.025% w/v, in 100 µM CaCl2) for 30 minutes. The stained roots were then washed three to four times with a sufficient volume of distilled water and viewed under a stereo zoom microscope [196].

2.3.7 Study of the stability of antibacterial activity

The objective of this study is to gain insight into the potential influence of three microorganisms, *Trichoderma* (T3) PGPR T7 and arbuscular mycorrhiza (AM), on the production of antimicrobial compounds in garlic. With the aim of identifying the most effective method for preserving the antimicrobial activity of garlic aqueous extract and facilitating its pharmacological application

2.3.7.1 Extract preparation and evaluation of the antibacterial potential of garlic extracts

The extraction process was conducted in accordance with the methodology described by Akullo, [189]. Following the extraction process, the resulting extract was divided into three equal portions, which allowed for the preparation of three distinct types of extract.

The aged aqueous extract: was stored in a refrigerator at 4°C for a period of six months.

The lyophilized aged extract: was lyophilized immediately, and then stored at 4°C for a period of six months.

The fresh extract: was used immediately after extraction without any further storage.

The antibacterial potential of the three types of aqueous extract of garlic was evaluated using the same protocol used in the previous studies, against five bacterial strains provided by DCQ-SAIDAL pharmaceutical company (Algiers, Algeria). The tests were conducted using the disc diffusion whereby the inhibition diameter around a disc impregnated with the extract was measured as previously described by Hemeg *et al*. [193]

2.3.8 Gel preparation from fresh aqueous extract and evaluation of the antibacterial efficacy

The formulated gel was prepared in accordance with the methodology proposed by Stancu *et al.* [197] with a minor modification. 3g of carbopol were incorporated into 100 ml of a fresh aqueous extract of garlic, and the solution was agitated continuously to prevent the formation of clumps. Subsequently, 10 percent of a sodium hydroxide solution was added drop wise to the carbopol dispersion, while stirring the pH was monitored meticulously with the objective of achieving a pH of approximately 6-7, which will result in the desired thickening of the gel.

The antimicrobial potential of the garlic fresh aqueous extract gel was evaluated against the same five bacteria provided by DCQ-SAIDAL using the disc diffusion method,

as previously described by Hemeg *et al*.[193].

2.3.9 Statistical analysis

The results were analyzed using Minitab version 18 software with the aim of providing a comprehensive and objective evaluation, in order to ensure the most accurate and reliable outcome. A one-way analysis of variance (ANOVA) was conducted with the aim of analyzing the potential impact of endemic microorganisms on plant growth, leaf pigmentation, antioxidant compounds AMF colonization and inhibition rate of germination and growth. Furthermore, a General Linear Model (GLM) was used to examine the potential effect of endemic microorganisms on garlic extract inhibition of pathogenic microorganisms. Moreover, Tukey test subsequently used to ascertain any significant differences ($p \leq 0.05$) [198].

CHAPTER: 3

Results and discussion

3. Results and discussion

3.1 Potential impact of endemic microorganisms on garlic growth

The objective of this research is to investigate the potential impact of endemic microorganisms on the growth and yield of garlic, with a view to provide an alternative biofertilizer to the excessive and unselective use of chemical fertilizers in agriculture.

3.1.1 The potential impact of mycorrhizal fungi on garlic growth cultivated in sterilized soil

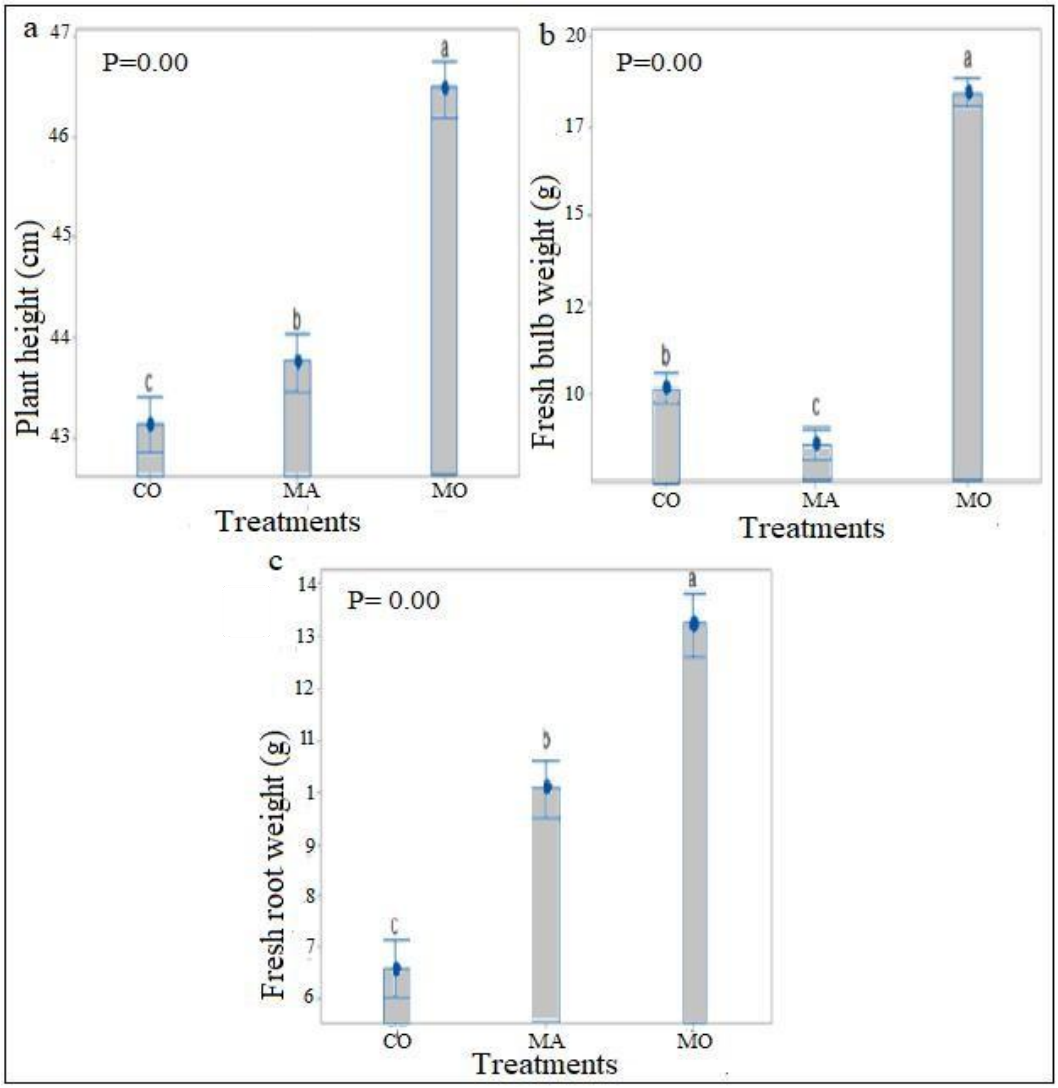
The impact of mycorrhizal fungi isolates on garlic growth was evaluated by assessing growth parameters and leaf pigment in relation to root colonization. The results of this study indicate that the inoculation of garlic with AMF isolates (MO and MA) exerts a variable influence on growth of cultivated garlic.

3.1.1.1 Growth parameters of garlic

AMF isolates were found to have a significant effect on garlic plant height, root weight, and bulb weight, as demonstrated in Figure 13.

The statistical analysis clearly demonstrates a significant difference, between AM isolates and the control on garlic plant height (P=0.00, F=190.61) (appendix 3), root weight (P=0.00, F=161.69) (appendix 4) and bulb weight (P=0.00, F=800.12), (appendix 5), providing strong evidence that the treatments have a distinct effect on the measured parameters related to garlic growth promotion. Isolates MO and MA dramatically increased plant height (46.46 cm and 43.74 cm), and root weight (13.2 g and 10.08 g), respectively compared to the control (43.12 cm and 6 g), (Figure 14 a, 14 c).

The isolate MO demonstrated a notable increase in bulb weight (18.42 g), while MA exhibited a distinct decline, with a weight (8.66 g) that was lower than that of the control (10.18g), (Figure 14 b).



Plant height (a), fresh weight of bulb (b) and fresh weight of root (13 c). (CO: Control, MO and MA: AMF isolates).

Figure 14: Effects of mycorrhizal isolates on growth parameters of garlic.

This finding is in harmony with those of Nacoon *et al*. [199] who demonstrated the beneficial impact of Arbuscular mycorrhizal colonization on the growth performance of two black-rice cultivars. Furthermore, Golubkina *et al*. [200] demonstrated that the application of Mycorrhizal formulations have been demonstrated to exert a considerable influence on the growth and development of shallot plants, Hyaman and Moss [201] also reported that the application of AMF to *Allium cepa* also resulted in a significant increase in bulb yield with a 2–18-fold increase observed, contingent on the initial phosphorus content of the soil. The results of our study suggest that mycorrhizal fungi may enhance plant growth by facilitating the absorption of water and mineral nutrients.

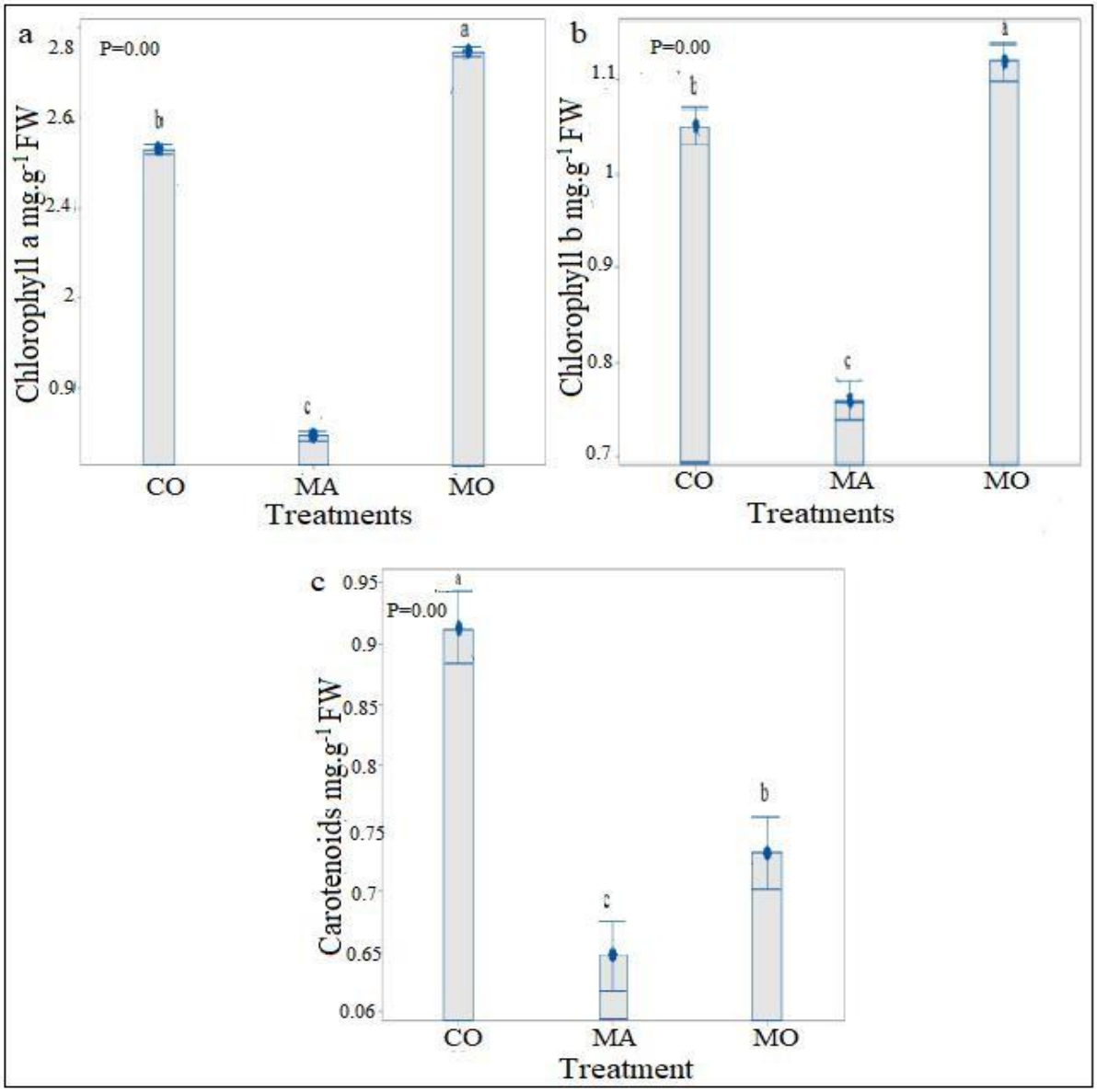
This hypothesis is corroborated by the findings of Karandashov and Bucher [202], who reported that one of the most significant effects of AMF inoculation on the host plant is the increase in P in the plant, which is primarily due to mycorrhiza absorbing phosphate

from the soil and transferring it to host roots. Additionally, Garcia and Mendoza [203] observed that the highest arbuscular colonization was associated with the greatest concentrations of nitrogen and phosphorus in plant tissue, indicating a correlation between an increase in the rate of nutrient transfer between the two organisms. As well as, the findings of Clark and Zeto [204] demonstrated that AMF plays a crucial role in the enhancement of uptake of nutrients, including phosphorus (P), nitrogen (N) and potassium (K). The isolate MA increased plant height and root weight, but reduced plant bulb weight. This result is consistent with the findings of Sari *et al*. [205] who have shown mycorrhizal inoculation did not show any significant effect on the fresh weight of bulbs or the number of cloves per bulb. The observed reduction in growth of colonized plants is attributed to an unbalanced trade between the host plant and Arbuscular mycorrhizal fungi [206].

3.1.1.2 Leaf pigment content

To evaluate the potential influence of AMF on garlic leaf pigmentation, the concentrations of chlorophyll a and b and carotenoids were determined.

The analysis of variance in leaf pigment content demonstrated significant variability in chlorophyll a, chlorophyll b and carotenoid (P=0.00, F=9678) (appendix 6), (P=0.00 , F=500) (appendix 7) and (P=0.00, F=1331), (appendix 8) respectively. The inoculation of garlic with the arbuscular mycorrhizal isolate MO resulted in an increase in chlorophyll a (2.74 $mg.g^{-1}$ FW) and chlorophyll b (1.1 $mg.g^{-1}$ FW) content and a decrease in carotenoids content (0.73 $mg.g^{-1}$ FW) in comparison to the control. In contrast, the isolate MA didn't show any increase in leaf pigment content, chlorophyll a (1.89488 $mg.g^{-1}$ FW), chlorophyll b (0.76 $mg.g^{-1}$ FW) and carotenoids (0.64 $mg.g^{-1}$ FW) compared to the control, chlorophyll a (2.53116 $mg.g^{-1}$ FW), chlorophyll b (1.0519 $mg.g^{-1}$ FW) and carotenoids (0.91 $mg.g^{-1}$ FW) (Figure 15 a, b and c).



a: Chlorophyll a, b: Chlorophyll b , c: Carotenoid, CO: Control,

MO and MA: different isolates of AMF

Figure 15: Effects of AMF isolates on the levels of garlic leaf pigment.

The increase in leaf pigmentation resulting from the application of arbuscular mycorrhizal fungi (AMF) has been observed in numerous studies. One such study, conducted by Jabborova *et al.* [207], indicated that the use of AMF alone led to a notable increase in the content of chlorophyll a, chlorophyll b and carotenoids in Spinach plant. Similarly, Ghani *et al.* [208] observed that the application of AMF appeared to enhance the synthesis of chlorophyll a, chlorophyll b, and carotenoid content at all growth stages. Additionally, Sulistiono *et al.* [208] have suggested that AMF inoculation may result in a notable increase in chlorophyll content in *Myristica fragrans* cultivars, Moreover, the studies conducted by Sulistiono *et al.* [210] and Mathur *et al.* [211] have demonstrated that elevated levels of leaf chlorophyll facilitate photosynthesis and enhance plant growth.

A similar result was observed in the present study, wherein the stimulation of chlorophyll corresponded to the stimulation of growth parameters, indicating that isolate MO may stimulate photosynthesis, which in turn may lead to growth stimulation in garlic.

By contrast, Shuab et *al*. [212] noted that the chlorophyll content remained relatively stable or showed only a slight increase after 20 days of plant growth of onion. However, at the subsequent 20 day interval, there appears to be a slight increase in the chlorophyll a and chlorophyll b content in AMF-inoculated plants compared to non-inoculated ones, which persists until 80 days.

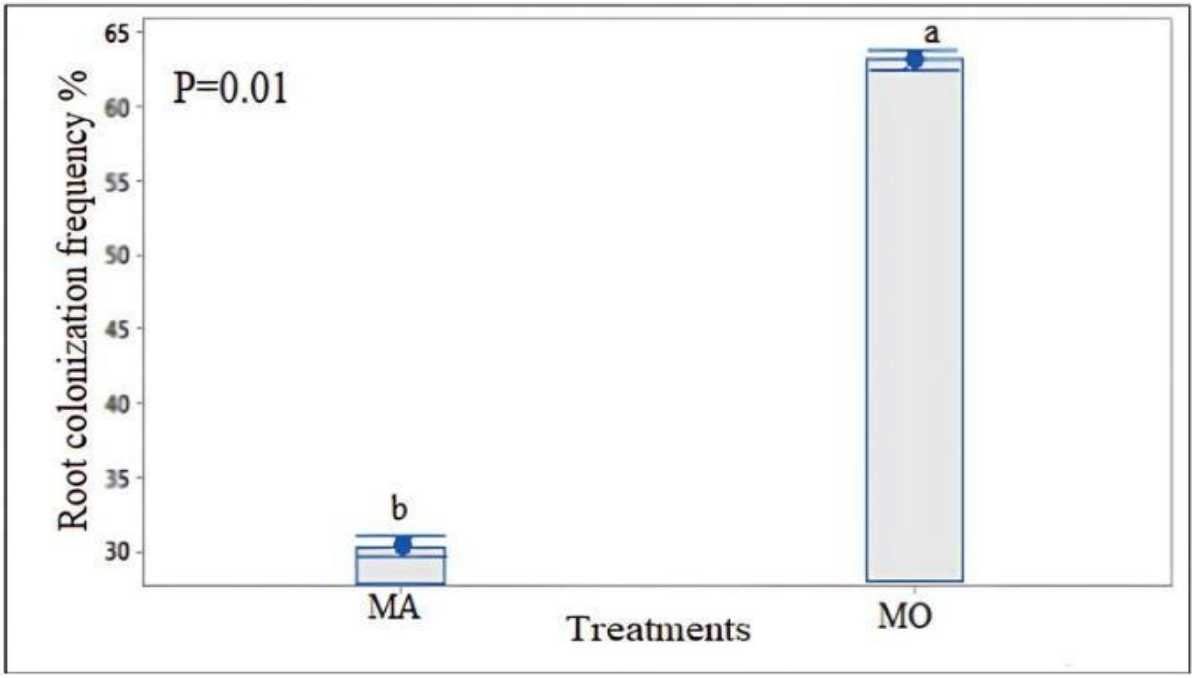
3.1.1.3 Arbuscular mycorhizal fungi Colonization

The result indicated that there was a notable variation in garlic root colonization frequency and mycorhizal external hyphal length of the isolate MO and MA.

- **Root colonization frequency**

Root colonization frequency was determined to investigate the response of garlic to arbuscular mycorrhizal isolates.

The inoculation of mycorrhizal isolates MO and MA had a significant variation on plant root colonization frequency (P=0.01, F=21.83)**,** (appendix 9), MO isolate exhibited the highest frequency of colonization (63.33%), while MA isolate exhibited a lower frequency (32.66%) compared to MO isolate (Figure 16), which reveals that garlic responded well to Arbuscular mycorrhizal fungi inocula MO than the inocula MA.



(MO, MA: different isolates of arbuscular mycorrhizal fungi).

Figure 16: Garlic root mycorrhizal colonization frequency.

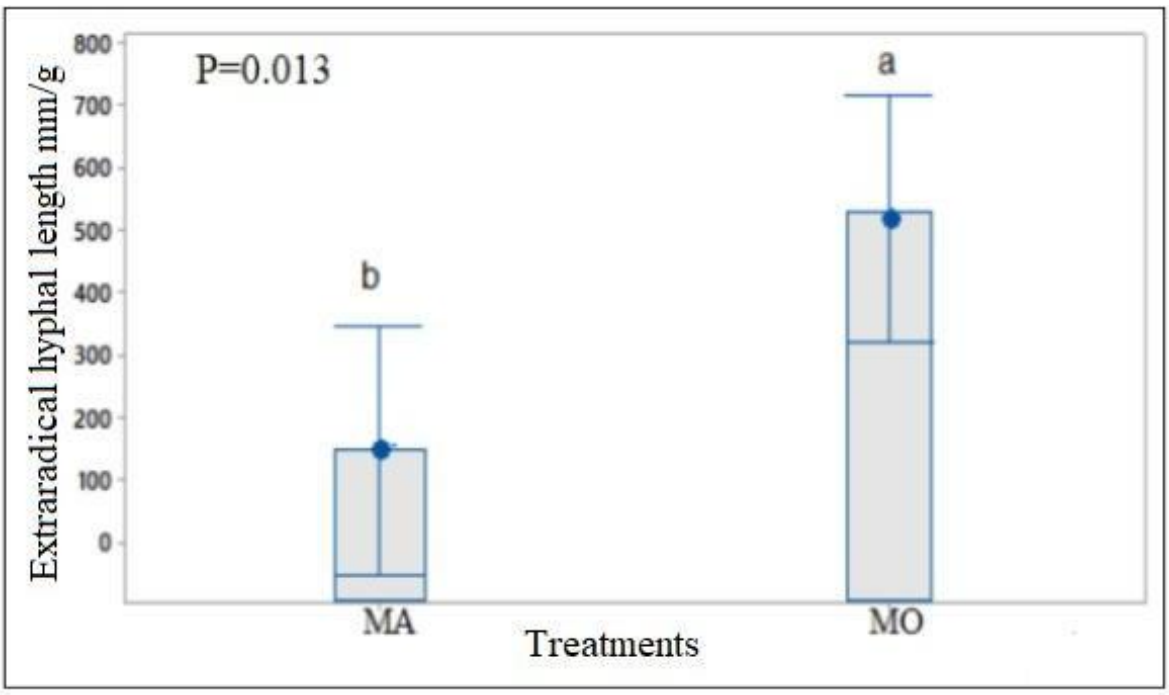
These findings are in accordance with those previously observed by Hart and Reader [213] who noted that the extent of root colonization by the 21 AMF isolates ranged from a mean of 3.2% to 84.8%. Moreover, significant differences were observed in the percentage of root colonization among AMF families, with distinct AMF colonization strategies related to taxonomic differences at the family level. Furthermore, Tommerup and Abbott [214] observed that an *Acaulospora* spp. was unable to establish a new infection from a root fragment containing hyphae. Conversely, they found that a *Glomus* spp. was able to successfully colonize root fragments. This finding suggests that the germination type of AMF also affects the percentage of root colonization.

Moreover, Sow [215] and Borde *et al.* [216] demonstrated that the arbuscular mycorrhizal fungi employed were highly efficacious and adaptable to environmental conditions, as evidenced by a notable increase in mycorrhization parameters. The low root colonization percentage of the isolate MA can be attributed to its inability to rapidly colonize roots (Parasitic' AMF), thereby exerting a greater demand on plant resources for a longer period than faster colonizers. In addition to the aforementioned factors, the extent of root colonization by AMF is contingent upon the number of spores per plant, the quality of the inoculum, the species of plant and the timing of inoculation, and to the growth conditions.

- **Mycorrhizal extraradical hyphal length**

Mycorrhizal extraradical hyphal length measured to investigate the response of garlic to arbuscular mycorrhizal isolates.

The analysis of variance of external mycorhizal hyphal length revealed a statistically significant difference between the two isolates (P=0.013, F=8.03),(appendix 10). The extraradical hyphal of the isolate MO were observed to be significantly longer (536 mm/g) than those of the isolate MA (165 mm/g) (Figure 17).



MO, MA: different isolates of arbuscular mycorrhizal fungi

Figure 17: Extraradical hyphal length of AMF isolates.

The observed differences in extraradical hyphal length among AMF isolates suggest that genetic variations among isolates influence hyphal growth rate. Similarly, Sanders *et al.* [217] reported that the extraradical hyphal length of isolates colonizing *P. vulgaris* under CO2 concentrations showed a significant difference at a distance of 1-2 cm from the nearest root in the experiment. The extraradical hyphal length of *Glomus* sp. (Basle Pi) was significantly greater than that of G*lomus* sp. (BEG 19). The discrepancies among the isolates were primarily attributable to variations in hyphal length. Moreover, *Glomus* sp. (Basle Pi) exhibited the formation of thicker extraradical hyphal, measuring 7.5 and 10 µm in diameter, which were not observed in the other two isolates. Nevertheless, at a distance of 13±14 cm from the nearest roots, the *Glomus* sp. (Basle Pi) isolate exhibited a significantly greater length of fine hyphae. Furthermore, the length of external AMF hyphae at elevated CO2 levels was found to be up to five times that observed at ambient CO2 levels, indicating that elevated CO2 levels promoted the allocation of AMF biomass to the extraradical hyphal. These findings indicate that, in addition to genetic variation, elevated CO2 and proximity to roots also exert an influence on growth rates and hyphal morphology.

Additionally, in an experiment conducted by Pepe and Sbrana [218], the three AMF isolates grown in symbiosis with *Cichorium intybus* in two *in vivo* systems demonstrated disparate abilities to the formation of appressoria on plant roots were observed in both experimental systems. The density of appressoria per unit root or colonized root length

produced by *Rhizoglomus irregulare* was significantly higher compared to the other AMF, *Funneliformis mosseae* and *Funneliformis coronatus.*

In our study, the isolate designated as MO, which exhibited the greatest length of hyphae, demonstrated the most pronounced enhancement in plant growth parameters in comparison to the isolate MA, which exhibited comparatively shorter hyphae. This observation may indicate that the degree of increasing growth of garlic plants depends on the length of mycorrhizal hyphae and nutrients uptake efficiency. This hypothesis is supported by the findings of Abou El Seoud *et al*. [219], which indicate that the response of wheat genotype V6 to low levels of soil phosphorus is closely associated with longer hyphae that readily absorb high quantities of phosphorus from the soil and obtain larger yields than those having shorter hyphal lengths (wheat genotype V4).

Moreover in a study conducted by Sawers *et al.*[220], the Oh43 maize line demonstrated the greatest phosphorus uptake when inoculated with arbuscular mycorrhizal fungi*,* this observation not correlated with the extent of root-internal colonization or the accumulation of ZmPt6 transcripts (predicted to encode the major periarbuscular membrane-associated PT transporter), but in the abundance of root-external hyphae.

Li *et al*. [221], observed in a Chinese fir (*Cunninghamia lanceolata*) plantation the extraradical hyphal length density in the rhizosphere soil and the ratio of hyphal length density to mycorrhizal colonization rate exhibited a notable decline with the addition of nitrogen (N), whereas these parameters remained unaltered with phosphorus (P) addition. Conversely, the extraradical hyphal length density in the in growth mesh bags demonstrated a substantial reduction in response to both N and P additions.

The results of this study may indicate that external hyphae remain a crucial factor in the acquisition of (P), maintaining a stable relationships despite fluctuating nutrient supply. Li *et al*. [221], observed also relative abundance of *Acaulosporaceae* and *Gigasporaceae* increased in response to phosphorus (P) addition, while that of *Glomeraceae* decreased. However, the addition of nitrogen (N) did not significantly impact the composition of arbuscular mycorrhizal fungi *(*AMF) communities.

In contrast, elevated CO2 levels were observed to stimulate external hyphal growth. However, there was no corresponding increase in phosphorus (P) acquisition by *Phaseolus vulgaris* plant, which suggests that hyphal growth may not always be a reliable indicator of enhanced P uptake.

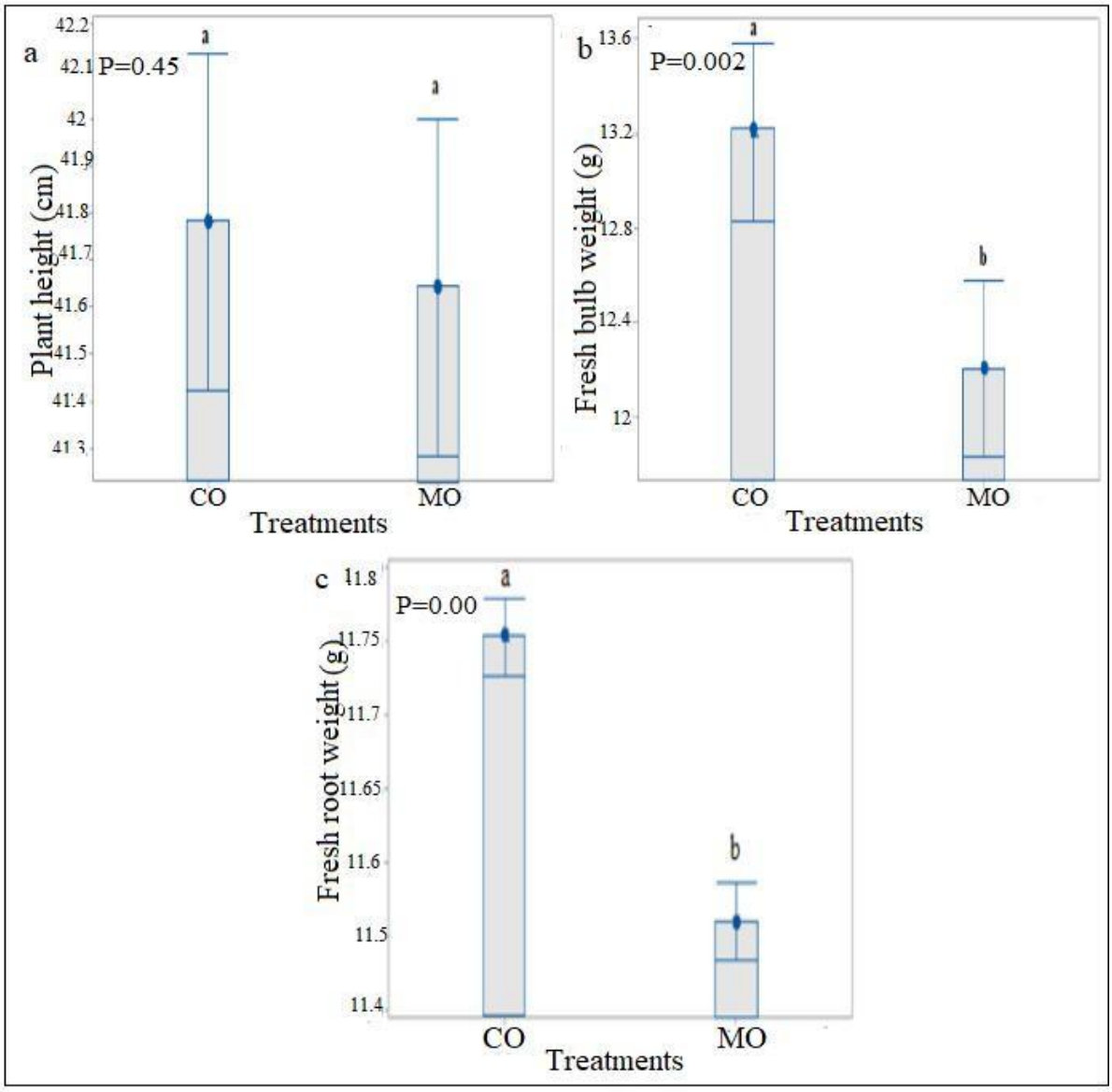
3.1.2 The potential impact of mycorrhizal fungi isolate on garlic growth cultivated in unsterilized soil

The impact of the mycorrhizal fungus isolate MO on the growth of garlic cultivated in unsterilized soil was evaluated by assessing growth parameters and leaf pigmentation in relation to root colonization.

The results of this study indicate that the inoculation of garlic with AMF isolates MO exerts a variable impact on growth of cultivated garlic

3.1.2.1 Growth parameters of garlic

The result revealed variability in growth parameters of garlic (Figure 18). The statistical analysis revealed that there was no statistically significant difference (P= 0.454, F=0.41), (appendix 11) in plant height between the inoculated garlic (41.64cm) and the control (41.78cm), (Figure 18a). However, the isolate MO exhibited a statistically significant effect on bulb (P=0.002, F= 19.14), (appendix 12) and root weight (P=0.00, F= 210.25), (appendix 13). The isolate MO exhibited no discernible positive effect on bulb weight (12.206 g) or root weight (11.56 g) in comparison to the control (13.21 g, 11.75 g), respectively (Figure b18, c18).



a: Plant height, b: fresh weight of bulb, c: fresh weight of root, CO: control, MO:AMF isolate

Figure 18: Effects of AMF isolates on growth parameters of garlic cultivated in unsterilized soil.

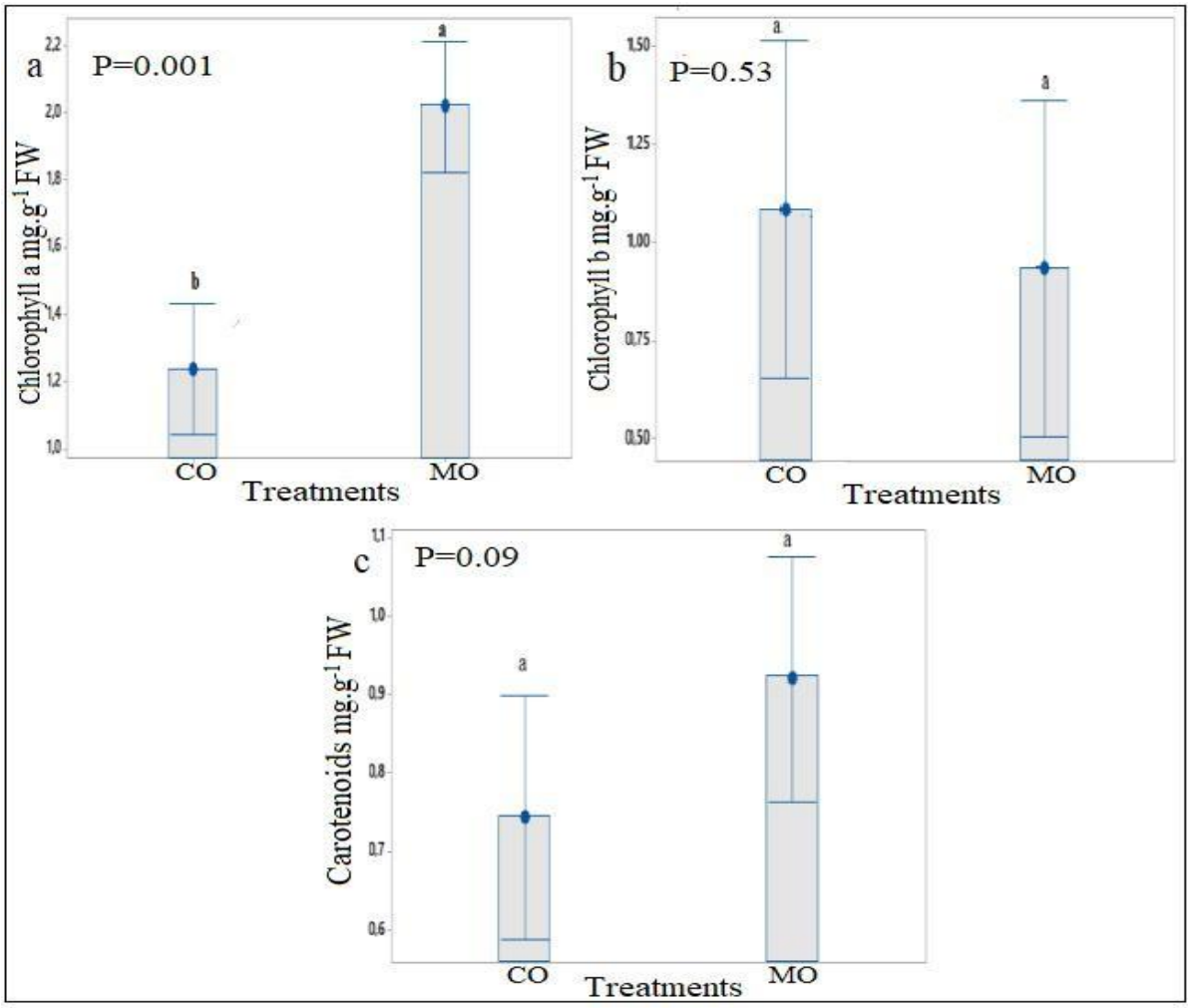
The application of arbuscular mycorrhizal fungi isolate did not yield any discernible positive effect in the parameters observed, this may be attributed to the presence of native arbuscular mycorrhizal fungi, which effectively neutralized the potential impact of the inoculated arbuscular mycorrhizal fungi. These results align with those reported by Janoušková *et al*. [222], in such cases, the growth of plants may not be significantly improved by inoculation as the native AMF community is already providing sufficient benefits to the plants. Furthermore, Frew [223] documented that the benefits of AMF inoculants in field conditions may be constrained if diverse native AMF communities are already present. Janoušková *et al*. [222] further postulated that the application of AMF inoculate may increase competition among the root-colonizing AMF, which can lead to

the suppression of plant growth or reduced biomass production in inoculated plants compared to non-inoculated or pre-inoculated plants.

3.1.2.2 Leaf pigment content

To evaluate the potential influence of AMF isolate MO on garlic cultivated in unsterilized soil on leaf pigmentation, the concentrations of chlorophyll a, b and carotenoids were determined.

The statistical analysis demonstrated that there was no statistically significant difference in chlorophyll b (P=0.53; F=0.47), (appendix 15) and carotenoids (P=0.09; F=6.2), (appendix 16) between the inoculated garlic and the control. However, the isolate MO demonstrated a statistically significant effect on chlorophyll a (P=0.001; F= 61.53), (appendix 14), the isolate MO demonstrated an increase in chlorophyll a (2.06 $mg.g^{-1}$ FW) in comparison to control (1.23 $mg.g^{-1}$ FW), (Figure 19 a). While no significant effect on chlorophyll b (0.93 $mg.g^{-1}$FW) and carotenoid (0.92 $mg.g^{-1}$ FW) compared to control (1.08$mg.g^{-1}$FW; 0,74 $mg.g^{-1}$FW) respectively (Figure 19b, 19c).



a: Chlorophyll a, b: Chlorophyll b, c: Carotenoid , CO: control, MO: AMF isolates.

Figure 19: Effects of AMF isolates on the levels of garlic leaf pigment

The inoculation of garlic with the isolate MO resulted in an increase in chlorophyll a, but no significant difference in chlorophyll b and carotenoid compared to the uninoculated control. Papoui *et al*. [224] observed that the chlorophyll content of lettuce and green onion was not significantly affected by the mycorrhizal treatment. Moreover, the mycorrhizal garlic plants exhibited higher chlorophyll content in comparison to the non-mycorrhizal garlic plants. Therefore, it can be concluded that the AM symbiosis has the potential to enhance the photosynthetic ability of garlic leaves.

Conversely, Zuccarini [225] reported that the inoculation of lettuce with *R. intraradices* resulted in an increase in chlorophyll content in lettuce. Chlorophyll a plays a crucial role in the process of capturing light for the purpose of photosynthesis. It may be the case that this process can be enhanced by increased availability of nutrients, in particular phosphorus, magnesium and nitrogen, all of which are essential for chlorophyll synthesis and proper photosynthetic function. It seems plausible to suggest that mycorrhizal fungi may facilitate the transport of nutrients to the host plant, particularly phosphorus and nitrogen. This could potentially enhance photosynthetic efficiency, which may lead to increased levels of chlorophyll a

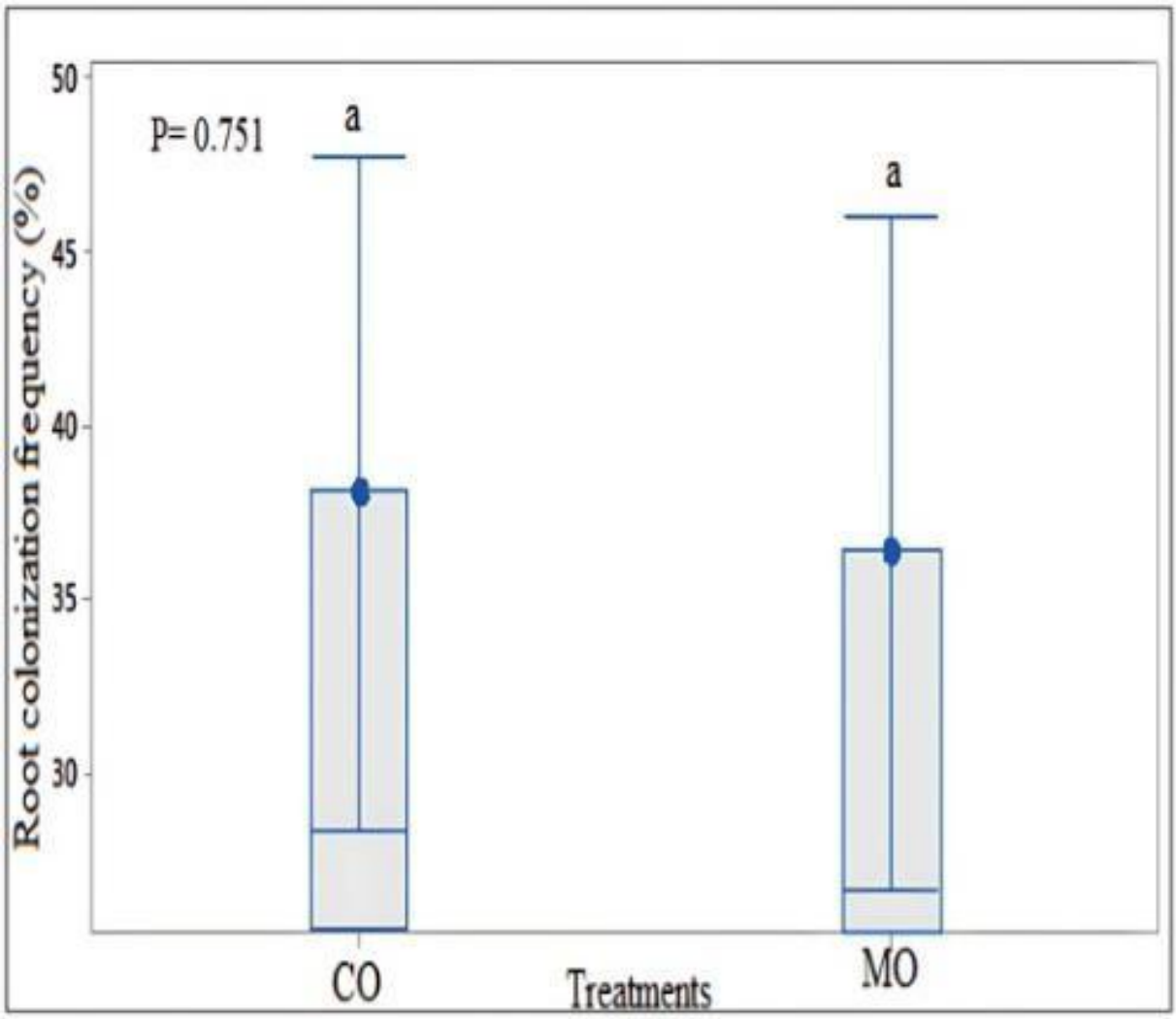
Our findings indicate that the integration of diverse mycorrhizal fungi may potentially enhance photosynthetic activity. Nevertheless, the underlying mechanism of interaction among the diverse AMF strains remains to be elucidated.

3.1.1.3 Root colonization frequency in unsterilized soil

Root colonization frequency was determined to investigate the response of garlic cultivated in unsterilized soil to arbuscular mycorrhizal isolates MO.

The results of the analysis of variance demonstrated that the root colonization by the arbuscular mycorrhiza isolate MO exhibited no statistically significant effect when compared to the uninoculated control (P=0.751; F=0.12), (appendix 17).

The results indicated that the garlic root inoculated with the MO isolate exhibited a root frequency colonization of approximately 36%, while the control showed a root colonization of approximately 38% (Figure 20).



MO: AMFisolate, CO: Control.

Figure 20: Garlic root mycorrhizal colonization frequency

The current findings indicate the presence of arbuscular mycorrhizal colonization in both the garlic root inoculated with the MO isolate and the control, thereby indicate that the native arbuscular mycorrhiza exiting in the substrate is already able to colonize the root system, which may limit the potential benefits that could be gained from the inoculated fungi. Similarly, Janoušková *et al*. [222] observed in some cases, the inoculated fungi may not achieve higher levels of root colonization than the native arbuscular mycorrhizal fungi (AMF). Moreover, in some instances, the overall root colonization may even decline as a consequence of intensified competition for resources, including space and carbohydrates.

Gazey *et al.* [226] reported that the inoculation of the West dale soil field with *G. invermaium* did not result in an overall increase in root colonization by mycorrhizal fungi. Instead, it replaced approximately 20% of the root length that had been colonized by the indigenous fungi. Conversely, in the soil field of South Carrabin (in the presence of native

mycorrhiza), the total root length colonized in the untreated field soil and in the inoculated field soil by *G. invermaium* was found to be similar. These findings highlight the necessity of assessing the compatibility between particular mycorrhizal isolates and the soil microbial environment when selecting inoculants for the purpose of promoting growth or enhancement.

3.1.3 The potential impact of *Trichoderma asperellum* on garlic growth.

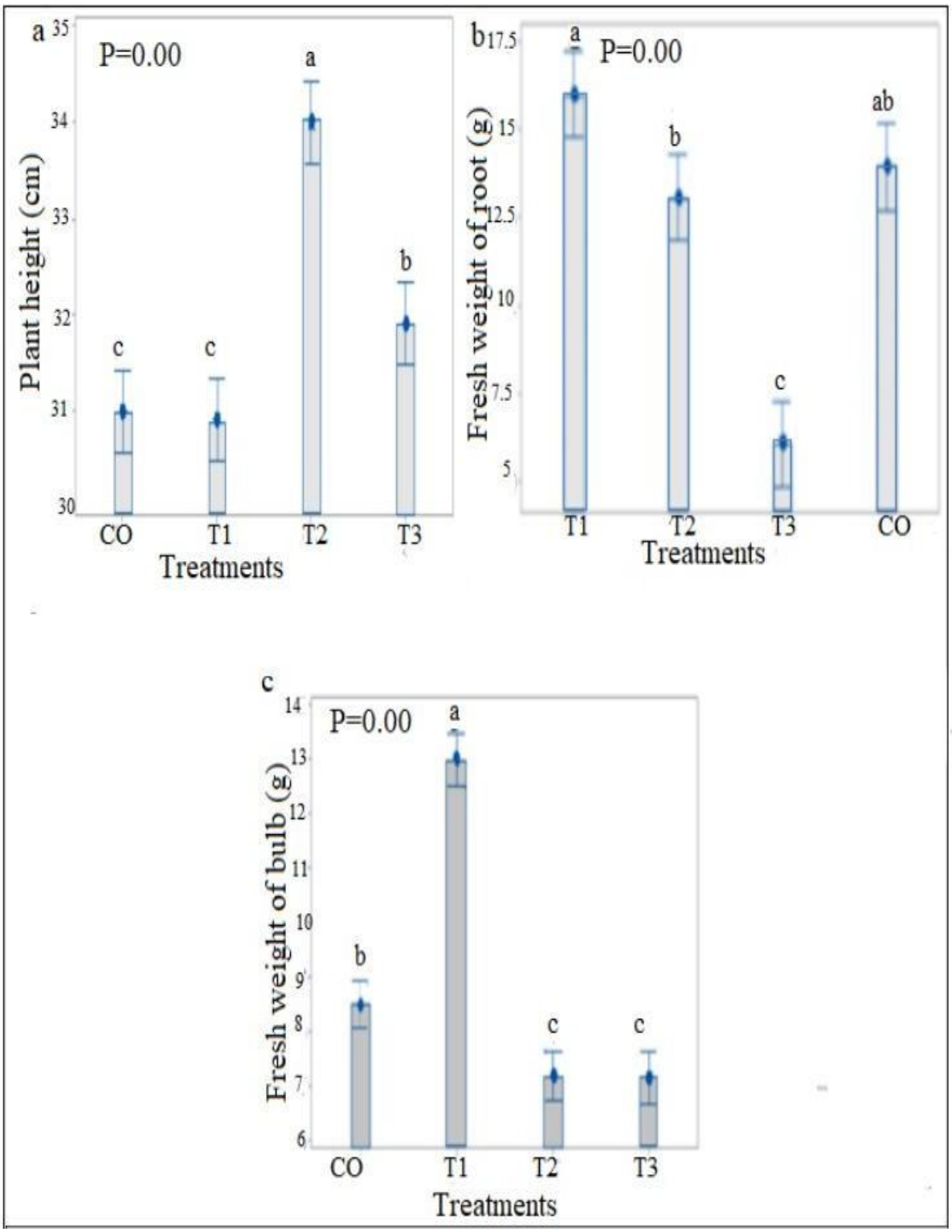
The impact of *T.asperellum* isolates on garlic growth was evaluated by assessing growth parameters and leaf pigment.

The results of this study indicate that the inoculation of garlic with *T.asperellum* strains (T1, T2 and T3) exerts a variable influence on growth and yield of cultivated garlic.

3.1.3.1 Growth and parameters

The effect of treatments of *Trichoderma* on plant high, the weight of garlic bulb, and root were presented in Figure 21. The statistical analysis revealed a notable disparity between the experimental treatments and the control group with regard to the Plant height (P=0.00; F=79.11), (appendix 19), fresh weight of root (P=0.00; F=65.31), (Appendix 20), and fresh weight of the bulbs (P = 0.00; F=146.54); (appendix 18), This indicates that the treatments have a discernible impact on the parameters associated with garlic yield.

The treatment T1 significantly increased the plant height (36 cm) the weight of fresh bulb (12.99 g) and fresh root weight (16 g) compared to control (33 cm), (8.50 g) and (13.5 g) respectively. This suggested that the application of *T.asperellum* TMSKOLDZ20 effectively promoted the yield of garlic bulbs. Conversely, treatments T3 and T2 showed no positive effect on the plant height (25.66 cm) and (32.66 cm) weight of bulb (7.14 g) and (7.18 g) respectively, compared to control (Figure 21), indicating *Trichoderma* TMSKOLDZ20 (treatment T1) was the most effective in promoting garlic yield.



a: Plant height, b:fresh weight of root, c: fresh weight of bulb, CO: control, T1, T2, T3: Trichoderma strains.

Figure 21: Effects of *Trichoderma asperellum* strains on growth parameters of garlic.

Our results revealed that all strains of *Trichoderma asperellum* had varying effects on garlic growth and yield parameters, among the treatments, only treatment T1 showed significant positive effects. This is consistent with previous research that has demonstrated the diverse effects of different *Trichoderma* strains on various host plants. For instance, a study conducted by Stewart *et al*. [227], four strains of *Trichoderma longipile* species were tested for their capacity to enhance the growth of lettuce seedlings. The results demonstrated a range of growth responses, with one isolate exhibiting no promoting effect. Moreover, Ortega-García [228] reported that both onion varieties exhibited an increase in bulb mass when treated with *T. asperellum* isolates (To and Tt).

However, *T. asperellum* isolate (Tm) did not affect the bulb mass of either onion variety. This confirmed that not all isolates are capable of promoting plant growth, and the degree of growth promotion achieved is also influenced by the genetic variability among *Trichoderma* isolates.

The beneficial effects of *Trichoderma* are likely due to its capacity to enhance nutrient uptake and transport in plants, stimulate the production of growth hormones and other beneficial compounds, and thereby promote plant growth and development. Furthermore, *Trichoderma* functions as a soil conditioning agent, enhancing the diversity and concentration of beneficial microorganisms in the soil [229, 230].

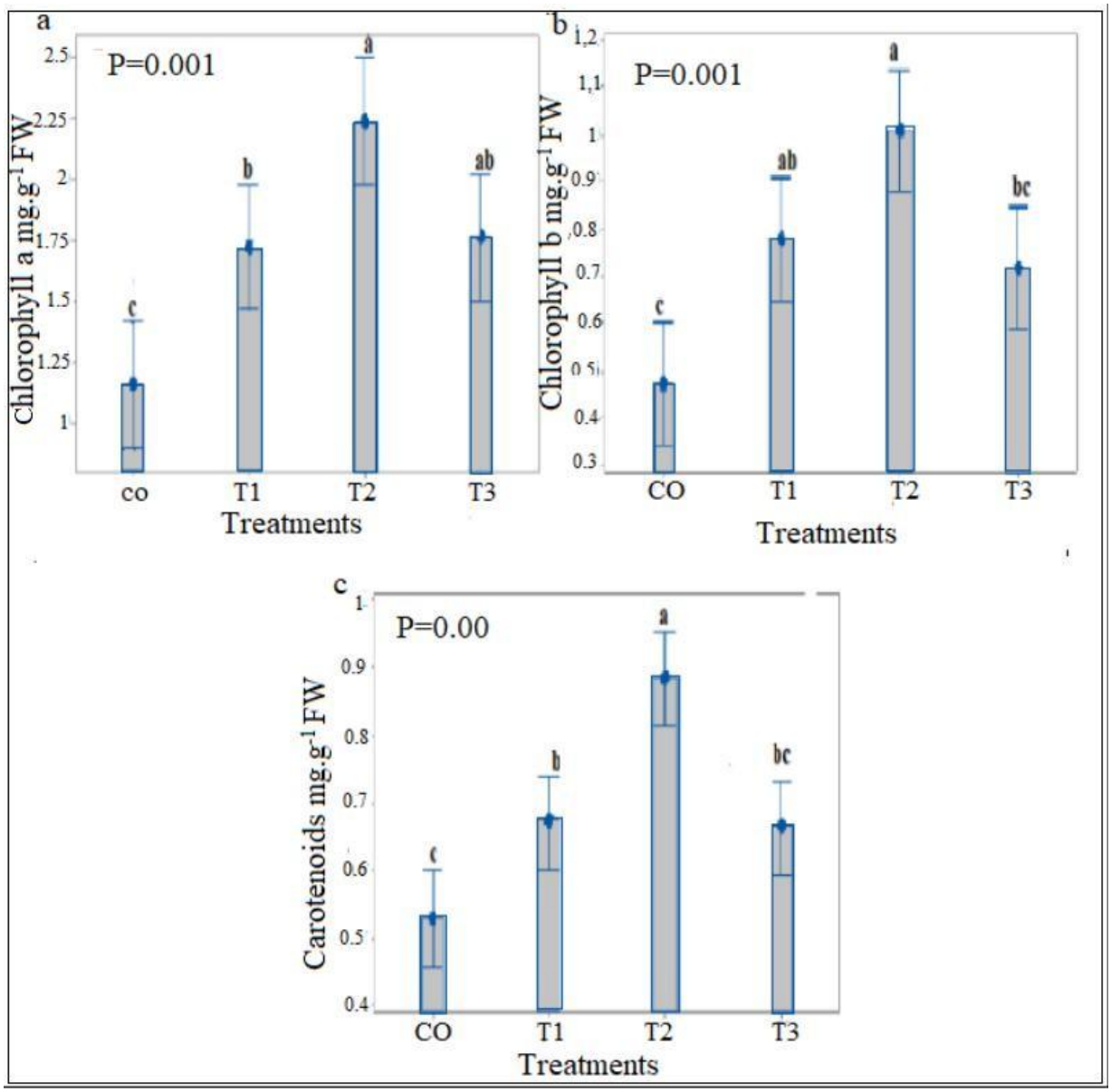
3.1.3.2 Leaf pigment

To evaluate the potential influence of *Trichoderma asperellum* on garlic leaf pigmentation, the concentrations of chlorophyll a, b and carotenoids were determined.

The analysis of variance in leaf pigment content revealed significant differences between the treatments and the control in chlorophyll a, chlorophyll b and carotenoids (P=0.001, F=15.95) (appendix 21), (P=0.001, F=15.42),(appendix 22) and (P=0.00, F=23.72) (appendix 23) respectively.

The *Trichoderma* strain T2 showed significantly higher values for chlorophyll a, chlorophyll b, and carotenoid of garlic leaves respectively (2.24 $mg.g^{-1}$ FW), (1.006 $mg.g^{-1}$ FW) and (0.88 $mg.g^{-1}$ FW).

Moreover, T3 and T1 *Trichoderma asperellum* strains increased chlorophyll a (1.76 $mg.g^{-1}$ FW), (1.72 $mg.g^{-1}$ FW), chlorophyll b (0.76 $mg.g^{-1}$ FW), (1.72 $mg.g^{-1}$ FW) and carotenoids (0.665 $mg.g^{-1}$ FW), (0.67 $mg.g^{-1}$ FW) respectively (Figure 22).



a: Chlorophyll a, b: Chlorophyll b, c: Carotenoid, CO: control, T1, T2 and T3: different treatment of *Trichoderma asperellum* strains.

Figure 22: Effects of *Trichoderma asperellum* isolates on the levels of garlic leaf pigment.

Previous research by Abdel-Fattah *et al.* [231] revealed that application of a conidia suspension of *Trichoderma harzianum* to *Oryza* plants resulted in a significant increase in photosynthetic pigments, including chlorophyll a + b and carotenoids. Da Silva *et al.* [232] found that specific *Trichoderma* strains, including *Trichoderma azevedoi* CEN1241, increased chlorophyll and carotenoid levels in the leaves of *Lactuca sativa*, Zapata-Sarmiento *et al.* [233] observed that plants inoculated with *T. asperellum* showed improved PSII efficiency and higher chlorophyll and carotenoid concentrations in Crystal White onion plants compared to uninoculated controls.

Rodríguez-Hernández *et al*. [234] also reported that *T. asperellum* helps to maintain chlorophyll and carotenoid levels in plants during *S. vesicarium* infection.

Chlorophylls and carotenoids are vital components of plant leaves, playing a crucial role in photosynthesis. Chlorophyll a is the primary photosynthetic pigment, while chlorophyll b and carotenoids serve as accessory pigments, protecting photosystems from photobiological damage. Mendes *et al.* [235] indicated that chlorophyll content is an indicator of plant nitrogen status; this is due to the fact that a lack of nitrogen can impact the synthesis of these pigments. However, the literature also indicates that the chlorophyll a and b ratio can increase when nitrogen availability is reduced, particularly under high light conditions.

The introduction of various endophytic *Trichoderma* resulted in an increase in the production of photosynthetic pigments and the up-regulation of genes regulating the biosynthesis of chlorophyll, light-harvesting complex proteins, and Calvin cycle components. This resulted in an improvement in the photosynthetic capacity of the plants [235].

3.1.4 The potential impact of PGPR on garlic growth

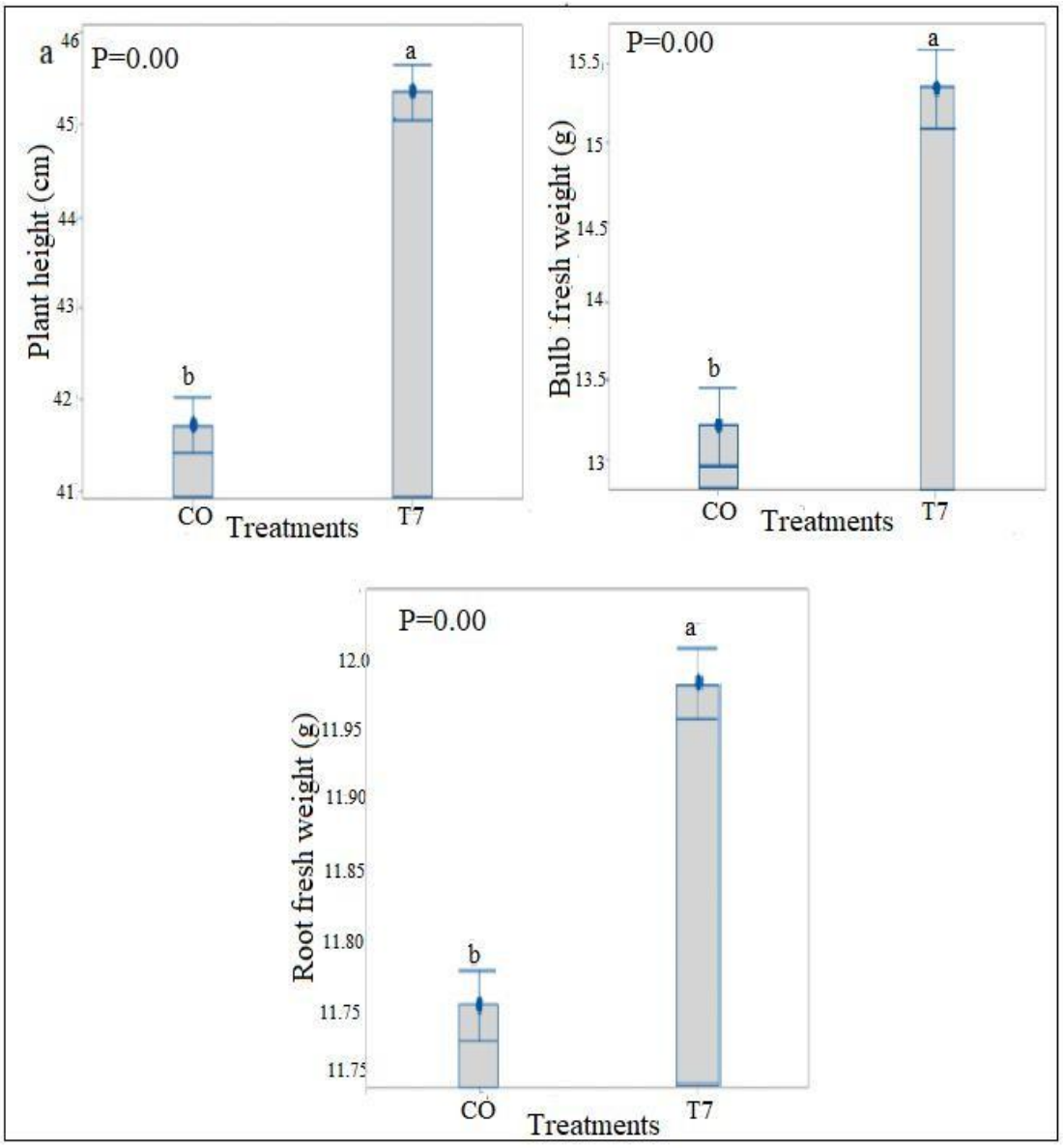
The impact of PGPR isolates on garlic growth was evaluated by assessing growth parameters and leaf pigment.

3.1.4.1 Growth parameters

The effect of *PGPR* treatment on plant high, the weight of garlic bulb and root were presented in Figure 23.

The statistical analysis indicated a notable difference between the inoculated garlic and the control plant in the following parameters: plant height (P=0.00; F=389.6), (appendix 24), bulb weight (P=0.00, F=1.96.85), (appendix 25) and root weight (P=0.00; F=323.57) (appendix 26).

The plants inoculated with PGPR demonstrated a promising increase in plant height (45.36cm) bulb weight (15.34g), and root weight (11.98 g) in comparison to the control (41.42cm); (13.21g) and (11.75g) respectively (Figure 23).



a: Plant height, b: fresh weight of bulb, c: fresh weight of root. CO: control, T7: PGPR.

Figure 23: Effect of PGPR on growth parameters of garlic.

The PGPR isolate was observed to exert a discernible impact on all examined growth parameters. As reported by Choi *et al*. [236], application of Bacterial species include those belonging to the genus *Bacillus* have been demonstrated to promote the growth of vegetable plants, including peppers cultivated in open fields. This has been demonstrated to result in increased root biomass and stem weight.

Moreover, the application of PGPR in the cultivation of tomato plants resulted significant enhancements in plant biomass and root length. As well as Xia *et al*. [237] mentioned that the PGPR enhances plant dry weight, chlorophyll and sugar content which ultimately enhance the growth of plants.

Rhizobacteria, including *Bacillus, Pseudomonas, Pantone and Arthobacter*, are capable of producing phytohormones and have been demonstrated to be effective in inducing

tolerance to abiotic stress [238]. The mechanisms of action of bacterial species differ from one species to another.

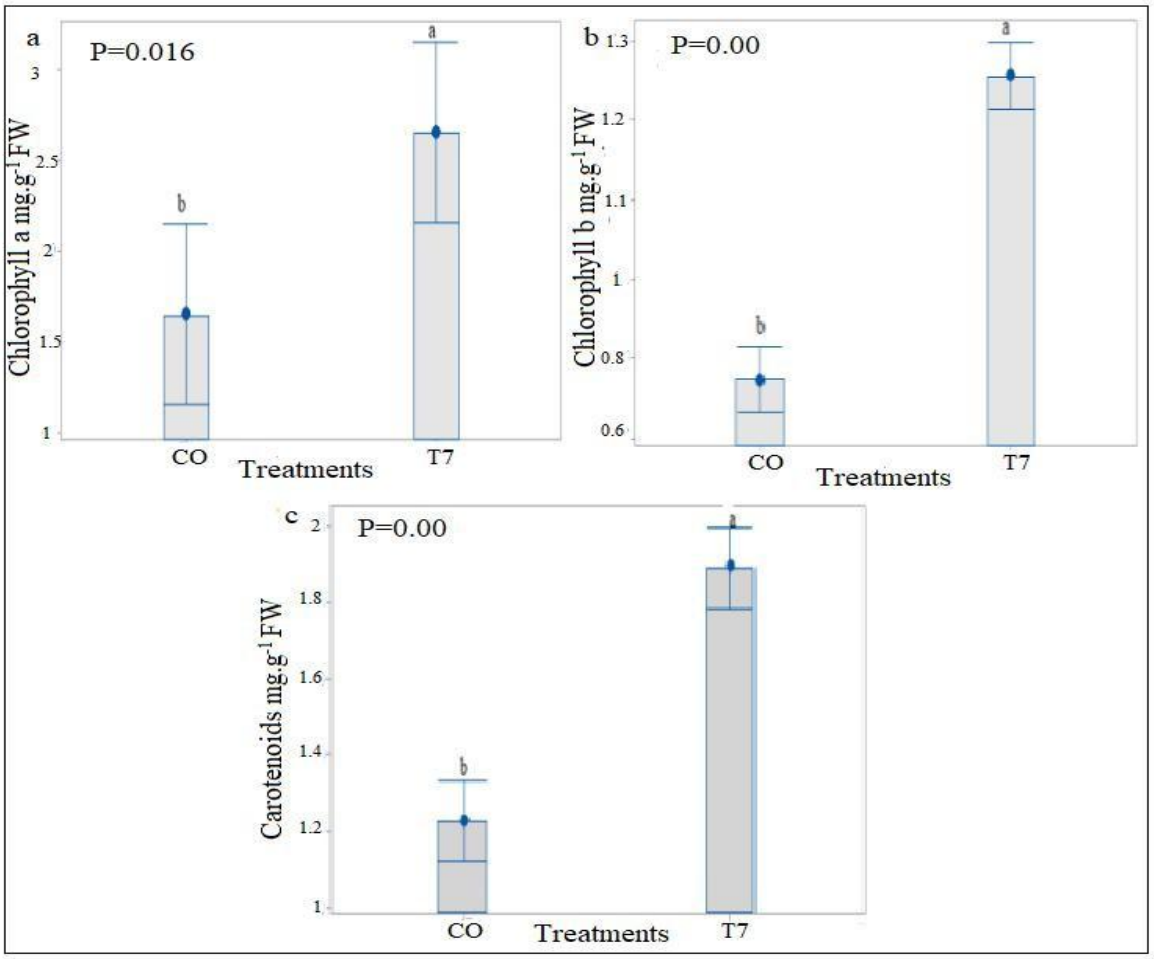
These differences include changes in phytohormone levels, production of volatile compounds, and increased availability of nutrients by the root and tolerance to abiotic stresses [238].

3.1.4.2 Garlic leaf pigment content

To evaluate the potential influence of PGPR on garlic leaf pigmentation, the concentrations of chlorophyll a and b and carotenoids were determined.

The results demonstrated a statistically significant difference in chlorophyll a (P=0.016; F=1570) (Appendix 27), chlorophyll b (P=0.00; F=321.81), (Appendix 28) and carotenoid (P=0.00; F=148.09), (Appendix 29) between the inoculated garlic and the control.

The inoculation of garlic with the PGPR isolate resulted in a notable increase in chlorophyll a (2.65 mg.g^{-1} FW) (Figure 24 a) and chlorophyll b (1.51 mg.g^{-1} FW) (Figure 24 b), as well as in carotenoids (1.89 mg.g^{-1} FW), (Figure 24 c), in comparison to the control (1,65 mg.g^{-1} FW), (0.74 mg.g^{-1} FW) and (1.22 mg.g^{-1} FW) (Figure 24 a, b and c).



a: Chlorophyll a , b: Chlorophyll b , c: Carotenoid , CO: control, T7:PGPR isolate.

Figure 24: Effects of PGPR isolate on the levels of garlic leaf pigment.

Our findings appear to be in line with those of numerous previous studies, including the research conducted by Jahangir *et al*. [239], which suggests that PGPR inoculation may have the potential to enhance the chlorophyll b and total chlorophyll content of onion leaves. Similarly, Prisa and Altimari [240] have also suggested that the PGPR bacteria contribute to an increase in the concentration of chlorophyll and water in the leaves, which could have a positive impact on the uptake of micro-nutrients. Additionally, Tariq *et al.* [238] have documented that PGPR inoculation resulted in a notable increase in chlorophyll a and b content in garlic.

Chlorophylls and carotenoids are the integral components of photosynthetic machinery that drive plant's photosynthetic activity and consequently the accumulation of plant biomass.

Mikiciuk*et al.* [241] and Younes *et al*. [242] have shown that higher carotenoid levels caused by AMF and PGPR colonization can improve plant resilience to biotic and abiotic stressors. This is achieved by protecting the photosynthetic apparatus from photodestruction and photoinhibition.

In light of the presented evidence, it can be posited that isolates MO, T1 and T7 enhance the production of garlic. This may be regarded as a growth promoter and an alternative to the excessive and unselective utilization of chemical fertilizers in agricultural contexts.

In unsterilized soil, the isolate MO demonstrated no discernible positive impact on the growth of the garlic plant. This highlights the necessity of assessing the compatibility between specific mycorrhizal isolates and the soil microbial environment before selecting inoculants as alternative to chemical fertilizers.

3.2 The potential impact of endemic microorganisms on chemical compounds of garlic

The aim of this research is to examine the potential influence of endemic microorganisms on the nutritional profile of garlic, with a view to enhancing its bioactive compounds and medicinal value.

3.2.1 The potential impact of mycorrhizal fungi isolates on chemical compounds of garlic cultivated in sterilized soil

The results of this study indicate that the inoculation of garlic with AMF isolates (MO and MA) exerts a variable influence on functional groups and phenolic compounds of garlic extract.

3.2.1.1 Fourier Transform Infrared (FTIR) analysis

The results of the infrared analysis demonstrated spectral variability, characterized by shifts in the spectra in terms of band position the disappearance or appearance of new peaks, and alterations in absorption intensity values. In comparison to the control, this variability exhibited a modification in the distribution of the functional groups within the samples.

Between 4000 and 400 cm^{-1}, nine distinct absorption regions were identified for both the MA and control samples, and ten absorption regions were identified for the MO sample, (appendix 68). Additionally, the absorption displayed was not located in analogous regions. The sample MO exhibited an absence of absorption in the region between 1650 -1550cm^{-1}, the sample MA exhibited no absorption between 1000-700 cm^{-1} and 1650 -1550cm^{-1} range, while the control exhibited an absence of absorption between 700-600 cm^{-1} and 1750-1650 cm^{-1} range.

With regard to the appearance and disappearance of peaks across different spectra, significant variability was observed in the spectra recorded for samples MO, MA and control.

In the range of 3600-3000 cm^{-1}, the sample MO exhibited vibrational peaks at wavenumbers 3588 cm^{-1}, 3564 cm^{-1} 3544 cm^{-1}, 3524 cm^{-1}, 3502 cm^{-1} 3480 cm^{-1} and 3443 cm^{-1}, the sample MA exhibited vibrational peaks at 3524 cm^{-1}, 3501 cm^{-1}, 3446 cm^{-1}, 3423 cm^{-1} and 3400cm^{-1}, while the sample CO displayed a single peak at 3417 cm^{-1}. In the range of 3000-2800 cm^{-1}, both the MO sample and the control exhibited vibrational peaks at 2929 cm^{-1}, while the MA sample displayed two vibrational peaks at 2965 cm^{-1} and 2925 cm^{-1}.

All the samples revealed the presence of a peak within the range of 2400-2000 cm^{-1}, the control sample exhibited a peak at 2346 cm^{-1}, 2305 cm^{-1} 2161 cm^{-1} and 2137 cm^{-1}, while the MO sample demonstrated a peak at 2346 cm^{-1}, 2305 cm^{-1}, 2161 cm^{-1} and 2137 cm^{-1}. In contrast, the MA sample exhibited a peak at 2346 and 2309 cm^{-1}. It was observed that only the samples MO and MA exhibited a peak within the 1750-1650 cm^{-1} range. Specifically, the sample MO demonstrated a peak at 1651 cm^{-1}, while the sample MA exhibited a peaks at 1738 cm^{-1} and 1651 cm^{-1} within this region, in contrast, only the control sample showed a peak at 1638 cm^{-1} between 1600 cm^{-1} and 1550 cm^{-1} region.

The analysis revealed a peak between 1550 and 1400 cm^{-1} in all samples. The control sample exhibited a peaks at 1458 and 1400 cm^{-1}, while the MO sample demonstrated a peaks at 1545 and 1459 cm^{-1}. The MA sample exhibited a peak at 1545 cm^{-1}, 1459 cm^{-1} and 1417 cm^{-1}.

In the range between 1450 and 1000 cm^{-1}, the control sample exhibited a peaks at 1335 cm^{-1}, 1265 cm^{-1}, 1107 cm^{-1}, 1062 cm^{-1}, 1028 cm^{-1}, while the sample MO demonstrated peaks at 1399 cm^{-1}, 1355 cm^{-1}, 1100 cm^{-1}, 1064 cm^{-1} and 1033 cm^{-1}, while the MA sample showed a peaks at 1335 cm^{-1}, 1271 cm^{-1}, 1108 cm^{-1} and 1070 cm^{-1}. Both samples MO and control showed a peak between 1000-700 cm^{-1}, the MO sample showed a peak at 946 cm^{-1}, while the control showed two peaks at 934 cm^{-1} and 814 cm^{-1}. In the range of 600-500 cm^{-1} , a peak was observed in all samples. The MO sample exhibited peaks at 596 cm^{-1}, 545 cm^{-1} and 521 cm^{-1}, while the MA sample displayed peaks at 581cm^{-1}, 547cm^{-1}, and 520cm^{-1} moreover the control sample exhibited peaks at 581 cm^{-1}, 547cm^{-1}, and 520 cm^{-1}. In addition, in the range below 500 cm^{-1}, the MO sample exhibited peaks at 473 cm^{-1} and 422 cm^{-1}, while the control sample exhibited a peak at 471 cm^{-1} and 417 $cm^{-1}$and the MA sample exhibited a peak at 472 cm^{-1}.(Table 7),

Table 7 : Allocation of infrared bands from the spectra of the various treatments.

Absorption	CO	MO	MA	Functional groups
3600-3000 cm^{-1}	3417	3588; 3564; 3544; 3524; 3502; 3480; 3443; 3421; 3398; 3362	3524; 3501; 3446; 3423; 3400	OH stretching hydroxyl groups (alcohol, phenol and polysaccharides)
3000-2800 cm^{-1}	2929	2929	2965; 2925	symmetrical and asymmetrical C-H stretching vibrations of methylene (CH2)
2400-2000 cm^{-1}	2346; 2305; 2161; 2137	2377; 2346; 2318; 2140; 2093	2346; 2309	stretching vibrations of -C≡N -N=N+=N- and -C≡C-
1750-1650 cm^{-1}	-	1651	1738; 1651	C=O
1650-1550 cm^{-1}	1638	-	-	the stretching of C=OO- and aromatic C=C groups (phenol compounds and organosulfur)
1550-1400 cm^{-1}	1458; 1400	1545; 1459	1545; 459; 1417	COO- asymmetric stretching was observed in all spectral samples related to carboxylates
1450-1000 cm^{-1}	1335; 1265; 1107; 1062; 1028	1399; 1355; 1100; 1064; 1033	1335; 1271; 1108; 1070	CH2/CH3 bending (aliphatic compounds, organosulfur compounds), C-O; stretching (polysaccharides, phenolics), S=O stretching (sulfoxides in allicin)
1000-700 cm^{-1}	934; 814	946	-	Aromatic C–H Bending, (phenolic compounds)
700-600 cm^{-1}	-	669; 620	692	C–S stretching vibrations (organosulfur compounds)
600-500 cm^{-1}	594; 520	596; 545 521,	581; 547; 520	S–S stretching vibrations (disulfide bonds in organosulfur compounds), C–S stretching vibrations (complex organosulfur
below 500cm^{-1}	471; 417	473; 422	472	C-C bending

All the samples exhibiting a broadband peaking between 3600 and 3000 cm^{-1} appear to be consistent with the presence of OH stretching hydroxyl groups. It is possible that these groups may be attributed to the presence of alcohol, phenol and polysaccharides, as has been previously reported by [243, 244, 245]. The samples designated MO, MA and control exhibited symmetrical and asymmetrical C-H stretching vibrations of methylene (CH2) within the frequency range of 3000-2800 cm^{-1}. These vibrations are predominantly ascribed to lipids, carbohydrates and organosulfur [246, 247, 248]. Similarly, within 2400-2000 cm^{-1} region, stretching vibrations of -C≡N, -N=N+=N- and -C≡C- were observed in the spectra of all samples [247, 249].

The spectral band at 1738 cm^{-1} in the control and at 1736 cm^{-1} in the MA sample could be attributed to the absorption of the C=O bonds of the ester groups, which is related to the presence of the fatty acids [246]. The bands around 1600 cm^{-1} could be associated with the stretching of C=OO- and aromatic C=C groups, e.g., phenol compounds and organosulfur for all samples [246, 250, 152]. Between the region 1550- 1400 cm^{-1}, COO- asymmetric stretching was observed in all spectral samples related to carboxylates [255]. Between the range of 1550 and 1000 cm^{-1}, all samples showed peaks related to CH2/CH3 bending (aliphatic compounds, organosulfur compounds), C-O stretching (polysaccharides, phenolics), S=O stretching (sulfoxides in allicin) [247, 252].

It is interesting to note that only the MO and control samples showed absorption between 1000 and 700 associated with Aromatic C–H bending, indicating the presence of phenolic compounds [246].

It appears that both samples MO and MA showed an absorption band between 700-600 associated to C–S stretching vibrations from its organosulfur compounds [253]. Moreover, all samples exhibited peaks between the region 600-500 associated to S–S stretching vibrations (disulfide bonds in organosulfur compounds), C–S stretching vibrations (in complex organosulfur compounds) and S–S disulfide stretch in proteins [254]. Additionally, the presence of C-C bending below 500 absorption region [247].

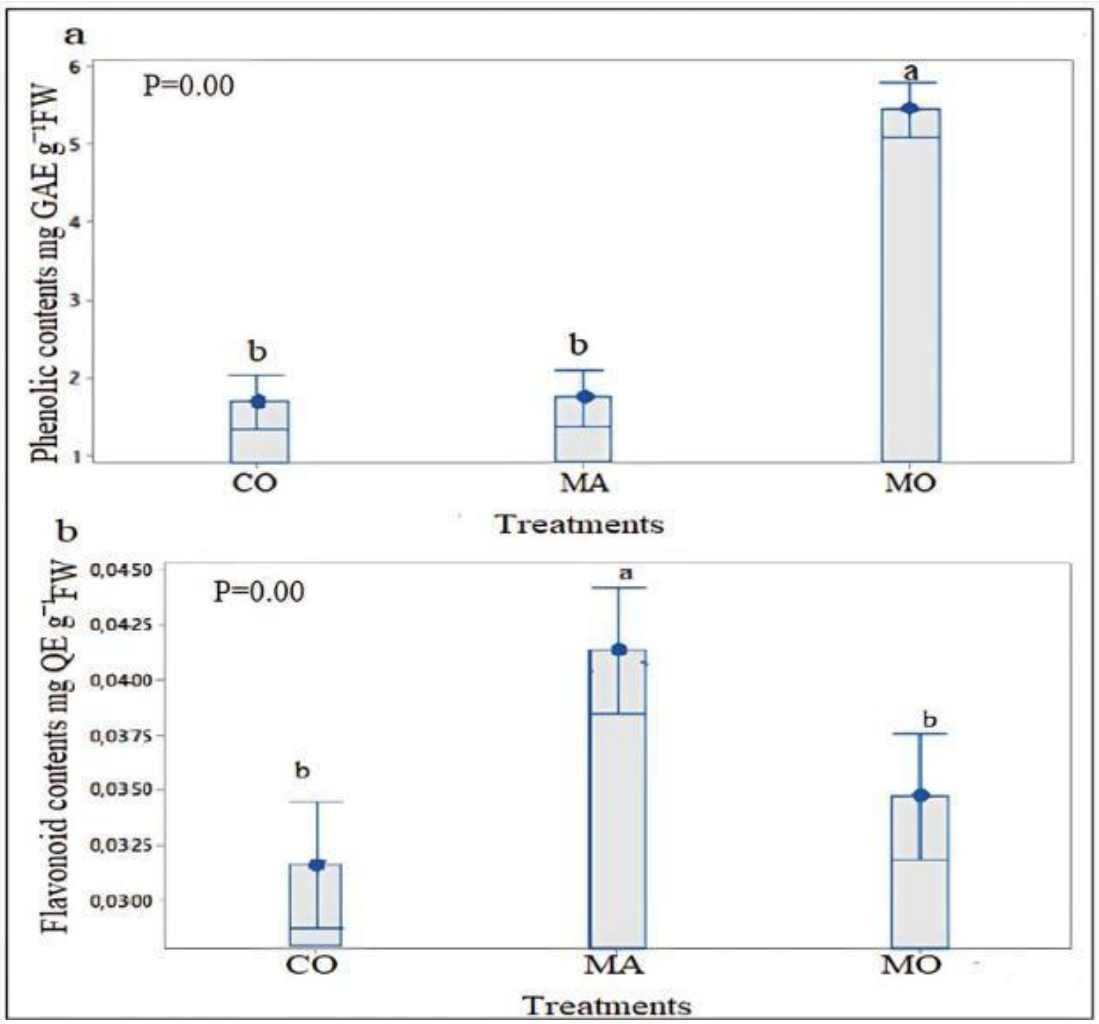
The garlic sample inoculated with the isolate MO clearly exhibited a high intensity of peaks. In contrast, the control sample exhibited the lowest intensity. Similarly, Dhalaria et al. [256] demonstrated that inoculated *Gomphrena globosa* plants with AMF exhibited a high intensity of peaks in comparison to the control, which indicates maximum metabolite accumulation [256]. Furthermore,Crișan et al. [257] found that, based on a detailed analysis of spectra, AMF has a positive influence on the development of *Iris germanica* L plants development and extractable compounds.

3.2.1.2 Total Phenolic and total Flavonoid contents

Total phenolic and total flavonoid contents were assessed to examine the impact of AMF isolates on the quality of garlic.

The inoculation of garlic plants with arbuscular mycorrhizal isolates was found to significantly affect the levels of total phenol (P=0.00, F=228.81), (appedix 30) and flavonoids content (P=0.03; F=18.26), (appendix 31). Garlic inoculated with the arbuscular mycorrhizal isolate MO exhibited the highest total phenol content (5.43 mg GAE g^{-1} FW), (Figure 25 a). In contrast, the MA isolate did not show any significant effect on total phenol content (1.75 mg GAE $g^{-1\ 1}$ FW) compared to the control (1.69 mg GAE g^{-1} FW), (Figure 25 a).

The analysis of garlic flavonoids indicated a relatively low quantity of these compounds. However, the isolate MA demonstrated the ability to enhance the flavonoid content (0,04 mg QE g^{-1} FW), (Figure 25 b), while the isolate MO (0.034 mg QE g^{-1} FW), (Figure 25 b) exhibited no discernible impact compared to the control (0.031 mg QE g^{-1} FW).



CO: control, MO, MA: different isolates of arbuscular mycorrhizal fungi.

Figure 25: Impact of arbuscular mycorrhizal isolates on the levels of phenolic (a) and flavonoids compounds (b) in garlic.

A substantial body of research indicates that symbiotic microorganisms exert a beneficial influence on the production of secondary metabolites in plants. This is exemplified by the findings of Mollaval *et al*. [258], who observed that the isolate of *Diversispora vermiformis* was effective in increasing the total polyphenol content in onions. Similarly, Lahbouki *et al.* [259] demonstrated that the total phenolic content was significantly enhanced by the application of AMF on the prickly pear cactus. The observed increase can be attributed to the enhanced uptake of macro- and micronutrients by arbuscular mycorrhizal fungi (AMF) due to their hyphal network within the root system. Lohse *et al*. [260] and Boutasknit *et al*. [261] have demonstrated the elevated concentration of total phenols can be attributed to the influence in the tricarboxylic acid cycle, which ultimately leads to the formation of sub-products that are employed in the integration of phenolic compounds. Additionally, Volpin *et al.* [262] demonstrated that AMF can induce the production of defence-related compounds (including phenolic compounds) in plants.

Conversely, Duc *et al.* [206] reported that arbuscular mycorrhizal fungi treatments did not significantly alter the content of proline and total phenols during plant growth of *Eclipta prostrata* L. In addition, Lee and Scagel [263] reported that inoculation of basil with AMF had no impact on the level of individual phenolic compounds or total polyphenolics, the results demonstrate that AMF does not consistently enhance the phenolic content. Instead, its impact is influenced by a multitude of variables, including cultivar, AMF isolate colonization, and mycorhiz*al* hyphal length.

The flavonoid content of the *Allium* extract exhibited considerable variation. In the present study, a low flavonoid concentration was identified, while the findings of Atif *et al.* [264] indicated the absence of flavonoids in garlic. Conversely, the results of Miean and Mohamed [265] indicated a high flavonoid concentration.

The results demonstrated that AMF isolate MA increased flavonoids compared to the control, while the isolate MO showed no effect. This finding is in line with the results reported by Perner *et al*. [266], who indicated that AMF may enhance flavonoids in bulb and root of onion. Furthermore, Mollavali *et* al. [268] demonstrated that plants inoculated with *Diversispora versiformis* exhibited an increase in QDG content (Quercetin-3,4'-O-diglucoside) compared to other AMF strains and the control treatment. However, further research is required to elucidate the relationship between AMF and the production of secondary metabolites in its host plant.

3.2.2 The potential impact of mycorrhizal fungi isolate on garlic chemical compounds cultivated in unsterilized soil.

The results of this study indicate that the inoculation of garlic with AMF isolate (MO) exerts a variable influence on functional groups and phenolic compounds of garlic extract.

3.2.2.1 Fourier Transform Infrared (FTIR) analysis of garlic

Fourier Transform Infrared (FTIR) spectroscopic analysis of the samples in the infrared range (4000-400 cm-1) indicated the presence of specific bands in each spectrum appendix 69)

The infrared region between 4000 and 400 cm^{-1} exhibited eight distinct absorption regions for MO and seven absorption regions for the control sample. A notable degree of variability was observed in the spectra recorded for the MO samples in comparison to the control sample with regard to the appearance and disappearance of peaks across different spectra, the sample MO exhibited a peak at 3251 cm^{-1} within the 3200-3400 cm^{-1} region, while the CO sample did not exhibit a peak in this region. Between the 2800-3100 cm^{-1} region, the sample MO showed two peaks at 2918 cm^{-1} and 2851 cm^{-1}, while the control sample showed one peak at 2928 cm^{-1}. The samples MO and CO both exhibited a peak at 1620 cm^{-1} and 1601cm^{-1} within 1600-1650 cm^{-1} region. Furthermore, the samples MO and CO both demonstrated peak at 1450 cm^{-1} withing the region 1615-1450 cm^{-1}, two peaks at 1128 cm^{-1} and 1125 cm^{-1} withing 1200-1020 cm^{-1}, and three peaks at 1020 cm^{-1} and 1016 cm^{-1} withing the region 990-1050 cm^{-1}. In 670-900 cm^{-1} region, the MO sample exhibited three peaks at 866 cm^{-1} and 816 cm^{-1} and 778 cm^{-1}, while the CO sample exhibited two peaks at 819.0 4 cm^{-1} and 781.36 cm^{-1}. In the range of 670 to 400 cm^{-1}, the MO sample exhibited peaks at 615, 592, and 421, while the CO sample displayed peaks at 781 cm^{-1}, 592 cm^{-1}, 527 cm^{-1}, 482 cm^{-1}, 462 cm^{-1}, 431 cm^{-1}, and 414 cm^{-1} (Table 8).

Table 8: Allocation of infrared bands from the spectra of the various treatments.

Absorption	CO	MO	Functional groups
3200-3400cm^{-1}	-	3215	O-H stretching hydroxyl (phenol)
2800-3100 cm^{-1}	2928	2918; 2851	C-H stretching vibration lipid
1600-1650 cm^{-1}	1620 1601	1620 1601	Stretching vibrations of carboxylic acids and amino groups
1615-1450 cm^{-1}	1450	1450	CH3 groups.
1200-1020 cm^{-1}	1128 ;1125	1128; 1125	C-OH (alcohol) stretch,
990-1050 cm^{-1}	1020; 1016	1020; 1016	Aliphatic phosphates (P-O-C stretch)
670–900 cm^{-1}	819.04 781.36	866;816; 778	Aromatic ring C-H
670- 400 cm^{-1}	781; 592; 527; 482; 462; 431	615, 592, 421	Aliphatic organohalogen

In the region between 3200-3400 cm^{-1}, the MO sample showed band at 3215.81cm^{-1}. This was attributed to the O-H stretching vibration indicating the presence of hydroxyl (alcohol and phenol) [268]. In contrast, the control sample showed no absorbance characteristics within this region. The control and MO samples showed a bands in the region 2800-3100 cm^{-1}, which was attributed to C-H stretching vibration indicating the presence of lipid [269].

In addition, the MO and control extracts showed peaks in the region 1600-1650 cm^{-1}, which was attributed to the presence of stretching vibrations of carboxylic acids and amino groups [270].

Furthermore both samples exhibited bands between 1615 cm^{-1} and 1450 cm^{-1} indicative of an aromatic ring stretch, this region presented CH3 groups [271, 272].

The samples MO and the control exhibited a band at 1128.94cm^{-1}, and 1125.92 cm^{-1} these correspond to the C-OH (alcohol) stretch, which has a typical frequency range of 1200-1020 cm^{-1} [271, 272].

Samples MO and the control, which were within the range of 990-1050cm^{-1}, exhibited a bands attributed to aliphatic phosphates (P-O-C stretch) [273].

Both samples demonstrated an absorbance within the range of 670–900 cm^{-1}, The aromatic ring C-H band in the MO and control samples was identified [272].

Samples within the range of 670 to 400 cm^{-1} exhibited bands that were indicative of the aliphatic organohalogen type [272].

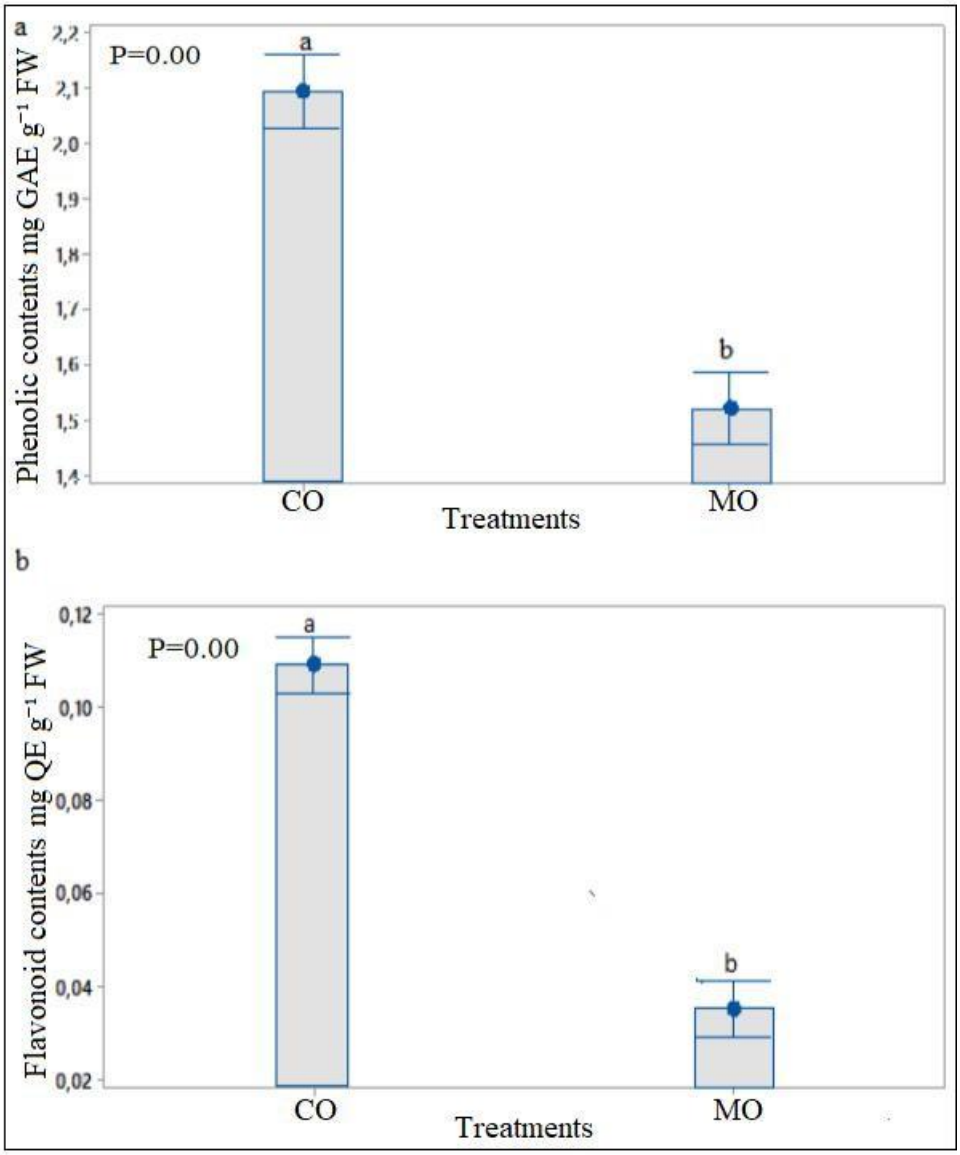
3.2.2.2 Total Phenolic and total Flavonoid Contents

Total phenolic and total flavonoid contents were assessed to examine the impact of AMF isolate MO on the quality of garlic.

The analysis of variance for total phenols (P=0.00; F= 295.38) (Appendix 32) and total flavonoids (P=0.00; F= 574.55), (Appendix 33) demonstrated a statistically significant variability between the inoculated isolates and the control.

The inoculation of garlic with the isolate MO resulted in a statistically significant reduction in the total phenolic content (1.521 mg GAE g^{-1} FW), (Figure 26 a) when compared to the control (2,09 mg GAE g^{-1} FW), (Figure 26 b).

Similarly, a reduction in the flavonoid content of garlic inoculated with MO isolates with value of (0,035 mg QE g^{-1} FW), (Figure 26 b) when compared to the control (0,109 mg QE g^{-1} FW), (Figure 26).



CO: control, MO, MA: different isolates of arbuscular mycorrhizal fungi.

Figure 26: Impact of arbuscular mycorrhizal isolate on the levels of total phenols (a) and flavonoids compounds (b) in garlic.

AMF has the potential to significantly enhance a plant's biological activity, which in turn could facilitate the exchange of nutrients between the two partners. This could potentially offer a number of benefits, including the accumulation of bioactive metabolites. Nevertheless, the current study did not provide any evidence indicating a positive impact of AMF on the accumulation of total phenol and flavonoid compounds in garlic bulbs.

These findings are consistent with those of Golubkina *et al*. [274], who observed that the inoculation of arbuscular mycorrhizal fungi in garlic plants had no effect on phenolic and flavonoid compounds in garlic. Furthermore, a comparable outcome was documented by Najar *et al*. [275], who observed that AMF treatment led to a decrease in flavonoids

content in *Spinacia oleracea* plant however a slight increase in total phenolic compounds was also observed

The authors propose that the observed variations in polyphenol and flavonoid content may be attributed to a combination of genetic differences, as well as the influence of disparate environmental stress conditions and agricultural practices, which collectively affect the chemical composition of plants [276, 277].

3.2.3 The potential impact of *Trichoderma asperellum* on chemical compounds of garlic

The results of this study indicate that the inoculation of garlic with *Trichoderma asperellum* isolates (T1, T2 and T3) exerts a variable influence on functional groups and phenolic compounds of garlic extract.

3.2.3.1 Fourier Transform Infrared (FTIR) analysis of garlic

The Fourier transform infrared analysis showed the presence of different functional groups, as evident from the distinct vibrations observed in the IR spectrum of garlic samples (appendix 70). The absorption region of functional groups exhibited spectral variability: shifts in band positions with appearance/disappearance of peaks, and variations in absorption intensity than control. All treated samples displayed eight absorption ranges, except the control and T3 samples. The control sample showed an additional absorption between 2400-2000 cm^{-1}, while the T3 sample showed an additional absorption between 1870-1650 cm^{-1} (Table 9).

In terms appearing and disappearing of peaks across different spectra, significant variability was observed in the spectra recorded for all samples, In the range of 3700-3100 cm^{-1}, both the control sample and the T1 sample exhibited four peaks, the control sample exhibited a peak at 3278.76 cm^{-1}, 3309.62 cm^{-1}, 3332.76 cm^{-1} and 33. 71.34 cm^{-1}, the T1 sample exhibited peaks at 3290.33 cm^{-1}, 3309.62 cm^{-1}, 3332.76 cm^{-1}, 3355.91 cm^{-1}, while the T2 and T3 samples displayed only two peaks at 3301.91 cm^{-1}, 3355.91 cm^{-1}, 3274.90 cm^{-1} and 3332.76 cm^{-1}, respectively. However, within the range of 3000-2800 cm^{-1} , the T3 sample exhibited two peaks at 2920.03 cm^{-1} and 2850.59 cm^{-1}. In contrast, the control, T1 and T2 samples displayed a single peak. at 2927.74 cm^{-1} for control and T1 while at 2931.60 for the sample T2.

In the range of 2400-2000 cm^{-1}, the control sample was the only one to show peaks at wave numbers 2113.84 cm^{-1}, 2306.7 cm^{-1}, 2345.28 cm^{-1} and 2376.14 cm^{-1}. Conversely, in the range of 1870-1650 cm^{-1}, only the sample T3 exhibited a peak at 1654.81 cm^{-1}.

The range of 1550-1300 cm^{-1} exhibited peaks at 1338.51 cm^{-1}, 1396.37 cm^{-1}, 1458.80 cm^{-1} and 1338.51 cm^{-1}, 1396.37 cm^{-1}, 1458.08 cm^{-1}. The T2 sample exhibited a peak at 1338.51 cm^{-1}, 1396.37 cm^{-1}, 1458.08 cm^{-1}, 1361.65 cm^{-1}, while the control sample exhibited a peak at 1411.80 cm^{-1}, 1458.08 cm^{-1}.

the samples T1, T2 and control exhibited peaks at 1026.06 cm^{-1}, 1056.92 cm^{-1}, 1130.21 cm^{-1}, 1218.93 cm^{-1} and 1265.22 cm^{-1} between the range 1300-1000 cm^{-1}. In contrast, sample T3 demonstrated a peak at 1022.20 cm^{-1}, 1056.92 cm^{-1}, 1130.21 cm^{-1} and 1218.93 cm^{-1}.

In the range of 1100-800 cm^{-1} , all samples exhibited the same peaks at wavenumbers 817.76 cm^{-1}, 867.91 cm^{-1}, 929.63 cm^{-1}, 1022.20 cm^{-1} and 1056.92 cm^{-1}.

In the range of 1000-650 cm-1, all samples exhibited peaks at 817.76 cm^{-1}, 867.91 cm^{-1}, 929.63 cm^{-1}, while the T3 sample displayed an additional peak at 659.6 cm^{-1}. Between 800-400 cm^{-1} range all samples showed same number of peaks, The sample T1 demonstrated peaks at 493.74 cm^{-1}, 528.46 cm^{-1}, and 590.18, while the sample T2 demonstrated peaks at 497.60 cm^{-1}, 536.17 cm^{-1}, and 594.03 cm^{-1}.

The sample T3 demonstrated peaks at 505.31 cm^{-1}, 528.46 cm^{-1}, and 659.61 cm^{-1}, and the control sample demonstrated peaks at 470.60 cm^{-1}, 532.32 cm^{-1}, and 601.75 cm^{-1}.

Table 9 :Allocation of infrared bands from the spectra of the various treatments.

Absorption	T	T1	T2	T3	Functional groups
3700-3100 cm⁻¹	3278.76 3309.62 3332.76 3371.34	3290.33 3309.62 3332.76 3355.91	3301.91 3355.91	3274.90 3332.76	O-H or N-H stretching vibrations (phenolic compounds proteins and polysaccharides).
3000-2800 cm⁻¹	2927.74	2927.74	2931.60	2920.03 2850.59	symmetrical and asymmetrical C-H elongation vibrations of methylene (CH2)
2400-2000 cm⁻¹	2113.84 2306.7 2345.28 2376.14	_	_	_	-C≡N, -N=N+=N- and -C≡C- stretching vibrations
1870-1650 cm⁻¹	_	_	_	1654.81	The amides stretching vibrations of the peptide and the vibrations of flavonoids and their derivatives
1650-1550 cm⁻¹	1546.80 1639.38	1616.24	1627.81	1569.95 1627.81	C=C, C=N, NH
1550-1300 cm⁻¹	1411.80 1458.08	1338.51 1396.37 1458.08	1338.51 1396.37 1458.08 1361.65	1338.51 1396.37 1458.80	C-H functional groups
1300-1000 cm⁻¹	1026.06 1056.92 1130.21 1218.93 1265.22	1026.06 1056.92 1130.21 1222.79 1269.07	1026.06 1056.92 1130.21 1222.79 1269.07	1022.20 1056.92 1130.21 1218.93	Functional groups CH3, CH2, C-O-C, C-OH, S=O, P=O, and C-F
1100- 800 cm⁻¹	817.76 867.91 929.63 1026.06 1056.92	813.90 867.91 929.63 1026.06 1056.92	817.76 867.91 929.63 1026.06 1056.92	817.76 867.91 929.63 1022.20 1056.92	Si-O and P-O
1000 -650 cm⁻¹	813.90 867.91 929.63	817.76 867.91 929.63 659.61	817.76 867.91 929.63	817.76 867.91 929.63 659.61	=C-H -NH Aromatic compounds Aliphatic group Alkenes
800 -400 cm⁻¹	470.60 532.32 601.75	493.74 528.46 590.18	497.60 536.17 594.03	505.31 528.46 659.61	C-halogen Aromatic rings

The preliminary phytochemical screening of the crude garlic powder revealed the presence of various phytochemicals, including phenolic compounds with alcohol bonds exhibiting O-H or N-H stretching vibrations, as well as proteins and polysaccharides

containing aliphatic primary amine. These were detected in the region between 3650-3200 cm^{-1} [247, 248].

Between 3000-2800 cm^{-1}, symmetrical and asymmetrical C-H elongation vibrations of methylene (CH2) were also detected. These vibrations are mainly attributed to lipids, carbohydrates, and nucleic acids [247]. -C≡N, -N=N+=N- and -C≡C- stretching vibrations were observed between 2400-2000 cm^{-1} region [247, 249].

The amides stretching vibrations of the peptide and the vibrations of flavonoids and their derivatives were detected between 1870-1650 cm^{-1} [249, 278, 279].

The bending vibrations of C-H functional groups, including CH3, CH2 and CH, were observed between the range of 1500-1300 cm^{-1} [280]. Additionally, the functional groups CH3, CH2, C-O-C, C-OH, S=O, P=O, and C-F were observed in the range of 1300-1100 cm^{-1} [247]. C=C, C=N and NH stretching vibrations were observed between 1650-1550 [278]. Elongation vibrations of bromo-aliphatic were observed between the region 700-600 cm^{-1}, according to Nandiyanto *et al.* [181] stretching vibration aliphatic iodinated alkyl halide compounds were observed between 600-500 cm^{-1}as well as C-C bending below 500 absorption region [247]. The IR spectrum of the treated sample T3 displayed an additional absorption region between 1870-1650 cm^{-1}, assigned to C=O stretching vibrations of the peptide (Amides I) and flavonoids and their derivatives which explains their high contents of phenolic compounds [279], which provides better antioxidant activity than other treatments and control. The garlic control sample also displayed an additional absorption region between 2400-2000 cm^{-1} assigned to nitrile and azide groups [249]. This could indicate the potential of *Trichoderma* strains in bio-remediating these compounds, which explain the high radiation absorption in the control sample, despite the low antioxidant activity compared to the treated sample T3 [282].

The infrared spectra similarities indicate similarities in chemical composition, differences in band shapes and absorption intensities can be explained by changes in chemical characteristics resulting from the application of *Trichoderma,* these results are supported by Wei *et al* [283], who reported changes in the FTIR spectra of treated wheat leaves attributed to the effect of static magnetic field (SMF) treatments on the molecular composition and structure of the leaves.

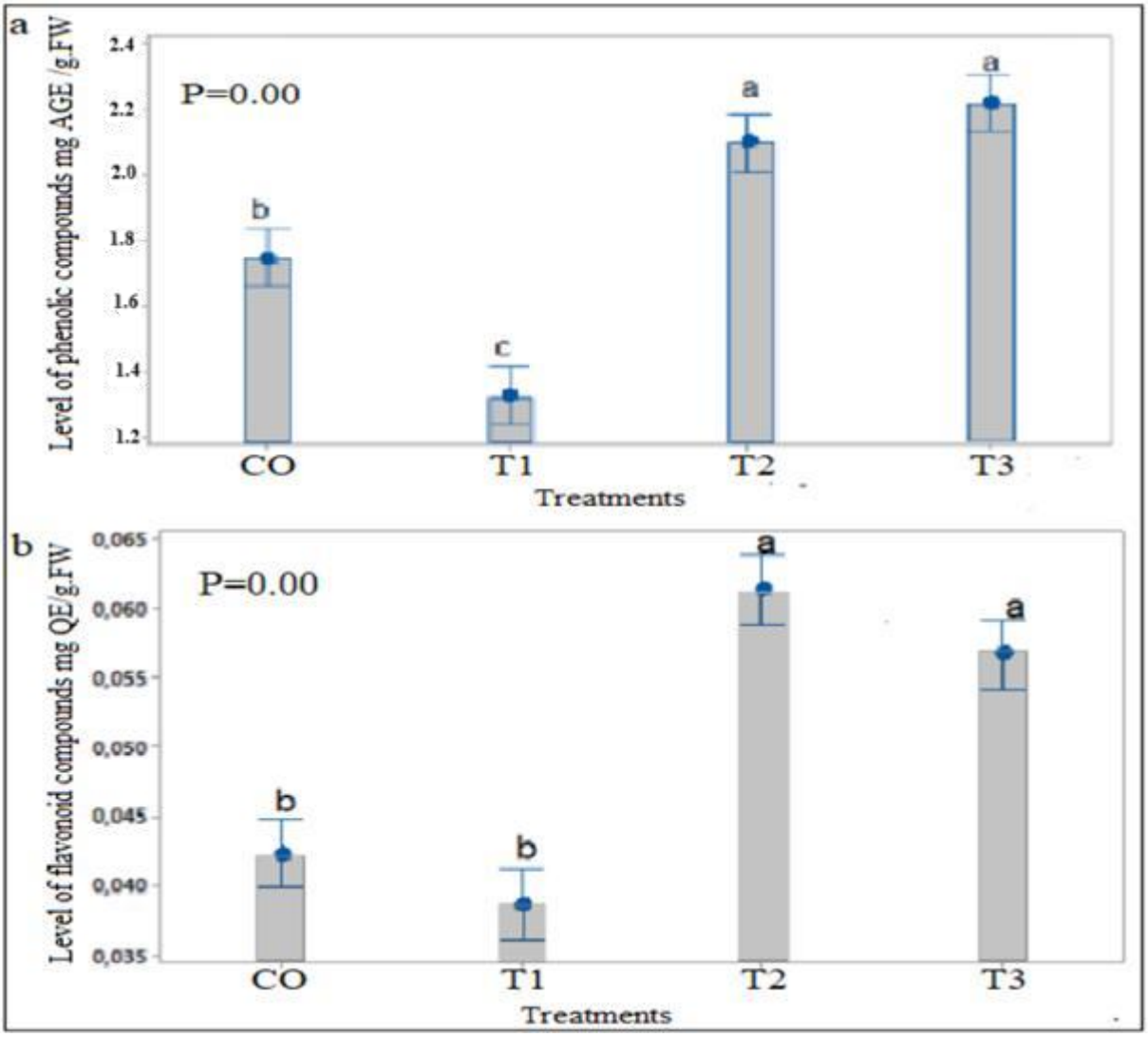
3.2.3.2 Total phenolic and total flavonoid contents

Total phenolic and total flavonoid contents were assessed to examine the impact of T*richoderma* isolates on the quality of garlic.

The statistical analysis indicated significant difference in the levels of total polyphenol (P= 0.00; F= 110.83) (appendix 34) and total flavonoids (P=0.00; F= 100.43) (appendix 35) between the *Trichoderma* isolates and the control sample.

The results of the study indicate that the application of treatments T2 and T3 on garlic plants led to a significant increase in their polyphenol and flavonoid contents. Specifically, the polyphenol content increased to 2.2 mg GAE g^{-1} FW and 2.9 mg GAE g^{-1} FW (Figure 27a), while the flavonoid content increased to 0.06 mg QE g^{-1} FW and 0.056 mg QE g^{-1} FW (Figure 27b), respectively.

Conversely, the application of T1 isolate significantly decreased the phenolic content 1.3 mg GAE g^{-1} FW , with no significant difference in flavonoid content 0.0386 mg QE g^{-1} FW in garlic bulbs than control (1.7 mg GAE g^{-1} FW, 0.042 mg QE g^{-1} FW) (Figure 27) respectively.



T: control, T1,T2,T3 different treatments of *Trichoderma asperellum*.

Figure 27: Impact of *Trichoderma asperellum* isolates on the levels of phenolic (a) and flavonoids compounds (b) in garlic extracts.

Inoculation with *Trichoderma asperellum* isolates T3 and T2 increased the synthesis of flavonoids and phenolic compounds in garlic bulbs. Several authors have reported the positive effect of *Trichoderma* on the accumulation of polyphenols and flavonoids in various plants, such as onion bulbs, *Passiflora caerulea*, beans, grapes, tomatoes, and olive leaves [284], in contrast to the *Trichoderma* isolate T2 showed a decrease in phenolic compounds and no effect on flavonoid compounds than control.

Vukelićet *al*.[284] research supports this finding, indicating that the use of *T. harzianum* reduces the total phenolic compounds in both types of tomato cultivars N*arvik* and G*ružanskizlatni,* and explained this variations in polyphenol and flavonoid contents of tomato fruit depend on the tomato cultivar.

This variation could be due to genetic differences, as well as different environmental stress conditions and agricultural practices that affect the chemical composition of plants. However, Ortega-García [228] reported that the accumulation of phenolic compounds in response to *Trichoderma* species has been associated with biochemical protection against plant pathogens.

3.2.4 The potential impact of PGPR on chemical compounds of garlic

Fourier Transform Infrared (FTIR) spectroscopic analysis of the two samples in the infrared range (4000-400 cm^{-1}) indicated the presence of specific bands in each spectrum. The infrared region between 4000 and 400 cm^{-1} exhibits six absorption regions for the control and T7 samples. With regard to the appearance and disappearance of peaks across different spectra, a notable degree of variability was observed in T7 sample spectra in comparison to the control (appendix 71).

The sample T7 exhibited a peak at 2928.68 cm^{-1} within the 2800-3100cm^{-1} region, while the CO sample did not exhibit a peak in this region. In contrast the CO sample showed a peak at 1601.05 between the 1600-1650 cm^{-1} region, the sample T7 showed two peaks at 1450.56 cm^{-1} and 1595.01 cm^{-1}, while the control sample showed one peak at 1450.56 cm^{-1} between the region 1615 -1450 cm^{-1} .

The samples T7 and CO both exhibited a peak at1125.51cm^{-1}, 1125.92 cm^{-1}, 1015.47 cm^{-1} and 1016.92 cm-1, within the 1200-1020 cm^{-1} and 990-1050 cm^{-1} regions. Furthermore, the T7 sample exhibited three peaks at 779.99 cm^{-1} , 870.39 cm^{-1}, 812 cm^{-1} while the CO sample exhibited two peaks at 819.04 cm^{-1} and 781.36 cm^{-1}.

In the range of 670 to 400 cm^{-1}, the T7 sample exhibited peaks at 779.99 cm^{-1}, 661.89 cm^{-1} and 591.80 cm^{-1} while the CO sample displayed peaks at 781.36 cm^{-1} , 592.56cm^{-1}, 527.83 cm^{-1}, 482.39 cm^{-1} , 462.33 cm^{-1} , 431.72 cm^{-1} and 414.12 cm^{-1} (Table10).

Table 10 :Allocation of infrared bands from the spectra of the various treatments.

Absorption	**CO**	**T7**	**Functional groups**
2800-3100cm^{-1}	-	2928.68	C-H stretching vibration indicating the presence of lipid
1600-1650 cm^{-1}	1601.05	-	stretching vibrations of carboxylic acids and amino groups
1615 -1450 cm^{-1}	1450.56	1450.56 1595.01	aromatic ring stretch
1200-1020 cm^{-1}.	1125.51 1125.92	1125.51 1125.92	C-OH (alcohol) stretch
990-1050 cm^{-1}	1015.47 1016.92	1015.47 1016.92	aliphatic phosphates (P-O-C stretch)
670–900 cm^{-1}	819.04 781.36	779.99 870.39 812	aromatic ring
670-400 cm^{-1}	781.36 592.56 527.83 482.39 462.33 431.72 414.12	779.99 661.89 591.80	aliphatic organohalogen type

Between 2800-3100 cm^{-1} region T7 and control samples showed a bands at 2928.68 cm^{-1} which was attributed to C-H stretching vibration indicating the presence of lipid [285]. In addition, the control sample showed peaks in the 1600-1650 cm^{-1} region at 1601.05 cm^{-1}, which was attributed to the presence of stretching vibrations of carboxylic acids and amino groups [270].

Both samples exhibited bands between 1615 and 1450 cm^{-1} indicative of an aromatic ring stretch at 1450.56 cm^{-1}, while T7 demonstrated an additional peak at 1595.01 cm^{-1} this region presented CH3 groups [271, 272].

The samples T7 and the control exhibited a band at 1125.51 cm^{-1} and 1125.92 cm^{-1}, these correspond to the C-OH (alcohol) stretch, which has a typical frequency range of 1200-1020 cm^{-1}.

Between 990-1050 cm^{-1} region samples T7 and the control, which were within the range of 990-1050 cm^{-1}, exhibited a band at 1015.47 cm^{-1} and 1016.92 cm^{-1} respectively, this was attributed to aliphatic phosphates (P-O-C stretch) [273].

Both samples demonstrated an absorbance within the range of 670–900 cm^{-1} at 779.99 cm^{-1} 870.39 cm^{-1}, and 812 cm^{-1} for the sample T7 and at 819.04 cm^{-1} and 781.36 cm^{-1} for the control sample, this region presented the aromatic ring [272].

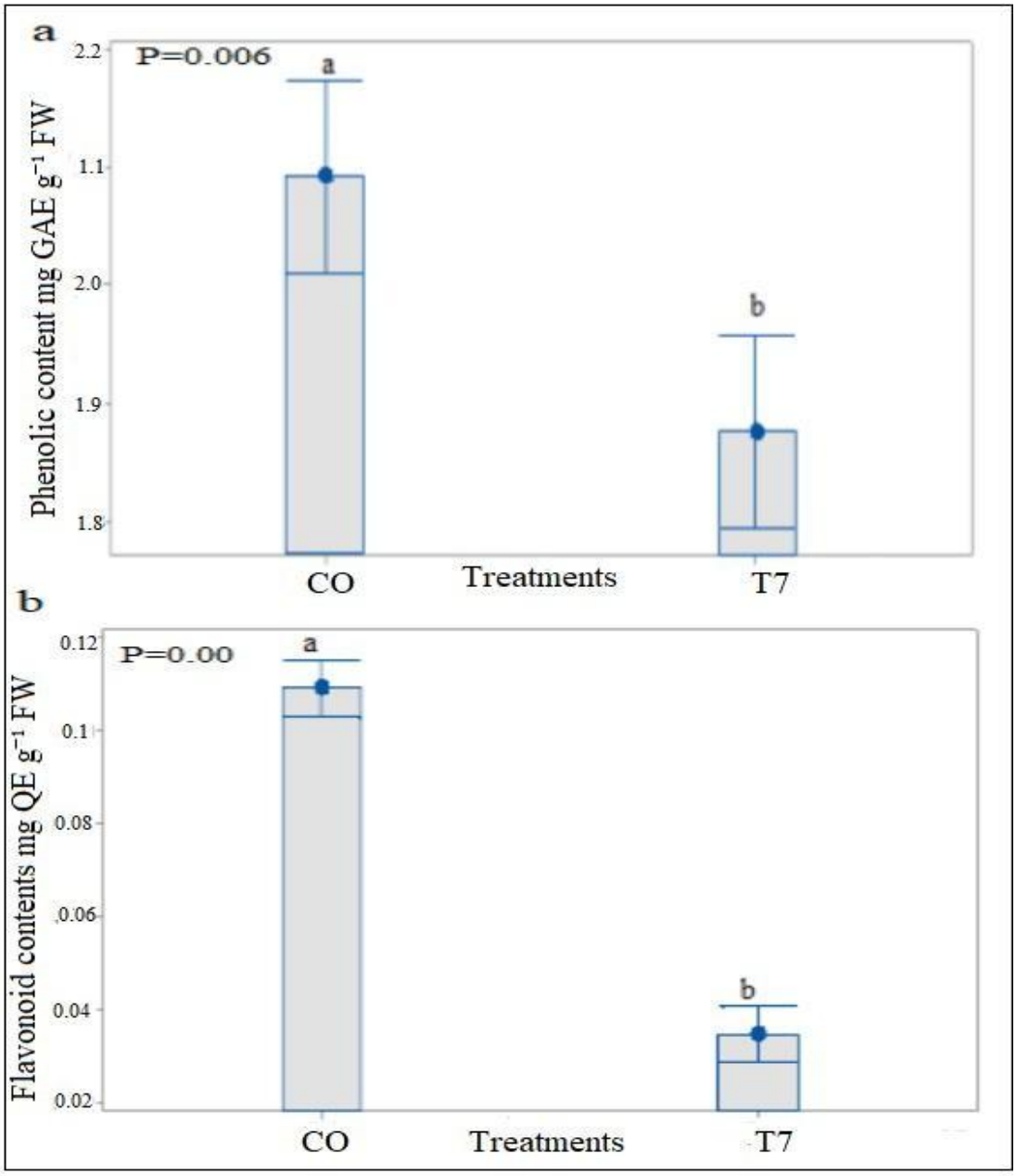
The sample M7 displayed peaks at 779.99 cm^{-1}, 661.89 cm^{-1}, and 591.80 cm^{-1} within the range of 670 to 400 cm^{-1} which exhibited bands that were indicative of the aliphatic organohalogen type while the control sample exhibited peaks at 781.36 cm^{-1}, 592.56 cm^{-1}, 527.83 cm^{-1}, 482.39 cm^{-1}, 462.33 cm^{-1}, 431.72 cm^{-1}, and 414.12 cm^{-1} [272].

3.2.4.2 Total phenolic and total flavonoid contents

Total phenolic and total flavonoid contents were assessed to examine the impact of PGPR isolate on the quality of garlic.

The analysis of variance for total polyphenols and total flavonoids revealed a significant discrepancy between the inoculated garlic and the control group, with a statistically significant variability in total phenol content (P=0.006; F= 27.29) (appendix 36) and in flavonoids content (P=0.00; F=584.75) (appendix 37).

The T7 isolates exhibited a reduction in phenolic content (1.87 mg GAE g^{-1} FW), (Figure 28 a) and flavonoid contents (0.03 mg QE g^{-1} FW); (Figure 28 b) in comparison to the control (2.09 mg GAE g^{-1} FW) and (0.1 mg QE g^{-1} FW) respectively (Figure 28 a,b).



a: Control sample, d: sample treated with treatment T7.

Figure 28: Impact of *PGPR* on the levels of phenolic (a) and flavonoids compounds (b) in garlic aqueous extracts.

The present study demonstrated a reduction in the total phenol and flavonoid content of garlic inoculated with PGPR, which is consistent with the findings of previous studies, including the study conducted by Miguel *et al.* [286], which showed that some PGPR inoculations result in a decrease in the concentration of these compounds in pepper seedling.

Conversely, the PGPR strains *Bacillus subtilis* and *Bacillus Licheniformis* promote flavonoid production enhance fruit quality, Furthermore, it was demonstrated that PGPR *B. pumilus* was more efficacious in the production of phenolic compounds when subjected to unfavorable environmental conditions.

Secondary metabolites, including polyphenols, flavonoids, and antioxidants, frequently constitute an integral component of a plant's intrinsic defense mechanisms, particularly in response to abiotic and biotic stressors. A reduction in antioxidant compounds in inoculated plants may be indicative of optimal nutrient conditions [287].

The presence of variations in band shapes and absorption intensities in the infrared spectra is indicative of alterations in chemical characteristics, consequent to the application of endemic microorganisms. In a similar manner, the antioxidant compounds present in the garlic extract were found to be enhanced by the isolates T2, T3 and MO, suggesting that these isolates may contribute to the enhancement of biochemical responses in plants, which could represent a valuable strategy for the improvement of plant health and productivity in sustainable agriculture.

3.3 Evaluation of antioxidant and antimicrobial activities of garlic inoculated with endemic microorganisms

The objective of this research is to gain a deeper understanding of the potential impact of endemic microorganisms on the antioxidant and antimicrobial properties of garlic to contribute in the development of natural alternatives to conventional antibiotics.

3.3.1 Evaluation of antioxidant and antimicrobial activities of garlic inoculated with mycorrhizal fungi cultivated in sterilized soil

The antioxidant and antimicrobial activity were assessed to examine the impact of arbuscular mycorhizal fungi cultivated on sterilized soil on garlic quality.

3.3.1.1 Antioxidant activity of garlic

DPPH scavenging activity and IC50 values were determined to examine the impact of mycorrhizal fungi isolates on the antioxidant activity of garlic.

The application of arbuscular mycorrhizal fungi isolates significantly impacted the DPPH scavenging activity (P=0.00 F=161.41), (Appendix 38) and IC50 value (P=0.00; F=9753.57), (Appendix 39). The DPPH scavenging activity of garlic plants at 2000 µg/ml treated with the MO and MA isolates was found to have increased to (56.74 %) and (54.43 %), respectively, in comparison to the control (46.82%), (Table11) with no significant difference between the two isolates. The garlic extract treated with the MA isolate exhibited the lowest and most significant IC50 value (0.56 mg ml^{-1}), followed by

the MO isolate (0.63 mg ml^{-1}) in comparison to the control (1.11 mg ml^{-1}). Nevertheless, these values did not reach the value of 0.01 µg ml^{-1} exhibited by ascorbic acid, (Table 11).

Table 11: **Impact of arbuscular mycorrhizal fungi isolates (MO and MA) on the DPPH scavenging activity and IC50 in garlic.**

Treatments	MO	MA	CO	Ascorbic Acid
DPPH scavenging activity %	56.74 b	54.43 b	46.82 c	99 a
IC50 mg/ml.	0.63 b	0.56 c	1.11 a	0.011 d

A number of researchers have reported comparable outcomes, indicating the impact of arbuscular mycorrhizal fungi on diverse plant species. This includes the study conducted by Rasouli *et al*. [288], which demonstrated that mycorrhizal inoculation elevated the levels of total phenols, chlorogenic acid, and antioxidant activity in artichoke leaves. Similarly, Albrechtova *et al*. [289] found that arbuscular mycorrhizal fungi significantly increased the total antioxidant capacity of onion bulbs. However, despite extensive research, no documented reports have been found which describe the beneficial effects of arbuscular mycorrhiza on the antioxidants found in garlic. Golubkina *et al*. [290] reported that a field trial conducted in the Chechen Republic (Russia) demonstrated an increase in antioxidant activity in *Allium cepa* bulbs. However, no significant impact on the antioxidant activity of garlic was observed. In a study conducted by Avio *et al*. [291], it was observed that the inoculation of lettuce plants with arbuscular mycorrhizal fungi resulted in the accumulation of greater quantities of antioxidant compounds. This phenomenon may be attributed to the interaction of the mycorrhizal fungi with the host plant's metabolism, leading to the increased production of phytochemicals and antioxidant molecules. Consequently, this alters the composition of the plant's secondary metabolites and enhances its antioxidant potential.

While in a study published by Jiang *et al.* [292] demonstrated that arbuscular mycorrhizal fungi influence the concentration of phytohormones, including jasmonic acid, gibberellic acid, and cytokinins. These changes enhance the absorption of essential nutrients and promote the production of antioxidant compounds.

Meriga *et al.*[293] and Mamun *et al.*[294] reported that the increase in phenol concentration is accompanied by an increase in antioxidant activity. The primary contributors to observed antioxidant activity are the phenolic constituents found in plants,

including flavonoids, phenolic acids, and phenolic diterpenes. However in our study the isolate MA was observed to enhance antioxidant activity concomitant with a reduction in polyphenol concentration. This finding provide a support to the results reported by Fredotović and Puizina [295], which it can be concluded that the observed antioxidant effects are primarily due to the presence of organosulfur compounds. Additionally, Albrechtova *et al.* [289] have reported that arbuscular mycorrhizal fungi may facilitate the production of organosulfur compounds in field conditions. Nevertheless, in the present study, the total antioxidant capacity was measured, rather than specific compounds. Consequently, it was not possible to determine which chemical compound arbuscular mycorrhiza was responsible for the observed increase in antioxidant activity.

3.3.1.2 Antimicrobial activity of garlic

The antimicrobial activity of aqueous garlic extracts was evaluated by measuring the diameter of the zones of inhibition of the strains under study. The results of the analysis of variance indicated that there were statistically significant differences in the inhibitory effect according to the treatments (P=0.00; F=37.92), the strains (P= 0.00; F=115.68) and the concentrations (P=0.00; F=110.68), (appendix 46). The treatment MO exhibited the most pronounced inhibitory effect, with a larger zone of inhibition than the control. While no significant difference was observed between the treatment MA and the control (Table 12). Furthermore, the yeast strain *Saccharomyces cerevisiae* exhibited greater sensitivity followed by *Candida albicans* yeast strains, suggesting that yeast strains may be more secretive than bacterial strains (Table 14, Figure 29). Furthermore, the degree of this activity increased in line with the concentration of the extracts (Table 13).

Table 12: Inhibitory effect of garlic extract inoculated with mycorrhizal isolates on microbial pathogens according to treatments.

Treatments	Inhibition zone
MO	28.22a(+++)
MA	24.27b(+++)
CO	24.16b(+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 13: Inhibitory effect of garlic extract inoculated with mycorrhizal isolates on microbial pathogens according to concentrations.

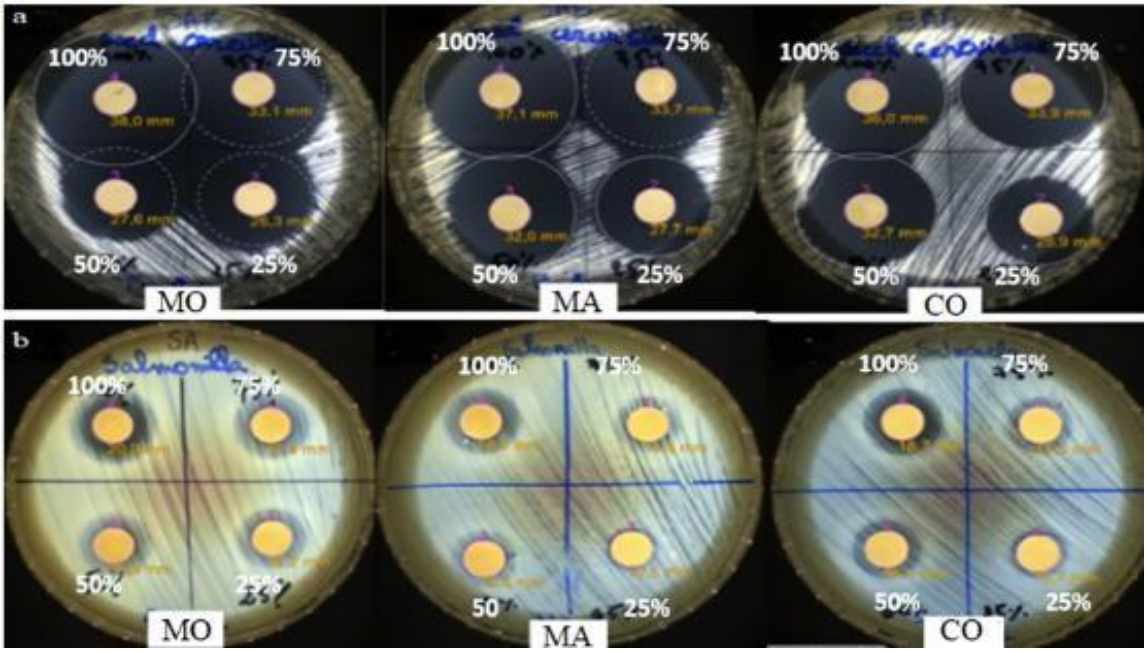
Concentrations %	Inhibition zone
100	31.2a (+++)
75	26.5b (+++)
50	24.01c (+++)
25	20.4d (+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 14: Inhibitory effect of garlic extract inoculated with mycorrhizal isolates on microbial pathogens according to strains.

Concentrations %	Inhibition zone
Saccharomyces cerevisiae	32.07a(+++)
Candida albicans	30.13ab(+++)
Bacillus subtilis	29.81ab (+++)
Escherichia coli	28.1b (+++)
Staphylococcus epidermidis	23.2c (+++)
Staphylococcus aureus	20.02d (+++)
Salmonella typhimyrium	15.3e (++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.



a: Saccharomyces cerevisiae b:Salmonella typhimyrium

Figure 29: Iinhibitory zones of the most and the least sensitive strains (a and b).

The results demonstrate that the aqueous extract of garlic, whether uninoculated or inoculated with arbuscular mycorhizal isolates MO and MA, is an effective treatment for microbial infections. However, the highest level of efficacy is observed at a concentration of 100%. These findings align with those of Emmanuel *et al.* [296], which demonstrate considerable antimicrobial efficacy against a range of bacterial strains, including *E. coli, S. typhi, S. aureus*, and *P. aeruginosa*, at a concentration of 100 mg/ml. Similarly, Uchida *et al.* [297] demonstrated that diverse garlic preparations exhibited a wide range of antibacterial activity against Gram-negative and Gram-positive bacteria, including *Escherichia* species, *Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, and Clostridium,* even fast-acidifying bacteria, such as *Mycobacterium tuberculosis*, have been observed to demonstrate sensitivity to garlic. Additionally, Ismai *et al*. [298] have shown that garlic possesses antimicrobial properties against a diverse array of gram-positive and gram-negative bacteria, as well as fungi and viruses.

In our study, Gram-positive bacteria demonstrated a greater sensitivity than Gram-negative bacteria to garlic extract. This result is also supported by the research of Emmanuel [296], who found that the bacteria *Escherichia coli*, *Salmonella typhirium*, and *Pseudomonas aeruginosa* exhibited lower sensitivity than *Staphylococcus aureus*. This may be due to the presence of an outer membrane that hinders access to antibacterial agents.

The findings of the present study demonstrate that the extract of garlic inoculated with the MO isolate has been shown to be more efficacious in the treatment of microbial infections

than the extract of garlic inoculated with the MA isolate. This suggests that the MO isolate enhances the phytochemical compound responsible for antimicrobial activity. This finding is corroborated by Dos Santos *et al.* [299], who reported that the methanolic extracts of *L. ferrea* fruits inoculated with *A. longula* exhibited superior *in vitro* antibacterial activity when compared to those from non-inoculated plants. This observation was made for all the bacterial strains under study. Conversely, Morelli *et al.* [300] demonstrated that the antimicrobial activity of the essential oil of the basil plant after AMF inoculation was not statistically significant. This may be attributed to an increase in phytochemicals that exert a relatively minor impact

3.3.2 Evaluation of antioxidant and antimicrobial activity of garlic inoculated with mycorrhizal fungi isolate cultivated in unsterilized soil

The antioxidant and antimicrobial activities were assessed to examine the impact of arbuscular mycorhizal fungi cultivated on unsterilized soil on garlic quality.

3.3.2.1 Antioxidant activity

DPPH scavenging activity and IC50 values determined the impact of the isolate MO on the antioxidant activity of garlic.

The application of arbuscular mycorrhizal fungi isolates significantly impacted the DPPH scavenging activity (P=0.001; F=88.23), (appendix 41) and IC50 (P=0.003; F=18.47) (appendix 42).

The inoculation of garlic with the isolate MO has been observed to exhibit the lowest antioxidant activity (50.39%) at concentration of 2000 µg/ml and the highest IC50 value (1.131 mg ml^{-1}) when compared to the control (56.31%) and (0.7 mg ml^{-1}) respectively and compared to acid ascorbic (99%) and (0.01 mg ml^{-1}) respectively (Table 15).

Table 15: **Impact of *arbuscular mycorrhizal isolates* (MO and MA) on the DPPH scavenging activity and IC50 in garlic cultivated in unsterilized soil.**

Treatments	MO	CO	Ascorbic Acid
DPPH scavenging activity %	50.39 b	56.31 c	99a
IC50 mg/ml.	1.13 a	0.7 b	0.011 c

Golubkina *et al*. [290] reported that a field trial conducted in the Chechen Republic (Russia) demonstrated no significant impact on the antioxidant activity of garlic was

observed. Moreover, Papoui *et al.* [303] observed that both *R. intraradicesand Diversispora* inocula significantly reduced the antioxidant content in lettuce by 20%. Furthermore, no significant differences were also found in the antioxidant capacity of green onion.

Conversely, study conducted by Dhalaria *et al.* [256] revealed that *Gomphrena globosa* plant inoculated with AMF exhibited the highest antioxidant activities when compared with the control plants. Similarly, Avio *et al*. [291] demonstrated that the inoculation of *Lactuca sativa* leaves with mycorrhiza resulted in an increase in antioxidant activity. Similarly, Rashidi *et al*. [304] observed elevated concentrations of phenol compounds and antioxidant activities in the reproductive organs and roots of *Ipomoea purpurea*, *Solanum nigrum* and *Digitaria sanguinalis* inoculated with AMF. In contrast, Charoonnart *et al.* [305] and Santander *et al*. [306] reported that inoculation of lettuce with AMF enhanced the antioxidant activity.

It might be the case that the reduction in antioxidant capacity of lettuce with AMF inoculation could be attributed to the different strains of AMF used in these experiments, or even the different cultivars combined with different AMF strains Papoui et *al*. [303]. It is also possible that lower antioxidant compound content may indicate less stressed plants, which could potentially reduce the positive effect of AMF [274]. However, it should be noted that the physiological mechanisms underlying the effect of AMF on antioxidant compound accumulation have not yet been fully identified.

3.3.2.2 Antimicrobial activity

The antimicrobial activity of aqueous garlic extracts was evaluated by measuring the diameter of the zones of inhibition of the strains under study. The results of the analysis of variance indicated that there were statistically significant differences in the inhibitory effect according to the strains (P=0.00; F=8.72) and the concentrations (P=0.016; F=3.55) Appendix 47.

However, no significant difference in the inhibitory effect according to treatments was observed (P=0.4; F=0.68) Appendix 47. The aqueous extract of garlic showed a notable inhibitory effect, although there were no significant differences between the inoculated garlic with the isolate MO and the control (Table 16). Furthermore, the bacterial strain *Staphylococcus aureus* was observed to exhibit the greatest sensitivity (36 mm), while bacterial strain *Salmonella typhimyrium* demonstrated the least sensitivity (11.8 mm) (Table 18, Figure 30). In addition, the extent of this activity was found to increase in accordance with the concentration of the extracts (Table 17).

Table 16: Inhibitory effect of garlic extract inoculated with mycorrhizal isolates on microbial pathogens according to treatments.

Treatments	Inhibition zone (mm)
MO	25.7a(+++)
CO	23.7a(++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 17: Inhibitory effect of garlic extract inoculated with mycorrhizal isolates on microbial pathogens according to concentration.

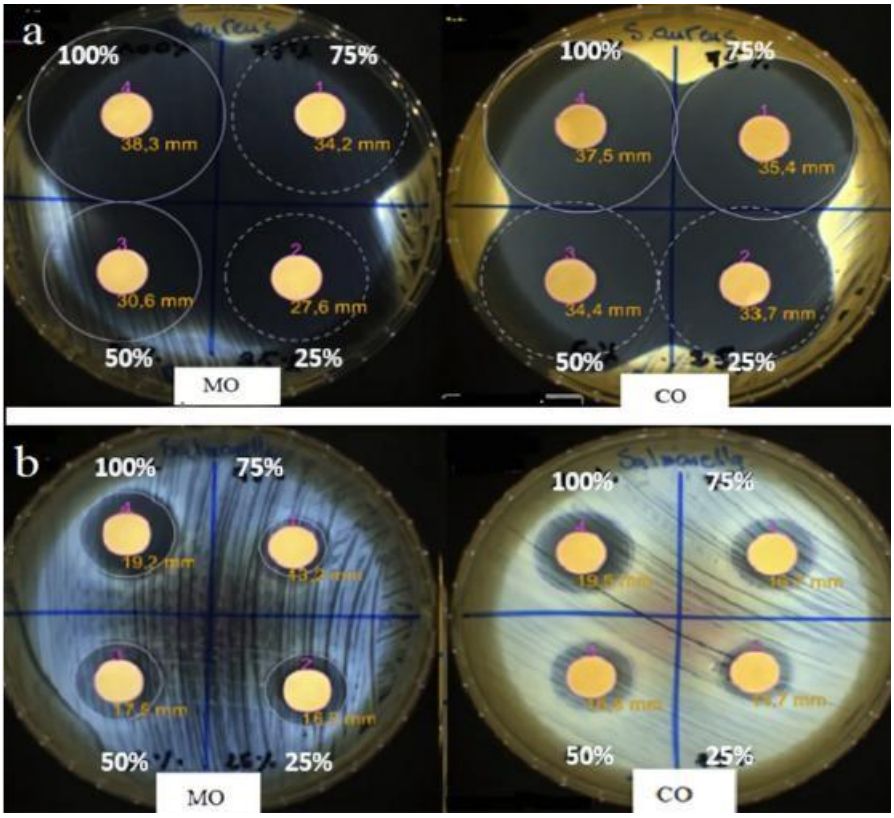
Concentrations %	**Inhibition zone (mm)**
100	31.08 a(+++)
75	24.5 b(+++)
50	23.01c(+++)
25	20.2 d (+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table18: The inhibitory effect of garlic extract on microbial pathogens, according to strains.

Strains	**Inhibition zone (mm)**
Staphylococcus aureus	36.7a(+++)
Staphylococcus epidermidis	31.5ab(+++)
Saccharomyces cerevisiae	30.5ab(+++)
Candida albicans	26.42ab(+++)
Bacillus subtilis	20.72bc(+++)
Escherichia coli	18.7bc(++)
Salmonella typhimyrium	11.8 (+)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.



a: ***Staphylococcus aureus, b: Salmonella typhimyrium***

Figure 30: Iinhibitory zones of the most and the least sensitive strains (a and b).

The findings indicate that the aqueous extract of garlic exhibited antibacterial properties against a diverse range of Gram-positive and Gram-negative bacteria and fungi. These findings are consistent with those of numerous other researchers, including Okunye *et al.* and Akullo *et al.* [307,189], Moreover, the results demonstrated that *S. aureus* exhibited the greatest sensitivity to garlic treatments. This finding is consistent with the observations of Yazgan *et al*. [308], who also reported that *S. aureus* was more susceptible to garlic. In contrast, the study by Anes *et al*. [309] indicated that *E. coli* was the most sensitive to garlic extract.

It has been demonstrated that AMF has the capacity to markedly enhance a plant's antimicrobial efficacy, including, Najar *et al.* [276] who reported AMF treatment also induced antibacterial properties of spinach plant against a range of fungi and bacteria, with the most potent effect observed against *Aspergillus flavus*, showing an increase of 38.5%. Conversely, dos Santos et al [310] observed that the extracts of *L. ferrea* fruits inoculated with *A. longula* exhibited greater potency in inhibiting the growth of Gram-negative bacteria. The zone diameters of inhibition exhibited a range of 2.48% to 7.56% greater than those observed in the non-inoculated *L. ferrea* fruit extracts. However, the presence of FMA does not consistently enhance the efficacy of plant extracts in inhibiting bacterial growth. The zones of inhibition produced by some of the methanolic extracts of

mycorrhizal *L. ferrea* fruits tested against *E. coli* were comparable to those formed by extracts of native *L. ferrea* fruits collected in the Caatinga region. Similarly, the results of the present study indicate that the isolate MO exhibited no significant difference in inhibitory effect compared to the control garlic. However in the present case it may be attributed to the presence of native arbuscular mycorrhizal fungi (AMF), which effectively neutralised the potential impact of the inoculated AMF.

3.3.3 Evaluation of antioxidant and antimicrobial activities of garlic inoculated with *Trichoderma asperellum*

Antioxidant and antimicrobial activities were assessed to examine the impact of *Trichoderma asperellum* on garlic quality.

3.3.3.1 Antioxidant activity

DPPH scavenging activity and IC50 values determined to examine the impact of the isolates of *Trichoderma asperellum* on the antioxidant activity of garlic.

The application of *T. asperellum* isolates significantly impacted the DPPH scavenging activity (P=0.00; F=3624.80) (appendix 42) and IC50 (P=0.00; F=9791.19) (appendix 43). Our study suggested that extract of garlic biomolecules possess a significant ability to reduce DPPH free radicals, indicating strong antioxidant activity. *Trichoderma* isolates T3 and T2 increased antioxidant activity of garlic extracts compared to control, while the isolate T1 decreased the antioxidant activity of garlic extract compared to control. Garlic extracts treated by isolates T3, T2, T1 and control inhibited the DPPH radical by 60.5 % and 59 % 53 % and 55 % respectively at 2000 µg/ml however the ascorbic acid used as reference inhibited the DPPH radical by 97.5 % at 2000 µg/ml (Table 19).

the analysis of variance revealed significant difference in the IC50 values of the antioxidant activity of the extract between the treatments and the control. The garlic extract treated with T3 isolate showed the lowest and most important IC50 value of 0.43 mg/ml, compared to the control's value 0.9 mg/ml. This was followed by T2 isolate with 0.65 µg/ml, and T1 isolate with 1.65 µg/ml. However those values couldn’t reach the value of ascorbic acid 0,01 µg/ml (Table 19).

Table 19: Impact of *Trichoderma* T1, T2 and T3 on the DPPH scavenging activity and IC50 in garlic cultivated in unsterilized soil.

Treatments	CO	T1	T2	T3	Ascorbic Acid
DPPH scavenging activity %	55% c	53% d	59% b	60% b	97.5 a
IC50 mg/ml.	0.9 b	1.65a	0.65c	0.43d	0.01e

In garlic extracts of control sample and inoculated samples, DPPH free radical scavenging inhibition and IC50 were significantly lower than the standard. These results correlated to many researchers funding, including Otunola and Afolayan [311] as well as akullo *et al.* [10].

The applied *Trichoderma asperellum* strains have a significant impact on the antioxidant activity of garlic.

This finding is in line with previous research. Singh *et al*. [312] provided definitive evidence that the extract of tomato fruit taken from a plant treated with *Trichoderma* exhibited significantly higher scavenging activity on DPPH radicals than the control. Similarly, Şesan *et al*. [313] reported that a plant inoculated with the highest concentration of *Trichoderma* consortium (108 cfu/mL) exhibited the highest antioxidant activity in DPPH assays.

Application of treatments T2 and T3 exhibited higher antioxidant activity than treatment T1 possibly due to increased total phenol and flavonoid accumulation. This result was supported by vukelić *et al.* [314] who reported that the use of *Trichoderma harzianum* as a biocontrol agent in tomato cultivation can have varying effects on tomato plant antioxidant activity depending on the specific compounds involved.

3.3.3.2 Antimicrobial Activity

The antimicrobial activity of aqueous garlic extracts was evaluated by measuring the diameter of the zones of inhibition of the strains under study. The results of the analysis of variance demonstrated a statistically significant difference in the inhibitory effect according to the various treatments (P=0.00; F=110.68), concentrations (P=0.00; F=115.68) and strains (P=0.00; F=37.92), (appendix 48). The treatments T3 and T2 exhibited the most pronounced inhibitory effect, with a larger zone of inhibition compared to the control (Table19, Figure 30).

Treatment T1 also demonstrated an important inhibitory activity, although it was not as pronounced as the other two treatments (Table 19). The results indicate that the bacteria *S. aureus* exhibited a greater sensitivity (32 mm), followed by the yeast strains

Saccharomyces cerevisiae (31 mm) and *Candida albicans (*28 mm) (Table 21, Figure 31). This suggests that *S. aureus* may be more secretive than fungal strains. Furthermore, the level of this activity increased in accordance with the concentration of the extracts (Table 20).

Table 20 : Inhibitory effect of garlic extract inoculated with *trichoderma* isolates on microbial pathogens according to treatments.

Treatments	Inhibition zone (mm)
CO	24,0145c (+++)
T1	27,1836b (+++)
T2	29,8511a (+++)
T3	29,2571a(+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 21: Inhibitory effect of garlic extract inoculated with *trichoderma* isolates on microbial pathogens according to concentration.

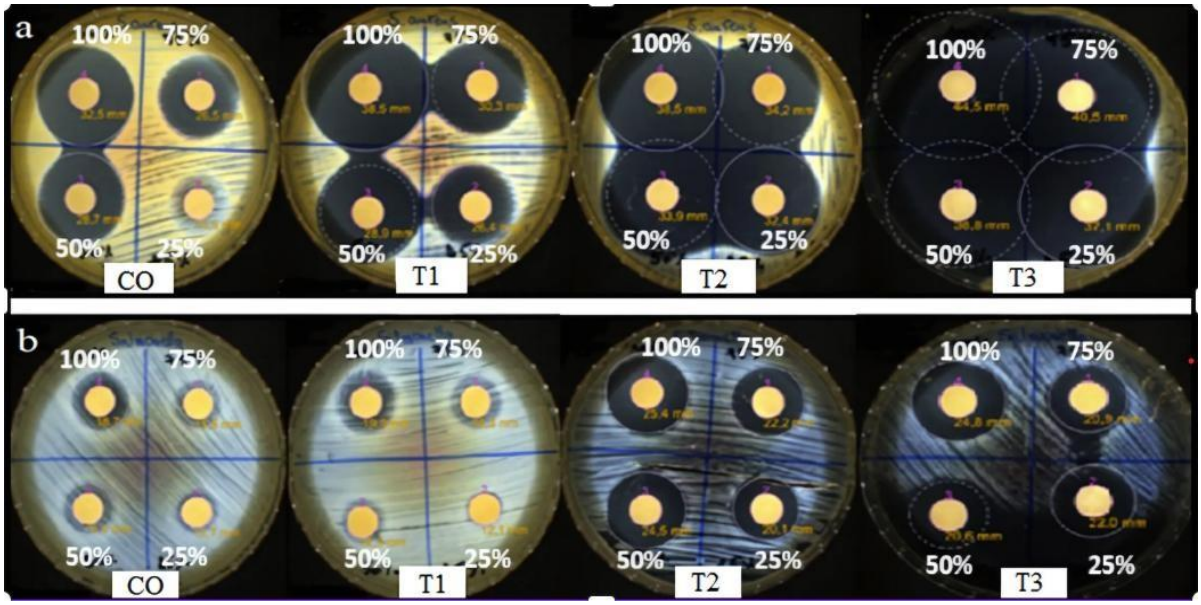
Concentrations %	Inhibition zone (mm)
100	32,6321a(+++)
75	28,5273b(+++)
50	26,7442c(+++)
10	22,4026d (+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 22: Inhibitory effect of garlic extract inoculated with *trichoderma* isolates on microbial pathogens according to strains.

Strains	inhibition zone
Staphylococcus aureus	32,6917a(+++)
Saccharomyces cerevisiae	31,2064ab(+++)
Candida albicans	28,8292bc(+++)
Bacillus subtilis	28,4275cd(+++)
Escherichia coli	26,9521cd(+++)
Staphylococcus epidermidis	25,9 d (+++)
Salmonella typhimyrium	19,0292e(++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.



a: ***Staphylococcus aureus, b: Salmonella typhimyrium***

Figure 31: Inhibitory zone of aqueous extracts of garlic inoculated with *T.asprellum* against the most and least sensitive strain

The findings suggest that the aqueous extract of garlic, whether inoculated with *Trichoderma asperellum* or not, may possess antibacterial properties against a range of Gram-positive and Gram-negative bacteria and yeast . These findings are consistent with those of numerous other researchers, including Akullo *et al.* [189], who observed that garlic extracts were highly effective against the three microorganisms tested in their study (*Staphylococcus aureus, Escherichia coli and Candida albicans*). Moreover, Yazgan *et al.* [308] demonstrated that garlic extracts were efficacious against all bacterial strains tested, including *Klebsiella pneumoniae, Salmonella paratyphi A, Staphylococcus aureus*, and *Enterococcus faecalis*. The present study demonstrates that the highest level of efficacy is observed at a concentration of 100%. This finding aligns with the conclusions of Safithri *et al*. [315] and Mohammed *et* al [316], who demonstrated that a higher concentration of garlic water extract exhibited a wider inhibition zone against *S. agalactiae, S. aureus*, and *E. coli*.

The findings revealed that the gram-positive bacteria exhibited heightened sensitivity in comparison to the gram-negative bacteria when subjected to the garlic extract. This outcome corroborates the observations documented by Nejad [317], which indicated that the antimicrobial efficacy of garlic is contingent upon the allicin compound. The compound was found to be threefold more potent against gram-positive bacteria than against gram-negative bacteria resulting in a limitation of the speed of RNA synthesis.

The aqueous extract of garlic inoculated with *Trichoderma asperellum* T5 and T11 exhibited the most pronounced inhibitory effect, followed by the extract of garlic

inoculated with MS in comparison to the control. This indicates that *Trichoderma asperellum* may facilitate the synthesis of the chemical compound responsible for antimicrobial activity, including allicin. Nevertheless, further analysis is necessary to substantiate this hypothesis. A substantial body of research has demonstrated that plants respond to *Trichoderma* colonization by producing and concentrating defensive compounds, including phytoalexins, flavonoids, terpenoids, phenolic by-products, aglycones, and additional antimicrobial compounds [318]. However, there is currently no evidence to confirm which antimicrobial compound *Trichoderma* affects to increase the antimicrobial activity of garlic.

3.3.4 Evaluation of antioxidant and antimicrobial activities of garlic inoculated with PGPR

Antioxidant and antimicrobial activities were assessed to examine the impact of PGPR on garlic quality**.**

3.3.4.1 Evaluation of the antioxidant activity

The impact of the isolate PGPR on the antioxidant activity of garlic was determined by measuring the DPPH scavenging inhibition and IC50 values.

The application of arbuscular mycorrhizal fungi isolates was found to have a significant impact on the IC50 (P=0.00; F=574.83) (Appendix 44), but no significant impact on the DPPH scavenging inhibition (P=0.67; F=0.2) (Appendix 45).

The inoculation of garlic with the isolate T7 has been observed to have no positive effect on DPPH scavenging activity (56%) and to exhibit the highest IC50 value (0.95 mg mL^{-1}), (Table 22), when compared to the control (56%) and (0.7 mg mL^{-1}) (Table 22) respectively as well as compared to acid ascorbic (99%) and (0.01 mg mL^{-1}) (Table 22) respectively.

Table 23: **Impact of PGPR isolates (T7) on the DPPH scavenging activity and IC50 in garlic.**

Treatments	T7	CO	Ascorbic acid
DPPH scavenging activity %	56 b	56 b	99 a
IC50 mg/mL.	0.95 a	0.7 b	0.01 c

The application of PGPR to garlic bulbs did not result in an enhancement of the antioxidant activity of the garlic, which is inconsistent with the findings of the previous review. Chandrasekaran *et al*. [319] observed that *B. subtilis* CBR05 exerted a net positive effect on the antioxidant activity of tomato fruit, as measured by the DPPH and ABTS scavenging capacity.

Moreover In a study conducted by Chiappero *et al*. [320], the antioxidant capacity of the DPPH radical scavenger was observed to increase by 80 and 100% in peppermint leaves inoculated with GB03 and WCS417, respectively. Additionally, Ochoa-Velasco [321] indicated that plants inoculated with *B.licheniformis* demonstrated elevated antioxidant profiles in tomato plants under greenhouse conditions.

3.3.4.2 Evaluation of the antimicrobial effect

The antimicrobial activity of aqueous garlic extracts was evaluated by measuring the diameter of the zones of inhibition of the strains under study.

The results of the analysis of variance indicated that there were statistically significant differences in the inhibitory effect according to the strains (P= 0.00; F=37.92), the concentrations (P= 0.00; F=115.68) and the treatments (P=0.00; F=110.68) (Appendix 49). The aqueous extract of garlic inoculated with PGPR T7 exhibited the most pronounced inhibitory effect, with a larger zone of inhibition than the control. The results demonstrated that MO exhibited the most pronounced inhibitory effect, with a larger zone of inhibition than the control (Table 23, Figure 32). Moreover, the yeast strain *Saccharomyces cerevisiae* demonstrated heightened sensitivity in comparison to the *Candida albicans* yeast strains. Furthermore, the bacterial strain *Staphylococcus aureus* displayed the greatest sensitivity among the bacterial strains (Table 25). Moreover, the extent of this activity was found to increase in accordance with the concentration of the extracts (Table 24).

Table 24 : The inhibitory effect of garlic extract on microbial pathogens, according to strains.

Treatments	Inhibition zone (mm)
T7	22.5a(+++)
CO	17.9b(++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 25: The inhibitory effect of garlic extract on microbial pathogens according to the concentrations.

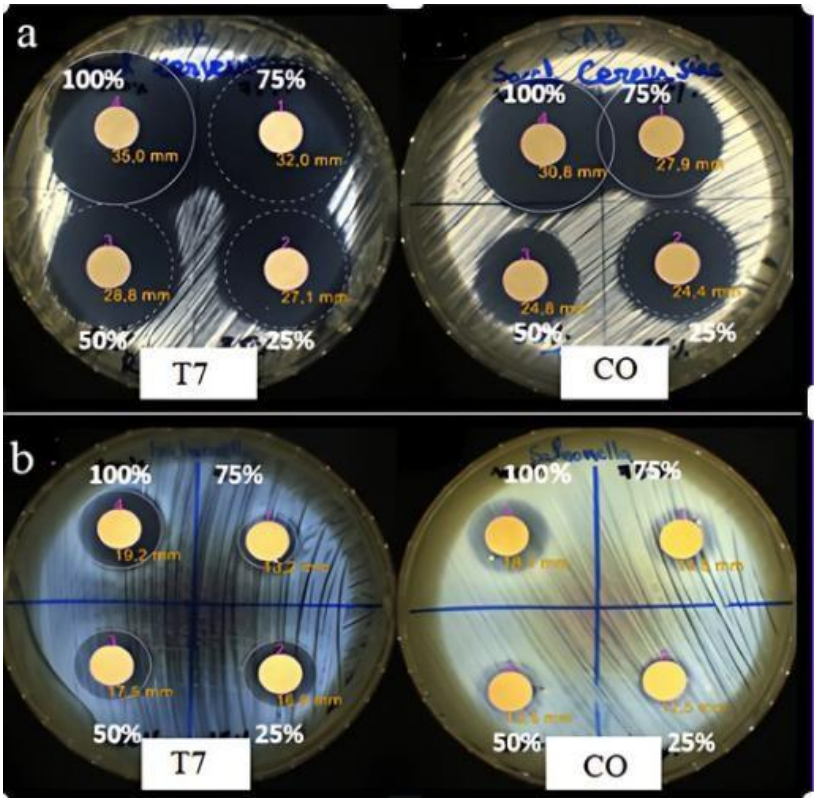
Concentrations %	Inhibition zone (mm)
100	31.5a(+++)
75	19.13b(++)
50	15.8c(++)
25	14.22c (+)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 26: The inhibitory effect of garlic extract on microbial pathogens according to strains.

Strains	inhibition zone (mm)
Saccharomyces cerevisiae	30.5a(+++)
Staphylococcus aureus	29.4a(+++)
Candida albicans	26.42ab(+++)
Staphylococcus epidermidis	24.34b(+++)
Escherichia coli	14.69c(+)
Bacillus subtilis	11.4c(+)
Salmonella typhimyrium	11c(+)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.



a: Saccharomyces cerevisiae, b:Salmonella typhimyrium

Figure 32: Inhibitory zone of aqueous extracts of garlic inoculated with PGPR against the most and least sensitive strains a and b

The antimicrobial activity of garlic aqueous extract against a wide range of pathogen strains has been extensively documented by numerous researchers, including Alirezaei *et al* and [322, 189].

The findings indicate that the aqueous extract of garlic exhibits antibacterial properties against a diverse range of Gram-positive and Gram-negative bacteria and yeast. However, the extract of garlic inoculated with PGPR showed the greatest antimicrobial activity, demonstrating a more pronounced effect than the extract of garlic that was not inoculated with PGPR. This finding corroborates the results reported by Ali Amin and Amin Hewedy [323], who observed that the application of PGPR increased the antimicrobial activity of *Moringa olifera*.

Moreover, the extraction of essential oils (EO) from the *Salvia officinalis* plant inoculated with different PGPR strains resulted in the production of EOs with varying inhibitory activities against distinct pathogen strains compared to untreated control plants. However, the highest inhibitory activity was observed for the EOs extracted from plants inoculated with Pp Ap14 than other strains against *Staphylococcus aureus*, exhibiting a maximum inhibition zone. The present study demonstrated that the 100% concentration exhibited the most pronounced inhibitory effect, indicating that the extract concentration exerts a

significant influence on the antimicrobial activity. This result has been observed by numerous studies, including that of Khalid et *al*. [214], whereas Houshmand *et al*. [324] reported that different concentrations of garlic extract (5, 10, 20, and 100%) have similar effects against a range of pathogens strains. The inhibitory activity of the extracts was observed to be generally higher against *Candida albicans* in comparison to the bacterial species [308]. However, in the present study, the extracts demonstrated greater sensitivity to *Saccharomyces cerevisiae* than *Candida albicans*. This discrepancy may be attributed to the preparation method and chemical composition of the garlic extracts, which are subject to significant influence from the soil and geographical location.

In conclusion, the T3 isolate has been demonstrated to exhibit superior efficacy in enhancing antioxidant and antimicrobial properties when compared to the other isolates under investigation. This finding suggests that T3 has the potential to serve as a promising source for the development of natural alternatives to conventional antibiotics.

3.4 Evaluation of the herbicide activity of aqueous extract of garlic inoculated with endemic microorganisms

The aim of this study is to examine the potential herbicidal properties of an aqueous garlic extract on *Lolium perenne* “monocot species” and *Amaranthus retroflexus* "dicot species" and to gain insight into the influence of microbial inoculation on the efficacy of garlic extract as an herbicide.

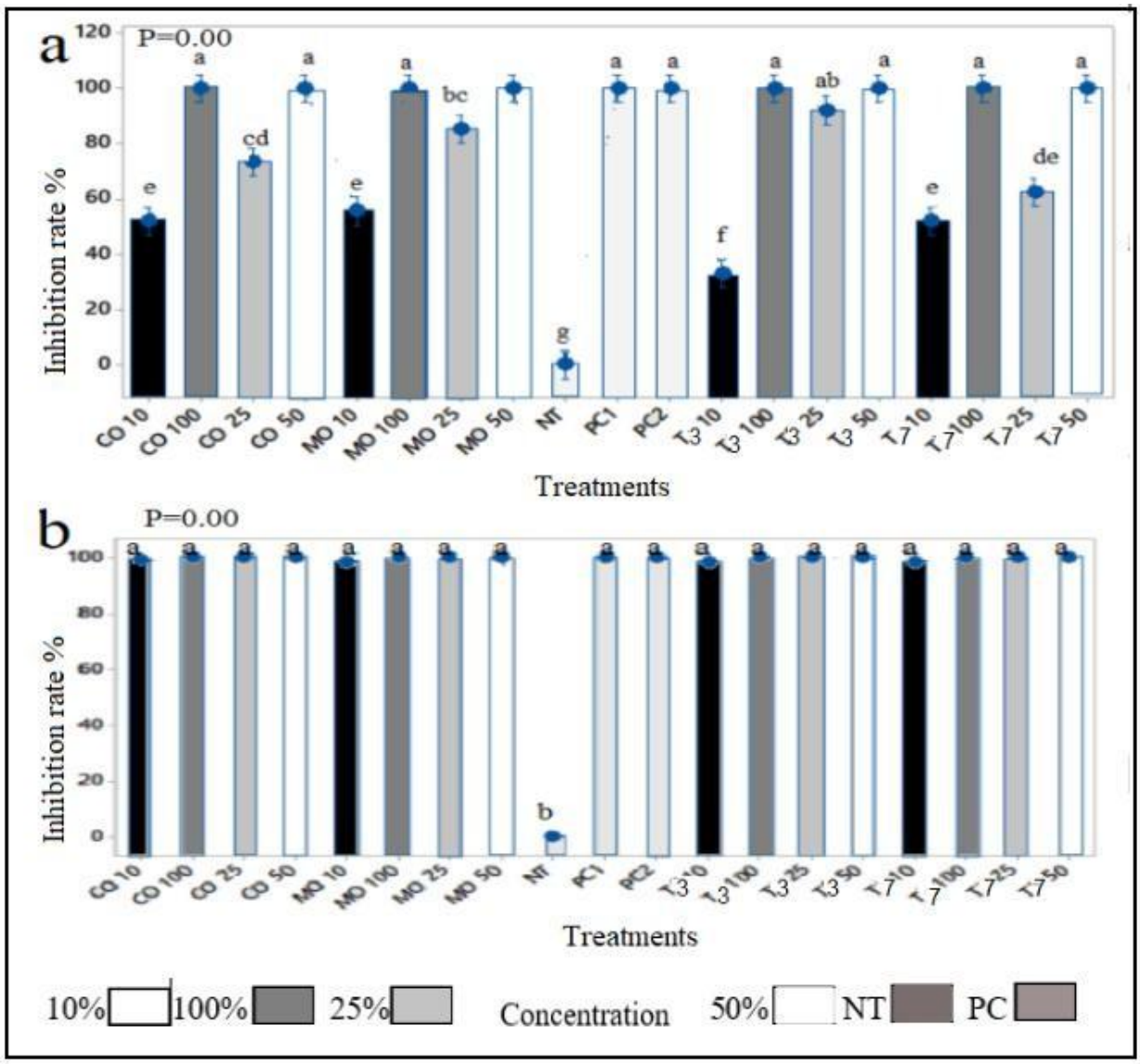
3.4.1 Garlic extract effect on seed germination

The herbicidal activity of fresh aqueous extracts of garlic bulbs inoculated with the selected isolates *T.asprellum* (T3), PGPR (T7) and AMF (MO) on the seed germination of *Lolium perenne* and *Amaranthus retroflexus* was evaluated and summarized in (Figure 33). The analysis of variance revealed that the application of aqueous garlic extracts exerted a significant impact on the seed germination of *L. perenne* (P= 0.00; F=136.14) and *A. retroflexus* (P = 0.00; F=24.27).

The impact of the aqueous garlic extracts on *L. perenne* exhibited variation according to the treatment and concentration. It was observed that all treatments at concentrations of 100% and 50% resulted in the complete inhibition of *L. perenne* seed germination, with an inhibition rate of 100%, which did not differ significantly from the positive control. However, at a concentration of 25%, treatment T3 exhibited the most significant effect, with an inhibition rate of 92%, followed by treatment CO (extract of uninoculated garlic)

with an inhibition rate of 85%, at a concentration of 10%, no significant effects were observed between the treatments, its inhibition rate ranged between 73% and 51%, with the exception of treatment T3, which exhibited the lowest inhibition rate (32%). Notwithstanding, it remains efficacious in comparison with the negative control (Figure 33 a)

Regarding *A. retroflexus* seeds, it was observed that all of the treatments demonstrated a high level of efficacy in the inhibition of *A. retroflexus* seed germination. No statistically significant differences were detected between the treatments, the positive control and the various concentrations (Figure 33 b).



a: *Lolium perenne*, b: *Amaranthus retroflexus*, NT: negative control, PC1 and PC 2: comercial herbicides

Figure 33: The inhibitory effect of a fresh aqueous extract of garlic inoculated with selected endemic microorganisms on the germination of seeds.

3.4.2 Garlic extract effect on seedlings' early growth

The herbicidal activity of fresh aqueous extracts of garlic bulbs inoculated with the selected isolate *T.asprellum* (T3), PGPR (T7) and AMF (MO) on seeds germination of *L. perenne* and *A. retroflexus* was evaluated and summarized in Figure 34.

The analysis of variance revealed that the application of aqueous garlic extracts exerted a significant impact on the growth inhibition of root seedlings of *L. perenne* ($P = 0.00$; $F = 10.65$) and *A. retroflexus* ($P = 00.0$; $F = 7.47$) as well as shoot seedlings of *L. perenne* ($P = 0.00$; $F = 24.08$).

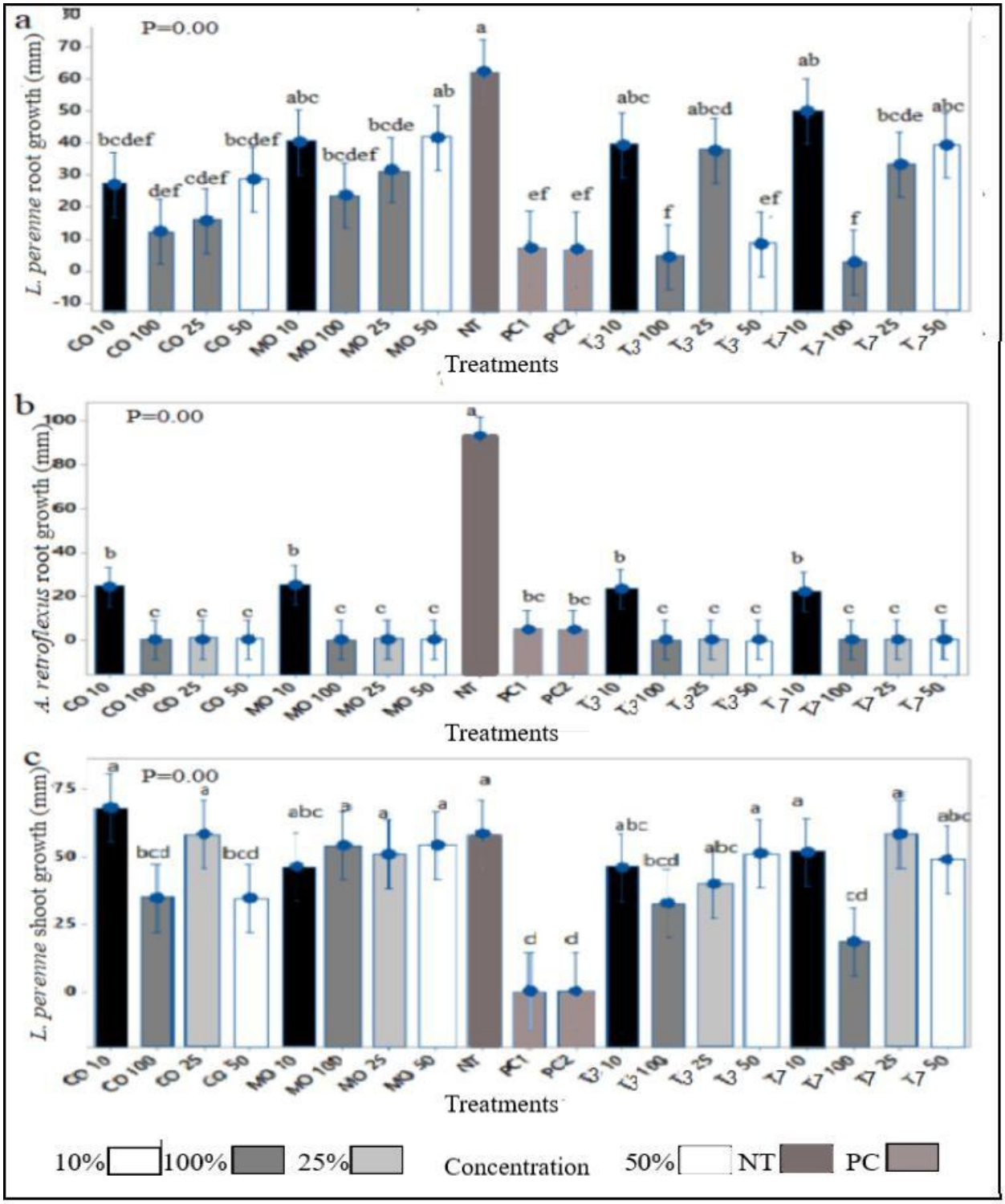
All of the treatments exhibited a significant effect on the reduction of root growth of *L. perenne* at all different concentrations. However, treatments T3 and T7 demonstrated the most significant inhibitory effect on the roots of *L. perenne* at a concentration of 100%, exceeding the positive control effect. At a concentration of 50%, treatment T3 exhibited the most effective inhibition, with no significant difference observed when compared to the positive control (Figure 34 a).

The extracts of inoculated garlic exhibited a low inhibitory effect at concentrations of 25% and 10% in comparison to the control extract CO (uninoculated garlic). However, the inhibitory effect of the treatments was comparatively significant in relation to the negative control (Figure 34 a).

For *A. retroflexus,* the results obtained demonstrated that all treatments exhibited a significant inhibitory effect on the roots of *A. retroflexus* at concentrations of 100%, 50% and 25%, which exceeded the positive control effect, with no discernible difference observed between the other treatments. However, the 10% concentration demonstrated the least inhibitory effect in comparison to the positive control, with no discernible difference observed between the other treatments. Notwithstanding, it remains efficacious in comparison with the negative control (Figure 34 b)

In contrast, the findings for *L. perenne* shoots revealed that treatment T7 exhibited the most significant inhibitory effect on shoot growth at concentrations of 100%, followed by treatments T3 and CO. However, this effect was not significant in comparison to the positive control (Figure 34 c).

At a concentration of 50%, treatment CO followed by treatment T7 demonstrated the most significant inhibitory effect, while treatments T3 and MO exhibited the lowest inhibitory effect, with no significant difference in comparison to the negative control. Notwithstanding, the treatments T3 and MO demonstrated the most inhibitory effect at 25% and 10%, respectively, in comparison to the other treatments (Figure 34c).



a: L. perenne root growth, b: *A. retroflexus* root growth and c: *L. perenne* shoot growth

Figure 34: Inhibitory effect of aqueous extracts of garlic inoculated with endemic microorganisms on seedling growth

Some studies have confirmed that garlic has growth inhibitory properties on various plants. Javed *et al*. [235] reported that aqueous extracts of three species, garlic, onion and ginger, showed significant herbicidal activity against the germination of *Parthenium* seeds.

However, aqueous extract of garlic was found to be the most effective, with 10% and 15% concentrations completely inhibiting seed germination, while the lowest 5% concentration significantly retarded the germination of the weed by 97%.

In a study conducted by Saraiva [226], it was observed that the aqueous extracts of garlic exhibited a notable inhibitory effect on the germination of *Taraxacum officinale*, as evidenced by the results of a pre-emergency bioassay. Moreover, the application of aqueous extracts of *Allium sativum* at concentrations of 5% and 7.5% resulted in considerable damage to *Taraxacum officinale* seedlings four weeks after germination, as evidenced by the post-emergency bioassay. Adeleke [2016] reported that in greenhouse experiments, the application of garlic extract at a concentration of 80% at 2 weeks after planting had a negative effect on the growth rate of both cowpea and groundnut plants, but the effect was more pronounced on the groundnut plants.

L. perenne root in the present study was found to be more susceptible to the effects of garlic extract than the shoot. This sensitivity to allelopathic plant extracts was observed in different species in many studies [235, 328, 329]. This may be due to the fact that roots are the first to absorb phytotoxic compounds [330].

The precise mechanism by which a single standard chemical or a combination of standard chemicals induces growth inhibition remains unclear. The T3 extract of the garlic plant inoculated by *Trichoderma* exhibited the most efficacious treatment in terms of its capacity to inhibit *L. perenne* seed germination and seedling growth. Similarly, the measurement of polyphenol content indicated the highest value in the garlic plant inoculated with *Trichoderma*, while there was no significant effect on flavonoid content between the treatments. Similarly, Li *et al.* [331] posited that the phenolics represent the primary allelopathic compounds that inhibit seed germination, plant growth, and the associated processes. Conversely, Jang *et al*. [329] observed that the total phenol and total flavonoid contents were higher in onion cultivars than in garlic cultivars, while the cucumber and barley plants growth inhibition was higher in garlic extracts than in onion extracts application. These findings are inconsistent with the present study, which indicate that total phenol or flavonoids are not the only compounds responsible for the observed herbicidal activity. Wu *et al*. [332] suggested that the phytotoxicity of garlic may be attributed to the presence of volatile compounds such as diallyl disulfide.

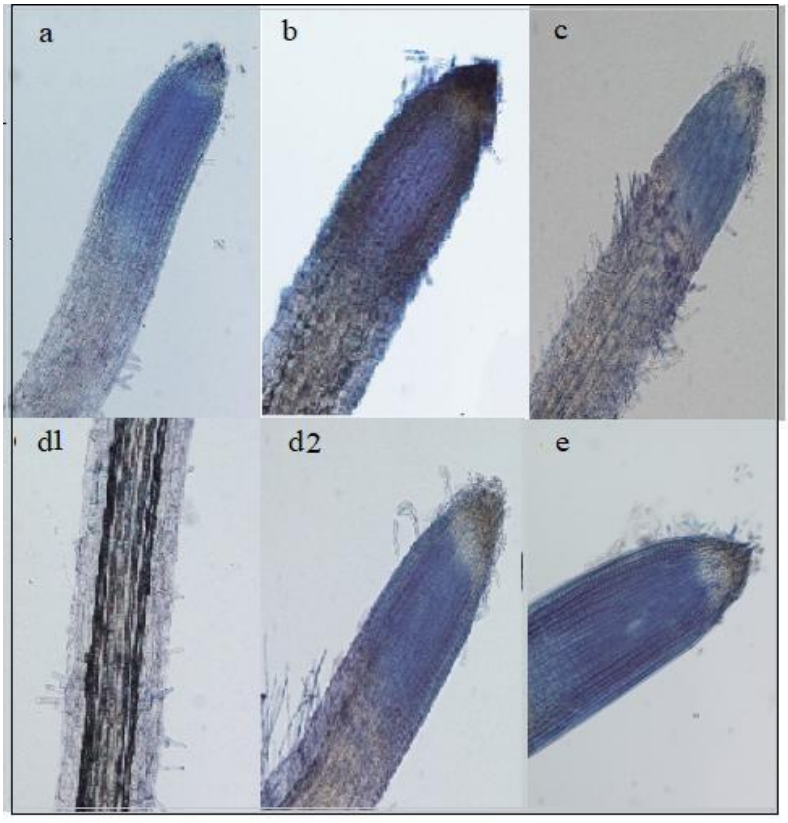
The application of all extracts of garlic at low concentration exhibited a diminished herbicidal effect on *L. perenne* in comparison to the positive control. Similarly, the study conducted by Ali *et al*. [333] revealed that the root length and fresh weights of the aerial parts and roots of the tomato plant increased when treated with DADS at low

concentrations. However, at elevated concentrations, a reduction in these parameters was observed. This provides a rationale for the observed reduction in the effect of the extract T3 in comparison to the other treatments. which indicates that the observed inhibitory effects were dependent on both the concentration of the extract and the effect of the inoculated microorganism on chemical compounds of garlic.

3.4.4 Effect of garlic extract on plasma membrane integrity

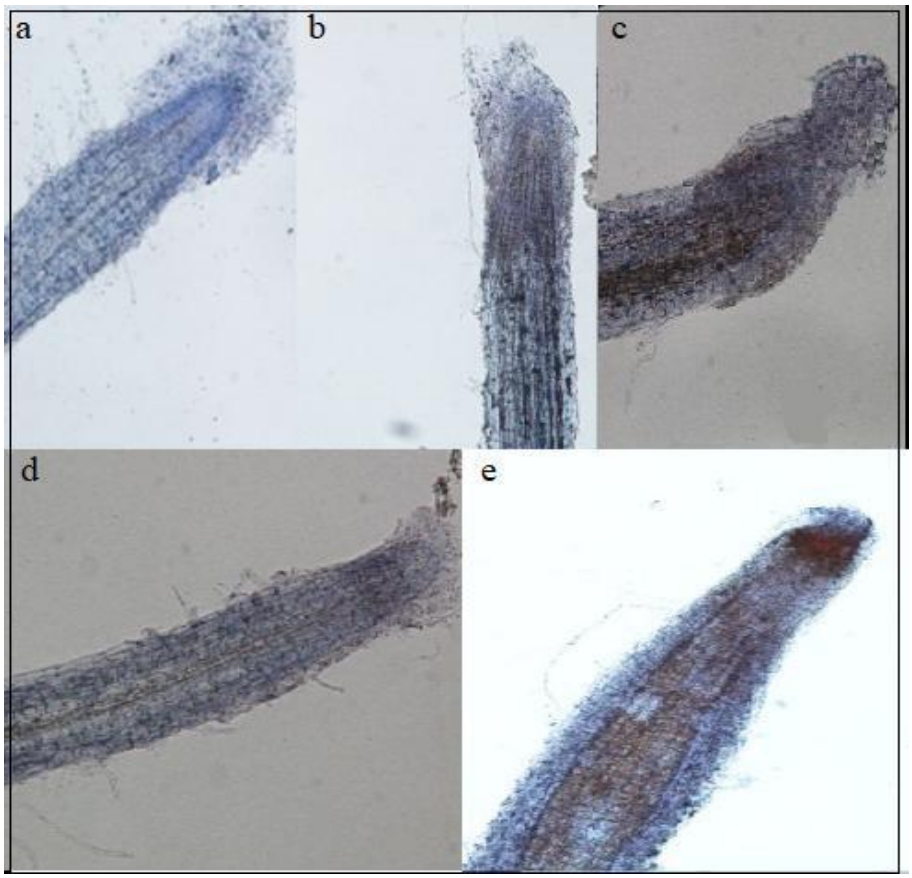
In order to gain insight into the impact of garlic extracts on plant roots, the integrity of the plasma membrane in roots was qualitatively assessed through Evans blue histochemical staining. As illustrated in the figure 40 and 41, the root of both species exhibited notable Evans blue absorption following the application of garlic extract.

In comparison to the negative control group that had not been treated with garlic extracts, the roots that had been treated with garlic extracts exhibited a notable increase in blue fluorescence intensity, This florescence was observed in the meristematic zone, where the active cell division occurs in roots and root cup (Figures 35: b, c, d2, e) and (Figure: 36 b,c,d,e) as well as in the transition zone, specifically in the membrane channel in this zone (Figures 35: b,c, d2,) and (Figure: 36 b, c, d, e).



a: NC, b: treatment T3, c: treatment T7, d1 and d2: treatment MO, e: treatment CO. NC: negative control.T3*: Trichoderma asperellum,* T7*:PGPR* , MO*:* Arbuscular mycorhizal fungi , CO: control.

Figure 35: Effect of the garlic extract treatments at 10% concentration on root plasma membrane of *Lolium perenne*.



a: NC(7a), b: treatment T3, c: treatment T7, d: treatment MO, e: treatment CO. NC: negative control.T3*: Trichoderma asperellum,* T7: PGPR , MO: Arbuscular mycorhizal fungi , CO: control.

Figure 36: Effect of the garlic extract treatments at 10% concentration on root plasma membrane of *Amaranth retroflexus*.

In comparison to the untreated control (NC), garlic extract exhibited an high intensity of blue color fluorescence, which clearly reflects Evans blue penetration into damaged cells indicating that a greater number of cells had been damaged. These findings suggest that the application of garlic extract may have resulted in cell death.

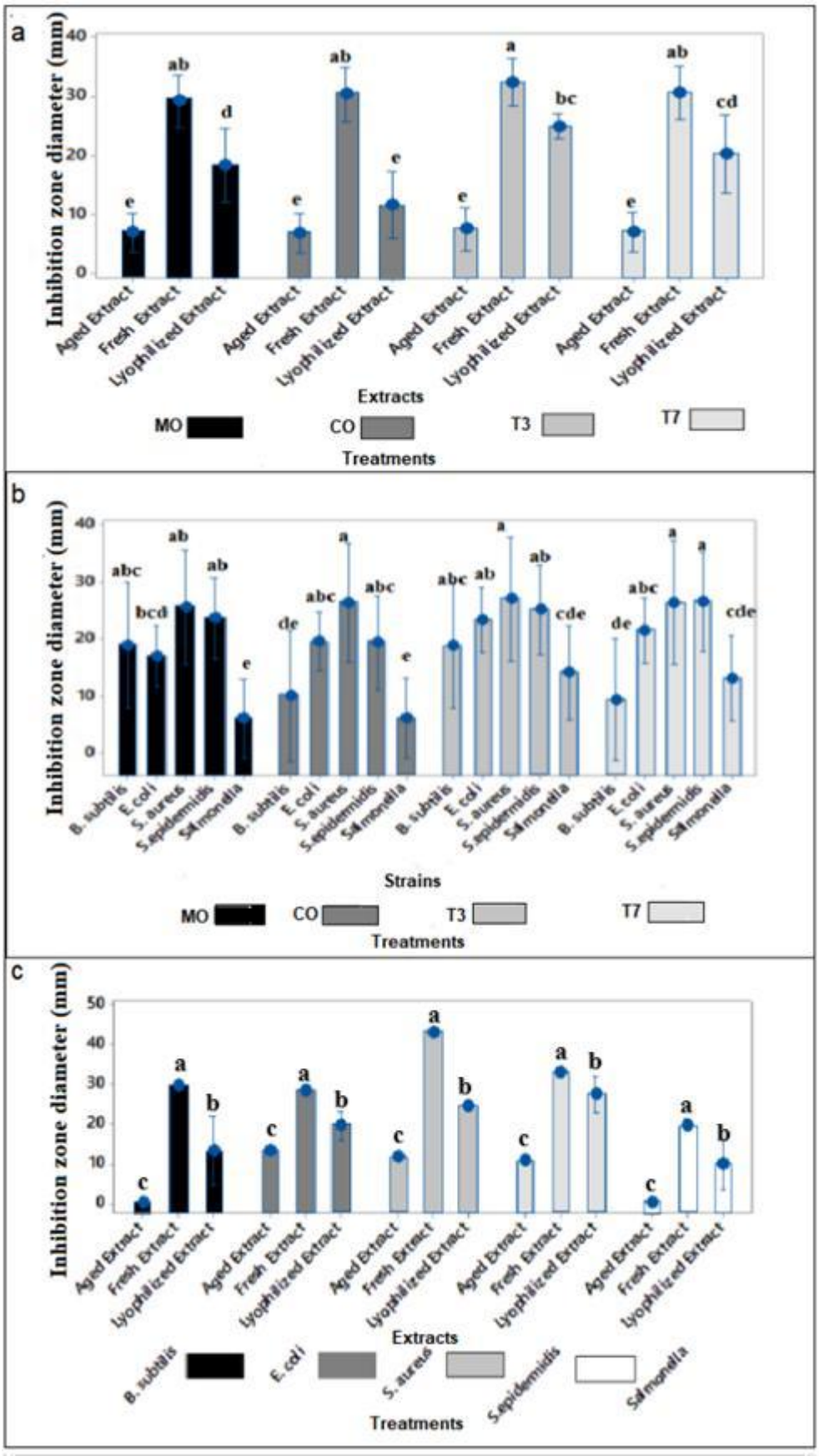
As posited by Pandir [334] and Shahid et al. [355], the exposure of plants to pesticides has been demonstrated to result in an augmentation of blue fluorescence when Evan's blue dye is utilized in comparison to untreated roots.

Moreover, Hatamleh et al. [336] demonstrated that the application of elevated pesticide concentrations resulted in plasma membrane integrity deficits, indicating that root cells lost their capacity to heal following exposure to pesticides.

3.5 Evaluation of the stability of antibacterial efficacy of garlic

The antimicrobial activity of fresh aqueous extracts, aged lyophilized extracts, and aged extracts of garlic bulb inoculates with the selected endemic microorganisms *T.asprellum* (T3) , PGPR (T7) and AMF (MO) were evaluated by measuring the diameter of the zones of inhibition of the strains under study. The results of the analysis of variance demonstrated a statistically significant difference in the inhibitory effect according to the microbial treatments (P=0.00; F=7.47) (Appendix 64), type of aqueous extract (P=0.00; F= 274) (appendix 65) and the strains sensitivity (P=0.00; F= 54.43) (Appendix 66).

Both the fresh extract and the aged lyophilized extracts of garlic showed significant inhibitory effects against all the bacteria tested. However, the fresh garlic extract demonstrated the most significant inhibitory effect. Moreover, the aged garlic extract demonstrate the lowest inhibitory effect against the bacterial strains under study (Figure 37 a, Table 29). The T3 treatment demonstrated the most significant inhibitory effect, exhibiting a larger zone of inhibition against the strains under study compared to the other treatments. The T7 treatment was observed to be the second most effective, followed by the control CO (Table 27). The bacteria demonstrated extreme sensitivity, with *S. aureus* exhibiting the most sensitivity to the treatments. (Table 28), Additionally the strain *Salmonella typhimurium* demonstrated the least sensitivity to the extracts of garlic inoculated with T3 and T7. In contrast, these strain exhibited resistance to the extract inoculated with AMF and to the uninoculated control (Figure 37 b)



(a): the type of extract , (b): bio-input treatment, (c) : pathogen strains

Figure 37: The inhibitory effect of garlic extracts on microbial pathogens according to (a) ,(b) ,(c) .

Table 27: Inhibitory effect of garlic extract inoculated with selected endemic bio input on microbial pathogens according to treatments.

Treatments	Inhibition zone
T3	21,6 a (+++)
T7	19,3 ab (++)
MO	18,21 bc (++)
CO	16,2 c (++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 28: Inhibitory effect of garlic extract inoculated with selected endemic bio-input on microbial pathogens according to strain.

Strains	inhibition zone
Staphylococcus aureus	26 a (+++)
Escherichia coli	20 b(+++)
Staphylococcus epidermidis	23 ab(+++)
Bacillus subtilis	14 c(+)
Salmonella typhimyrium	9,80 d (+)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 29: Inhibitory effect of garlic extract inoculated with selected endemic bio-input on microbial pathogens according to type of extract.

Treatments	Inhibition zone
Fresh garlic	30.69 a(+++)
Aged freeze dried garlic	18.83 b(+++)
Aged garlic	7.10 c(+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

The fresh aqueous garlic extract and freeze-dried garlic extract, whether uninoculated or inoculated with microorganisms, has been demonstrated to be an effective treatment for bacterial infections.

However the freeze-dried garlic extract exhibited a lower antibacterial activity in comparison to the activity of the fresh garlic extract. Our findings are consistent with those of Shafiur Rahman *et al*. [337], who reported that fresh garlic exhibited the greatest

inhibitory effect, followed by the freeze-dried powder extract. Additionally, Olivas-Flores *et al.* [338] have proposed that the freeze-drying of garlic extract may have a pronounced antimicrobial impact even at low concentrations.

In the present study, the aged garlic extract demonstrated a comparatively reduced efficacy when compared to both fresh and freeze-dried extracts. A number of researchers, including De Pauw *et al.*[339] have indicated that sulfur compounds are the primary cause of this activity. Furthermore, they have suggested that the limits of stability of sulfur compounds could potentially lead to a decrease in antimicrobial efficacy over time.

Suciu *et al.* [340] observed that the lyophilized extract stored at -20 °C lost 15% of its initial quantity over a period of 31 days. In comparison, the lyophilized extract stored at 4 °C exhibited a loss of 26% of its allicin content over the same period, while the extract stored at room temperature demonstrated a loss of approximately 63%. Furthermore, following a storage period of 90 days, the extract stored at 20 °C exhibited a loss of approximately 23% of its allicin content, the extract stored at 4 °C demonstrated a loss of approximately 45%. The extract stored at room temperature exhibited the largest loss, with a reduction of approximately 83%. These findings indicate that both time and temperature have a significant impact on the stability of allicin.

The study conducted by Al-Waili *et al.* [341] demonstrated that the antimicrobial efficacy of garlic juice was significantly diminished after 24 hours of storage at 4°C. Furthermore, Hughes and Lawson [342] illustrated that the antimicrobial activity of garlic is entirely abolished when the thiosulfinates (e.g., allicin) are removed from the aqueous extract. Additionally, the antibacterial activity is significantly diminished when allicin is degraded to diallyl disulfide, these findings elucidate the lowest activity of aged garlic observed in our study.

The antimicrobial activity of aqueous garlic extract demonstrated that *Staphylococcus aureus* exhibited the greatest sensitivity. This finding is corroborated by the results of a number of research studies including Shafiur Rahman *et al.* [337] who reported that in general, the results indicated that *Bacillus cereus* and *Staphylococcus aureus* were the most sensitive, while *Salmonella typhimurium* demonstrated the greatest resistance to garlic. In contrast, the present study demonstrated that the strain *Salmonella typhimurium* exhibited sensitivity to the extracts of garlic inoculated with *T. asperellum* (T3) and PGPR (T7), in comparison to the extracts of garlic inoculated with AMF (MO) and those that were uninoculated (control). Moreover, all the strains studied were more susceptible to the extracts of inoculated garlic, in particular the extract inoculated with *Trichoderma asperellum,* which indicates that the inoculation of garlic with the endemic

microorganisms T3, PGPR and MO can stimulate the chemical compounds of garlic, in particular the chemical compounds responsible for antimicrobial activity.

Bayoumi *et al.* [343] and Coşkuntuna and Özer [344] indicated that *trichoderma spp.* have the ability to stimulate a chemical compound in onion in the presence of the pathogen. The present study indict for the first time that the inoculation of garlic with PGPR (T7), AMF (MO) and *T. asperellum* (T3) in particular increase the antimicrobial activity of garlic extract and the lyophilized extract represents an optimal preservation method for sensitive extracts such as garlic and is readily adaptable for use in capsules.

3.6 Evaluation of the antibacterial efficacy of the formulated gel

The fresh aqueous extract of the garlic bulb, inoculated with *Trichoderma asperellum* T3, was transformed into a gel in order to enhance the stability of its antimicrobial activity.

The antimicrobial activity of the gel was evaluated by measuring the diameter of the zones of inhibition against five strains of bacteria: *S. aureus*, *S. epidermidis, E. coli, B. subtilis* and *S. typhimurium.* Subsequently, the results were compared with those obtained from the fresh extract to ascertain the efficacy of the gel.

The results of the analysis GLM demonstrated a statistically significant difference in the inhibitory effect of the treatments according to the strains (P=0.00; F=56.69) (appendix 67) while no significant difference between the treatments (fresh extract and gel) (P=0.63; F=0.24)

The formulated gel and fresh extract exhibited notable inhibitory activity against a diverse range of bacterial species, including *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Bacillus subtilis*, with no significant inter-strain variation with zones of inhibition diameters ranged from 28.16 mm to 29.5 mm, which were categorized as extremely sensitive respectively (Table 31, Figure 38). Conversely, the S. *typhimurium* strain exhibited the lowest degree of susceptibility 20 mm (Table 30).

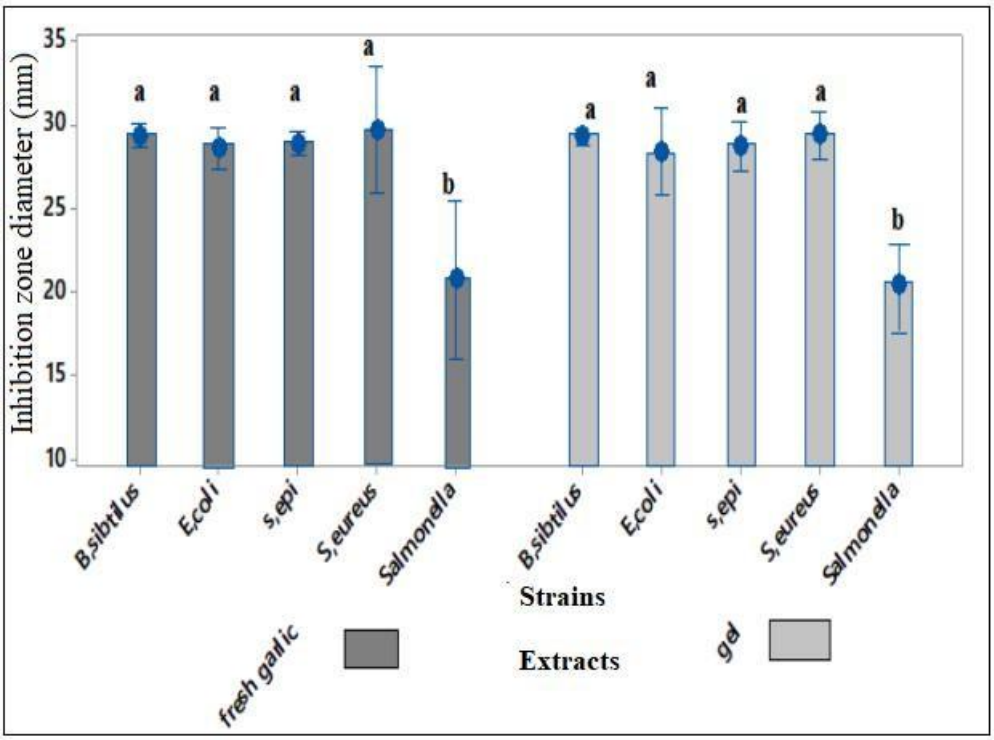


Figure 38: Inhibition zone by formulated gel according the strains and type of treatment.

Table 30: Inhibition zone of microbial pathogens according the strains.

Strains	inhibition zone
Staphylococcus aureus	29.5a (+++)
Escherichia coli	29.25a(+++)
Staphylococcus epidermidis	28.75a(+++)
Bacillus subtilis	28.41a(+++)
Salmonella typhimyrium	20.5b(+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

Table 31: Inhibition zone of bacterial pathogens according to the type of treatments.

Treatments	Inhibition zone
Fresh extract of garlic	27,5267a(+++)
Formulated gel	28,5067 a (+++)

Resistant (-), Sensitive (+), Very sensitive (++), Extremely sensitive (+++). Values followed by the same letter do not differ significantly.

The formulated gel exhibited notable inhibitory activity against a diverse range of bacterial species, including *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Bacillus subtilis.* This makes it an appropriate solution to guarantee the maintenance of its antimicrobial properties and facilitate its use in pharmaceutical applications. The present result is in accordance with those of Fitriana *et al.* [345], who observed that gel preparations with a 100% concentration exhibited significantly enhanced antibacterial efficacy, Shaqra *et al*. [346] demonstrated that freshly prepared gels with 2 different concentrations were active inhibitor to the pathogen studied. Furthermore, Ilić *et al.* [347] presented evidence suggesting that the microbiological analysis of pure allicin and allicin incorporated in gel exhibited comparable activity against the tested microbes. Additionally, the study identified *Staphylococcus aureus* ATCC 6538 as a bacterial strain that exhibited a particularly high level of susceptibility. This result is in accordance with our finding that the *Staphylococcus aureus* was the high sensitive to the formulated gel.

The formulated gel has the potential to become a valuable and convenient option for a range of medicinal and cosmetic applications, offering a promising avenue for further research and development.

General conclusion and research prospects

The aim of this research is to gain a deeper understanding of the potential impact of inoculated endemic microorganisms on garlic cultivation with view to valorize it in a range of agricultural and medical applications, our research has focused on the impact of specific microorganisms on garlic cultivation in pots, with the objective of identify the most promising candidates.

In order to achieve the stated objective, our study was based on the cultivation of garlic in pots and under greenhouse using endemic microbial bio-input, including two isolates of arbuscular mycorrhizal fungi (MO, MA), three isolates of Trichoderma asperellum (T1, T2 and T3) and one isolate of PGPR (T7).

To this ends, a number of parameters were evaluated, including growth, yield, and the analysis of phytochemical compounds by FTIR in garlic (*Allium sativum* L.). Moreover, the aqueous extracts prepared from samples of plants grown under the influence of the aforementioned bio-input were evaluated for their phenolic compound content and their antioxidant, antimicrobial (antibacterial and antifungal) and herbicidal activities. Additionally, two natural pharmaceutical formulations derived from garlic extract have also been developed.

The results obtained are promising and demonstrated the potential for improving the growth and yield of garlic. In particular, the isolate T1, MO and T7 showed the most notable increase across the majority of the examined parameters, including plant height, fresh weight of bulb and root, chlorophyll a and b, and carotenoid, which may be regarded as a growth promoter and an alternative to the excessive and unselective utilization of chemical fertilizers. However, no discernible positive impact was demonstrated by the inoculants MO on the growth of the garlic plant cultivated on unsterilized soil. This highlights the necessity of assessing the compatibility between specific mycorrhizal isolates and the soil microbial environment before selecting inoculants as alternative to chemical fertilizers

The optimization process also revealed a change in the functional group of chemical compounds of garlic. Furthermore the isolates T2, T3 and MO were observed to enhance the phenolic compounds of aqueous extracts derived from cultivated plants, including polyphenols and total flavonoids. This indicates that these isolates may play a role in enhancing the biochemical responses of plants, which could be a valuable strategy for boosting plant health and productivity in sustainable agriculture. Furthermore, the optimization process also affected the antioxidant and antimicrobial properties of the aqueous extracts. In particular, the isolates MO, MA, T2 and T3 exhibited an increase in

antioxidant activity. With regard to antimicrobial activity, all the examined isolates displayed an increase in this activity. However, the isolates MO, T7 and T3 were particularly efficacious, which could potentially contribute to the development of natural alternatives to conventional antibiotics. In addition, the aqueous extract prepared from garlic plants inoculated with the isolate T3 demonstrated notable inhibitory potential on seed germination and seedling growth of *L. perenne*.

Moreover, the application of garlic extract may have resulted in the death of cells in the plasma membrane of the seedling root. This, in turn, may have determined the mechanisms of action of the allelochemicals present in the extracts at the cellular level. Similarly, the extract prepared from garlic plants inoculated with *Trichoderma asperellum* isolate (T3) was found to possess potent antimicrobial properties, even retaining its efficacy in freeze-dried form for a period of six months. Based on these findings, antimicrobial capsules and gel were formulated for use in a pharmaceutical context.

The isolates T1 MO and T7 have been identified as the most promising in terms of biomass optimization, particularly with regard to bulb weight. It can be posited that these organisms may act as growth stimulators and as an alternative to the excessive and non-selective use of chemical fertilizers in agricultural contexts. However, when we considerthe potential of antioxidant compounds and their ability to act as antioxidants, antimicrobial agents and herbicides, *Trichoderma asperellum* isolate (T3) has shown particular promise for industrial use in organic garlic cultivation.

In view of the findings presented in this research, it might be beneficial to consider further investigation into the role of symbiotic microorganisms in garlic cultivation. This could include the identification and determination of the concentration of chemical compounds responsible for the antimicrobial activity of the extract of garlic inoculated with *Trichoderma* T3. It may be beneficial also to investigate the potential of utilizing lyophilized garlic powder as a natural preservative in food products. This could entail examining its efficacy in prolonging shelf life and preventing spoilage, as well as assessing its influence on diverse food types and determining optimal concentrations that maintain antimicrobial activity while preserving food quality. Furthermore, conducting safety studies of the formulated products would be recommended in order to ascertain the optimal dosages that are effective against pathogens while ensuring the safety of human subjects.

The application of T3 was found to exert a significant inhibitory effect on the seedling growth of *L. perenne*, representing a pioneering investigation into the potential of microbial applications to enhance the herbicidal bioactive compounds of garlic. This topic

has not yet been addressed in any published paper, which presents an opportunity for further research to identify the natural compounds responsible for the bioherbicidal activity. This could potentially pave the way for the development of new bioherbicides. Additionally, it may be advantageous to assess the long-term impact of these natural herbicides on crop safety and soil health.

It would be also advantageous to evaluate the influence of the combined inoculation of the microorganisms isolated as T7, MO and T3, under both biotic and abiotic conditions. It would be beneficial to examine the influence of microbial inoculation on a diverse range of garlic varieties. This may facilitate the identification of genotypes that may demonstrate enhanced efficacy when combined and are more productive.

It would be advantageous to determine whether the observed benefits of microbial inoculants in garlic can be extended to other crops, particularly those with economic or medicinal value.

This research respectfully proposes a few suggestions for ways in which yield, quality and bioactivity could be enhanced in a sustainable manner. It is our sincere hope that this research will contribute to a comprehensive approach to addressing agricultural requirements while promoting food safety and enhancing the therapeutic potential of crops in the long term.

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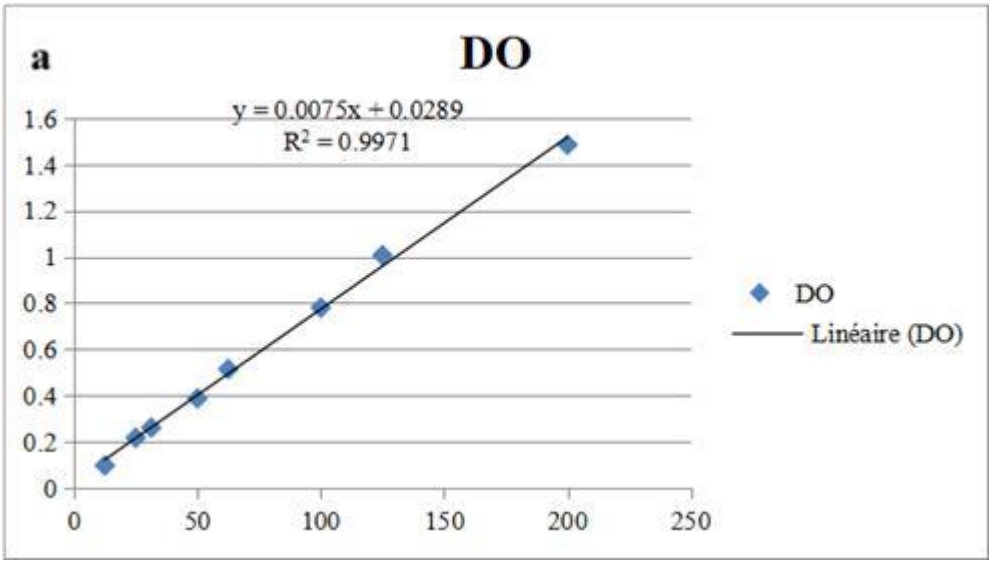
Appendix

Appendix 1: The recipe for making PDA medium from fresh potatoes follows that described by Gams et al. (1998)

Reagent	Quantity (g/l)
Potatoes extract	230ml
glucose	20 g
agar	15g

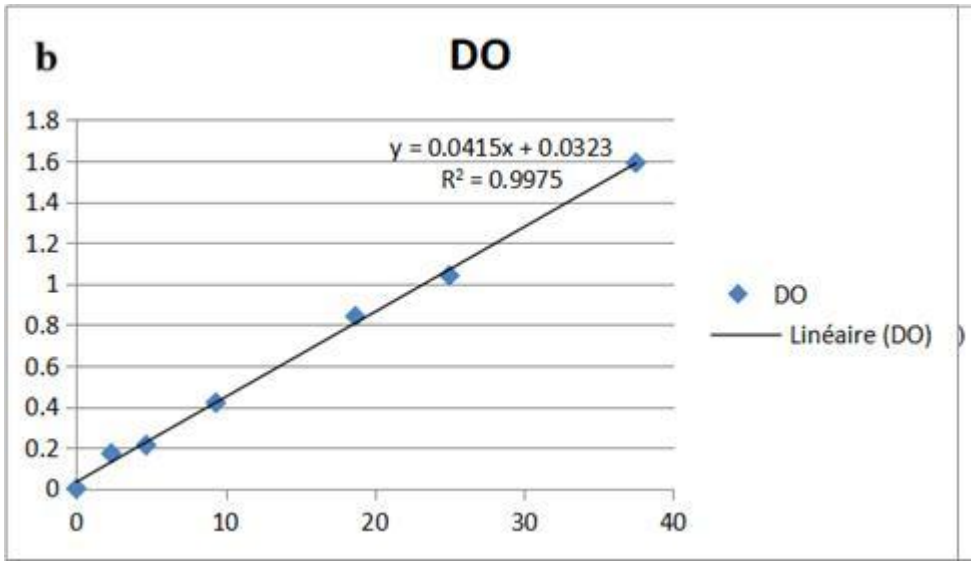
Appendix 2 :The calibration curve for acid galic (a) and quercetin (b).



DO = 0.0075 C+0.0289

C=(DO-0.0289)/0.0415

C= concentration



DO=0,0415C+0,0323

C=(DO-0,0323)/0,0415

C= Concentration

Appendix 3: Analysis of variance ANOVA test of the plant height of garlic plants cultivated in sterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	31,55	15,775	190,61	0
Error	12	0,9931	0,0828		
Total	14	32,5431			

Appendix 5: Analysis of variance ANOVA test of the weight of bulb of garlic plants cultivated in sterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	275,776	137,888	800,12	0
Error	12	2,068	0,172		
Total	14	277,844			

Appendix 6: ANOVA test of chlorophyll a of garlic leaves cultivated in sterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	1,17671	0,588353	9678,83	0
Error	6	0,00036	0,000061		
Total	8	1,17707			

Appendix 7: ANOVA test of chlorophyll b of garlic leaves cultivated in sterilized soil according to the effect of the isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	0,216705	0,108353	500,64	0
Error	6	0,001299	0,000216		
Total	8	0,218004			

Appendix 8: Analysis of variance ANOVA test of carotenoid of garlic leaves cultivated in sterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	0,111667	0,055803	132,24	0
Error	6	0,00253	0,00042		
Total	8	0,114200			

Appendix 9: ANOVA test of the root colonization frequency of garlic by AMF in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	1600,7	1600,67	21,83	0,01
Error	4	293,3	73,33		
Total	5	1894			

Appendix 10: ANOVA test of the hyphae length of AMF in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	549169	549169	8,03	0,013
Error	14	957936	68424		
Total	15	1507105			

Appendix 11: ANOVA test of the plant height of garlic plants cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,049	0,049	0,41	0,541
Error	8	0,96	0,12		
Total	9	1,009			

Appendix 12: ANOVA test of the weight of bulb of garlic plants cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	2,52	2,52	19,14	0,002
Error	8	1,053	0,1317		
Total	9	3,573			

Appendix 13: Analysis of variance ANOVA test of the weight of root of garlic plants cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,056067	0,056067	210,25	0
Error	4	0,001067	0,000267		
Total	5	0,057133			

Appendix 14: Analysis of variance ANOVA test of chlorophyll a of garlic leaves cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,90940	0,90940	61,53	0.001
Error	4	0,096852	0,01478		
Total	5	0,96852			

Appendix 15: Analysis of variance ANOVA test of chlorophyll b of garlic leaves cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,03375	0,03375	0,47	0,53
Error	4	0,28833	0,07208		
Total	5	0,32208			

Appendix 16: Analysis of variance ANOVA test of carotenoid of garlic leaves cultivated in unsterilized soil according to the effect of isolates of AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,046	0,046	4,97	0,090
Error	4	0,037	0,009		
Total	5	0,084			

Appendix 17: Analysis of variance ANOVA test of the root colonisation frequency of garlic cultivated in unsterilized soil by AMF.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	4,167	4,167	0,12	0,751
Error	4	144,667	36,167		
Total	5	148,833			

Appendix 18: Analysis of variance ANOVA test of the weight of bulb of garlic plants cultivated in sterilized soil according to the effect of isolates of *Trichoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	140,583	46,861	146,54	0
Error	22	7,035	0,3198		
Total	25	147,618			

Appendix 19: Analysis of variance ANOVA test of plant height of garlic according to the effect of isolates of T*richoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	178	59,3333	79,11	0
Error	8	6	0,75		
Total	11	184			

Appendix 20: Analysis of variance ANOVA test of root weight of garlic according to the effect of the isolates of T*richoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	0.7917	0.2639	0.54	0.662
Error	20	9.8333	0.4917		
Total	23	10.625			

Appendix 21: Analysis of variance ANOVA test of Chlorophyll a of garlic leaves according to the effect of isolates of T*richoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	1,7853	0,59511	15,59	0,001
Error	8	0,3054	0,03817		
Total	11	2,0907			

Appendix 22: Analysis of variance ANOVA test of Chlorophyll b of garlic leaves according to the effect of isolates of T*richoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	0,43428	0.14476	15.42	0.001
Error	8	0.07512	0.00939		
Total	11	0.5094			

Appendix 23: Analysis of variance ANOVA test of Carotenoids of garlic leaves according to the effect of isolates of T*richoderma.*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	0.19267	0.064224	23.72	0.00
Error	8	0.02166	0.002708		
Total	11	0.21433			

Appendix 24: Analysis of variance ANOVA test of plant height of garlic according to the effect of isolates of PGPR.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	33,124	33,124	389,69	0
Error	8	0,68	0,085		
Total	9	33,804			

Appendix 25: Analysis of variance ANOVA test of bulb weight according to the effect of isolates of PGPR.

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	11,4404	11,4404	196,85	0
Error	8	0,4649	0,0581		
Total	9	11,9054			

Appendix 26: Analysis of variance ANOVA test of root weight according to the effect of isolates of PGPR.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	1	0,082134	0,082134	323,57	0
Error	4	0,001015	0,000254		
Total	5	0,083149			

Appendix 27: Analysis of variance ANOVA test of chlorophyll a according to the effect of isolates of PGPR

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	5,05537	5,05537	6369,26	0
Error	4	0,00317	0,00079		
Total	5	5,05855			

Appendix 28: Analysis of variance ANOVA test of chlorophyll b according to the effect of isolates of PGPR

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments.	1	0,87423	0,874227	321,81	0
Error	4	0,01087	0,002717		
Total	5	0,88509			

Appendix 29: Analysis of variance ANOVA test of carotenoids according to the effect of isolates of PGPR

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,65841	0,658413	148,09	0
Error	4	0,01778	0,004446		
Total	5	0,6762			

Appendix 30 : Analysis of variance ANOVA test of phenolic content according to the effect of isolates of AMF in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	27,5368	13,7684	228,81	0
Error	6	0,361	0,0602		
Total	8	27,8978			

Appendix 31 : Analysis of variance ANOVA test of flavonoids content according to the effect of isolates of AMF in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	0,000148	0,000074	18,26	0,003
Error	6	0,000024	0,000004		
Total	8	0,000172			

Appendix 32 : Analysis of variance ANOVA test of Total Phenolic contents in garlic according to the effect of AMF isolates in unsterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,489796	0,489796	295,38	0
Error	4	0,006633	0,001658		
Total	5	0,496429			

Appendix 33 : Analysis of variance ANOVA test of flavonoids content according to the effect of isolates of AMF in unsterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,008128	0,008128	574,55	0
Error	4	0,000057	0,000014		
Total	5	0,008184			

Appendix 34 : Analysis of variance ANOVA test of phenolic content content according to the effect of Trichoderma isolates

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatemts	3	143,155	47,7185	110,83	0
Error	8	3,445	0,4306		
Total	11	146,6			

Appendix 35 : Analysis of variance ANOVA test of flavonoid content content according to the effect of Trichoderma isolates

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatemts	3	0,00108	0,00036	100,43	0
Error	8	0,000029	0,000004		
Total	11	0,001108			

Appendix 36 : Analysis of variance ANOVA test of phenolic content according to the effect of PGPR isolates

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
C3	1	0,07063	0,070634	27,29	0,006
Error	4	0,01035	0,002588		
Total	5	0,08099			

Appendix 37 : Analysis of variance ANOVA test of flavonoids content according to the effect of PGPR isolates

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,008272	0,008272	584,75	0
Error	4	0,000057	0,000014		
Total	5	0,008328			

Appendix 38 : Analysis of variance ANOVA test of DPPH scavenging activity according to the effect of AMF isolates on garlic cultivated in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	191,536	95,7678	161,41	0
Error	6	3,56	0,5933		
Total	8	195,096			

Appendix 39 : Analysis of variance ANOVA test of IC50 according to the effect of AMF isolates on garlic cultivated in sterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	0,5462	0,2731	9753,57	0
Error	6	0,000168	0,000028		
Total	8	0,546368			

Appendix 40 : Analysis of variance ANOVA test of DPPH scavenging activity according to the effect of AMF isolates on garlic cultivated in unsterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	52,735	52,7348	88,23	0,001
Error	4	2,391	0,5977		
Total	5	55,125			

Appendix 41: Analysis of variance ANOVA test of IC50 according to the effect of AMF isolates on garlic cultivated in unsterilized soil

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	2,0686	1,03432	18,47	0,003
Error	6	0,336	0,05601		
Total	8	2,4047			

Appendix 42: Analysis of variance ANOVA test of DPPH scavenging activity according to the effect of T. asperellum isolates on garlic

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	4	4833,07	1208,27	3624,80	0,000
Error	10	3,33	0,33		
Total	14	4836,40			

Appendix 43: Analysis of variance ANOVA test of IC50 according to the effect of T, asperllum on garlic

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	4	4,44128	1,11032	9791,19	0,000
Error	10	0,00113	0,00011		
Total	14	4,44242			

Appendix 44: Analysis of variance ANOVA test of DPPH scavenging activity according to the effect of PGPR isolates on garlic

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	1,42923	0,714614	574,83	0
Error	6	0,00746	0,001243		
Total	8	1,43669			

Appendix 45: Analysis of variance ANOVA test of IC50 according to the effect of PGPR on garlic

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	0,1332	0,1332	0,2	0,675
Error	4	2,6171	0,6543		
Total	5	2,7503			

Appendix 46 Analysis of GLM of the antimicrobial activity of aqueous extracts of garlic inoculated with AMF cultivated in sterilized soil against microbial pathogen

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	2	896,5	448,25	37,92	0,000
Strains	6	8204,7	1367,44	115,68	0,000
Concentrations	3	3902,3	1300,76	110,04	0,000
Error	240	2837,0	11,82		
Lack-of-Fit	72	2607,1	36,21	26,46	0,000
Pure Error	168	229,9	1,37		
Total	251	15840,4			

Appendix 47 Analysis of GLM of the antimicrobial activity of aqueous extracts of garlic inoculated with AMF cultivated in sterilized soil against microbial pathogen

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	1	169,2	169,2	0,68	0,412
Strains	6	13075,9	2179,3	8,72	0
Concentration	3	2664,6	888,2	3,55	0,016
Error	157	39252,7	250		
Lack-of-Fit	45	12510,1	278	1,16	0,258
Pure Error	112	26742,6	238,8		
Total	167	55162,4			

Appendix 48 Analysis of GLM of the antimicrobial activity of aqueous extracts of garlic inoculated with T. asperllum against microbial pathogen

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	3	1750,3	583,43	29,07	0,000
Concentration	3	4529,8	1509,94	75,22	0,000
Strains	6	5658,5	943,08	46,98	0,000
Error	323	6483,6	20,07		
Lack-of-Fit	99	6305,4	63,69	80,04	0,000
Pure Error	224	178,2	0,80		
Total	335	18423,2			

Appendix 49 Analysis of GLM of the antimicrobial activity of aqueous extracts of garlic inoculated with PGPR against microbial pathogen

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Strains	6	14243,6	2373,93	98,27	0
Treatments	1	876,8	876,8	36,3	0
Concentrations	3	7731,3	2577,1	106,68	0
Error	157	3792,5	24,16		
Lack-of-Fit	45	3732,8	82,95	155,46	0
Pure Error	112	59,8	0,53		
Total	167	26644,2			

Appendix 50 Analysis of variance ANOVA test of the effect of garlic extract on the seed germination of *L. perenne*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	18	45936,5	2552,03	136,14	0,000
Error	38	712,3	18,75		
Total	56	46648,8			

Appendix 51 Analysis of variance ANOVA test of the effect of garlic extract on the seed germination of *A. retroflexus*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	19	53873	2835,4	24,27	0,000
Error	94	10982	116,8		
Total	113	64855			

Appendix 52 Analysis of variance ANOVA test of the effect of garlic extract on the seedling root growth of *L. perenne*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	18	40438	2246,6	10,65	0,000
Error	129	27220	211,0		
Total	147	67658			

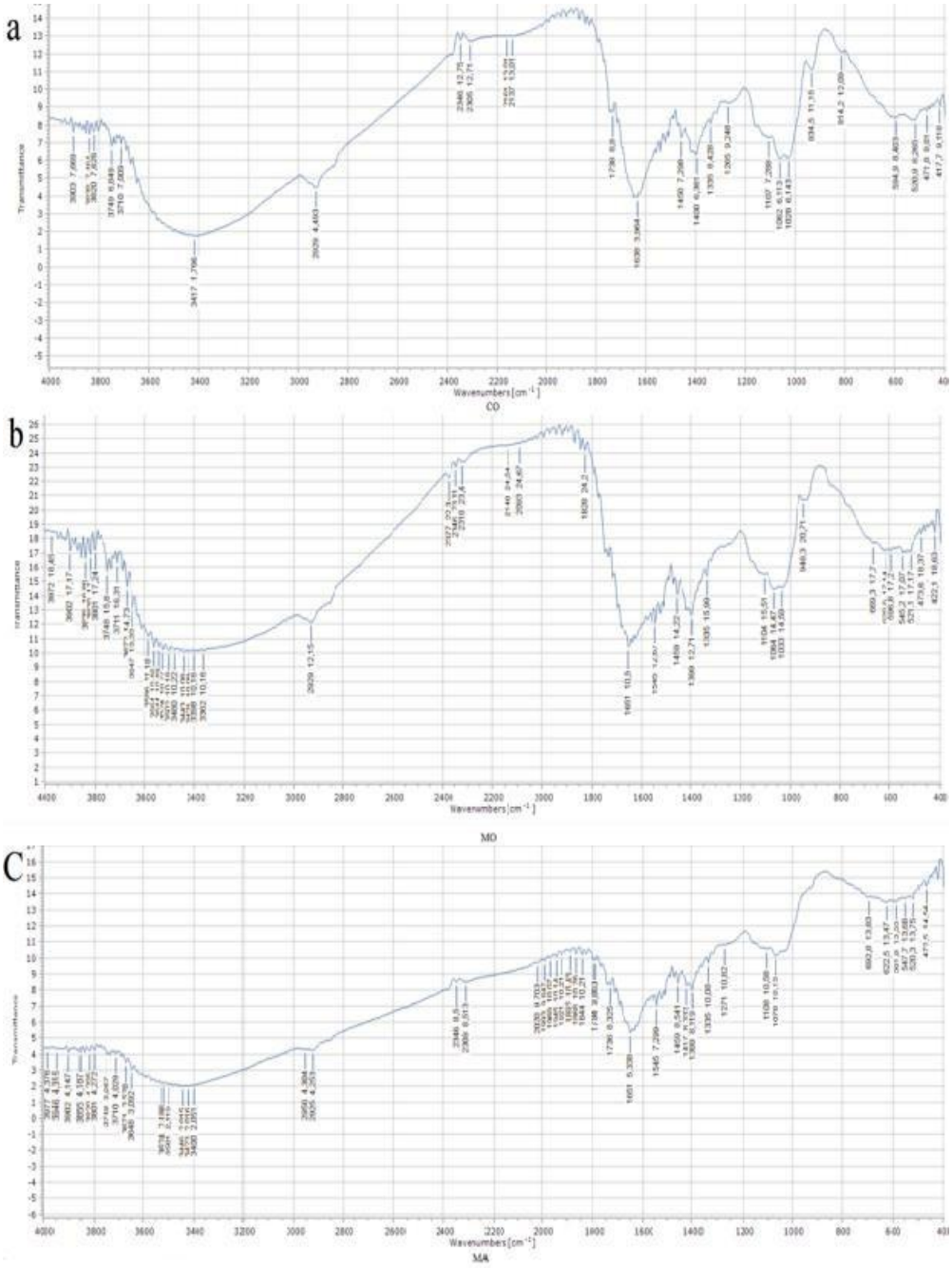
Appendix 53 Analysis of variance ANOVA test of the effect of garlic extract on the seedling root growth of *A. retroflexus*

Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	18	43796	2433,1	7,47	0,000
Error	129	42000	325,6		
Total	147	85797			

Appendix 54 Analysis of variance ANOVA test of the effect of garlic extract on the seedling shoot growth of *L. perenne*

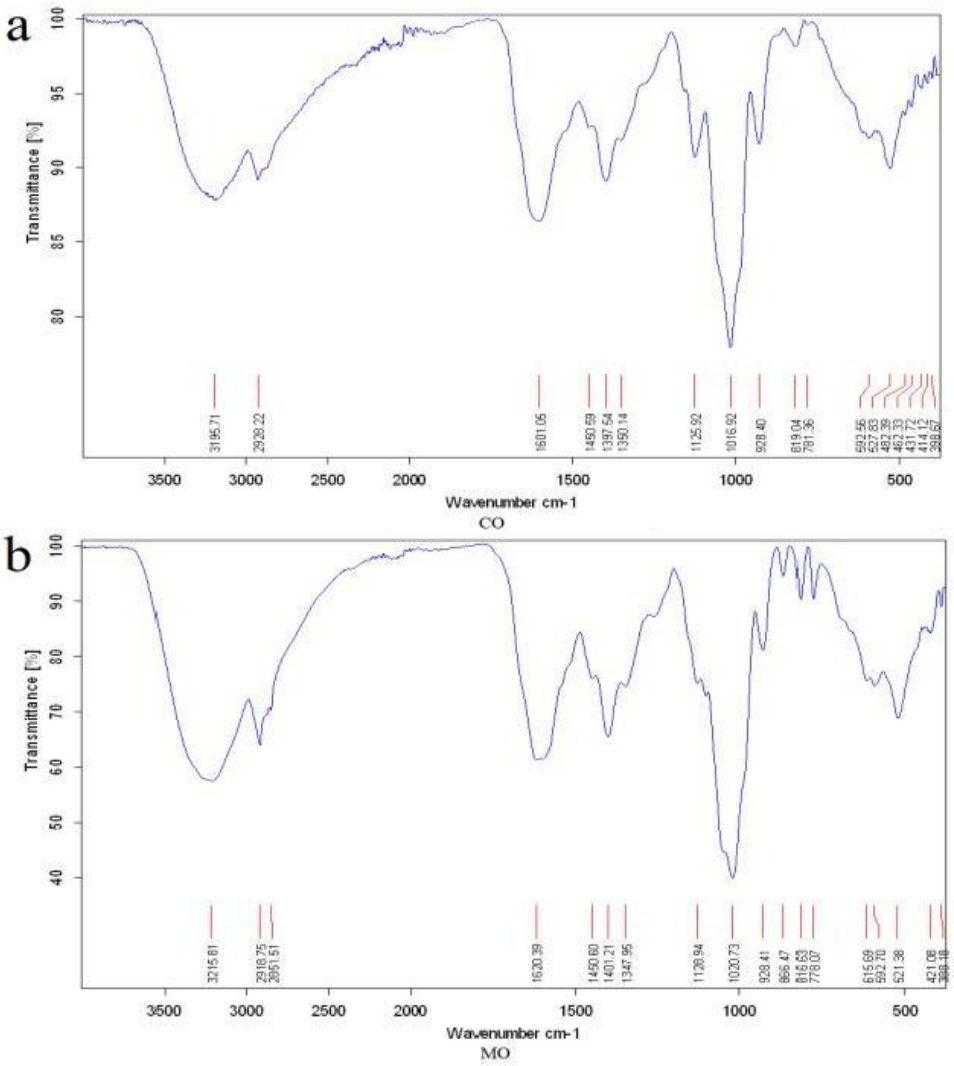
Source	DF	Adj SS	Adj MS	F-ratio	P-Value
Treatments	18	53392	2966,2	24,08	0,000
Error	95	11704	123,2		
Total	113	65096			

Appendix 54: Spectrograms of garlic samples.



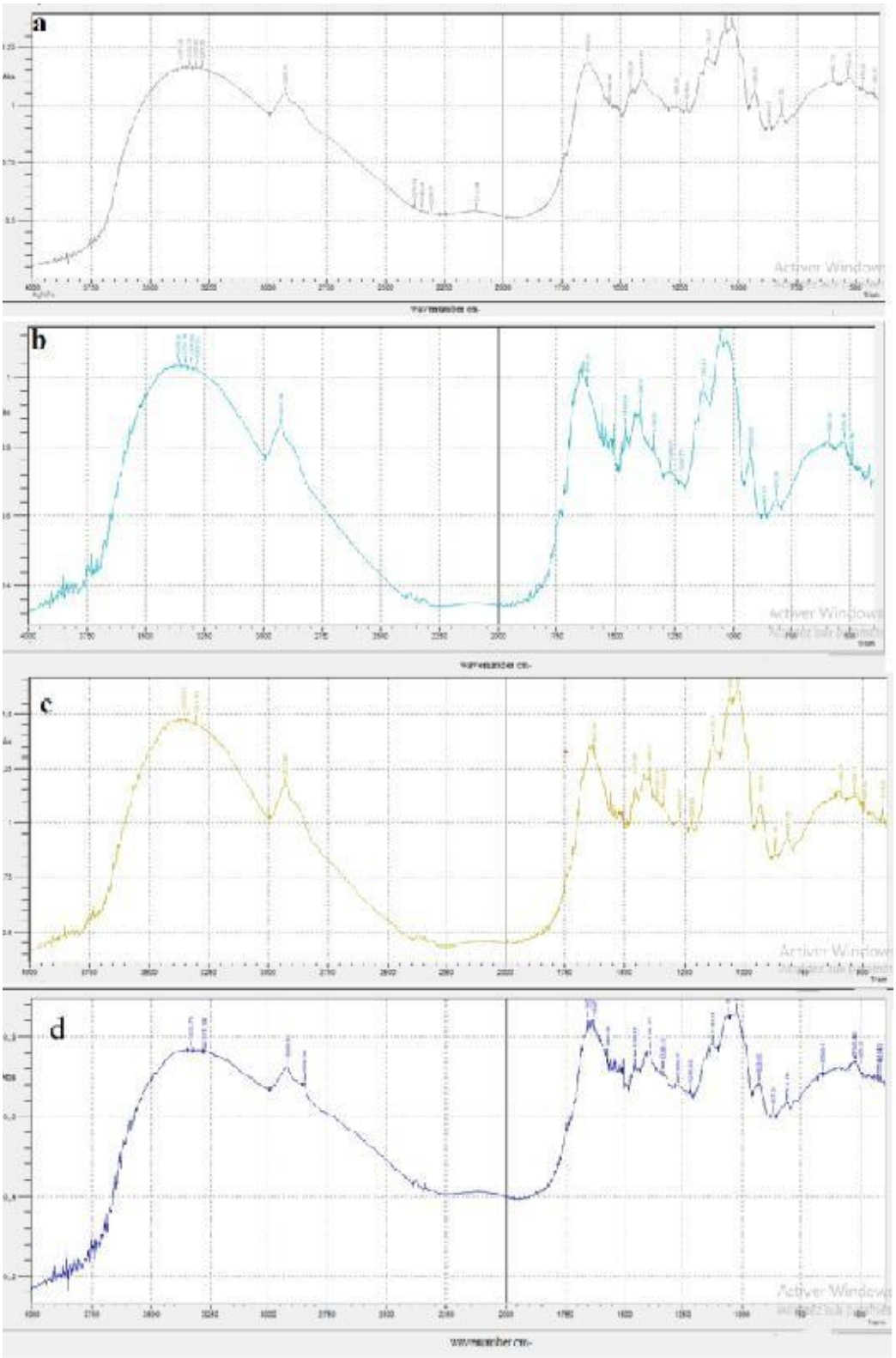
a: Control sample, b: sample treated with treatment MO , c: sample treated with treatment MA ,

Appendix 55: Spectrograms of garlic samples.



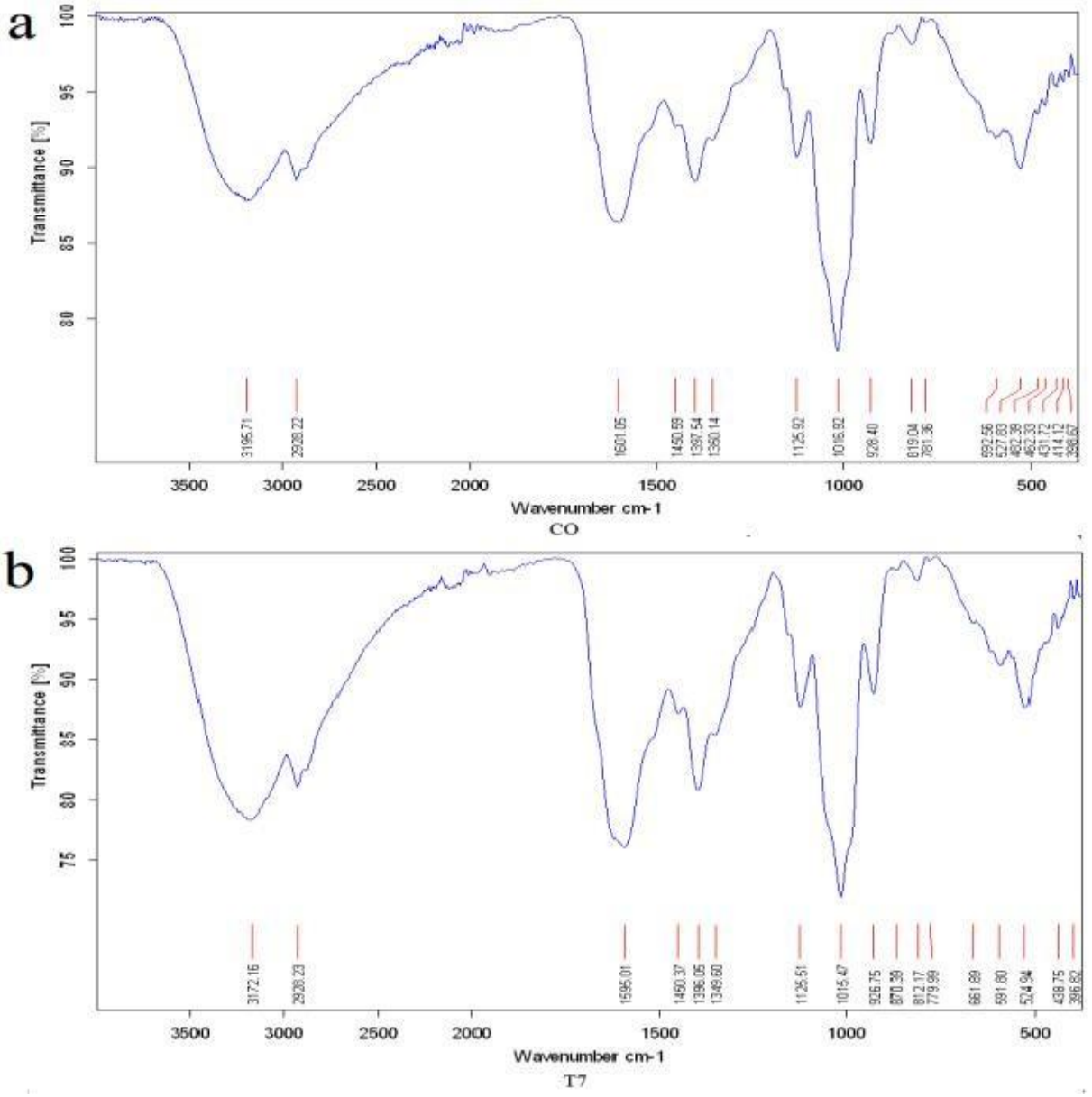
a:control sample, b: sample treated with MO treatment.

Appendix 56:Spectrograms of garlic samples.



a: Control sample, b: sample treated with treatment T1 , c: sample treated with treatment T2 , d: sample treated with treatment T3.

Appendix 57: Spectrograms of garlic samples.



a: Control sample, b: sample treated with treatment T1 , c: sample treated with treatment T2 , d: sample treated with treatment T3.