Democratic and Popular Republic of Algeria

Ministry Of Superior Education and Scientific Research

University Ammar TELIDJI- Laghouat

Faculty of Sciences

Department of Biology

Master's Dissertation for obtaining the diploma of

Masters in Applied Microbiology

Theme

Study of the interaction of two soil fungal isolates with *Arabidopsis thaliana*

by:

Safaâ FAGGOUS & Nour-el-Houda KERKABI

Members of the Jury

Dr. Meriem MARFOUA	Maître de Conférences	PRESIDENT
Mrs. Djamila AMEUR	Maître Assistant	EXAMINER
Mrs. Djalila TAKHI	Maître Assistant	DISSERTATION SUPERVISOR

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Dedications

بسم الله الرحمن الرحيم

قُلْ بِفَضْلِ اللَّهِ وَبِرَحْمَتِهِ فَبِذَٰلِكَ فَلْيَفْرَحُوا هُوَ خَيْرٌ مِّمَّا يَجْمَعُونَ

فالحمد لله أولاً وأخيراً، الذي بفضله وتوفيقه وحده تمّ ما تمّ، وكانت هذه الثمرة

This thesis is first dedicated to the courageous people of Gaza. To the students who dream of a future, to the teachers who uphold knowledge in the face of destruction, and to all those who stand for justice—your resilience fuels our collective conscience.

To my pillars of strength, my father and mother, who sacrificed endlessly, believed in me unconditionally, and whose love and prayers have been my guiding light. No words can truly express my gratitude.

To my sister, my confidante and personal cheerleader. Your empathy and endless belief in me often outweighed my own.

To my brothers, thank you for your constant encouragement, and for having my back no matter what. This achievement is ours as much as it is mine.

To my dear grandmother Asmaa, whose loving prayers and cherished stories have been a wellspring of comfort and strength. May Allah preserve her and grant her a long, healthy life.

To the memory of my beloved grandmother Talia who passed away. Her legacy of kindness and her whispered prayers continue to protect and guide me from above.

To my sisters-in-law, thank you for your love and support.

To my dearest friends, thank you for the much-needed distractions, and for standing by me through the challenges.

Finally, to myself, for the late nights, the resilience, and the unwavering belief that this day would come. This is for us.

Safaâ

Dedications

﴿ و آخر دعواهم أن الحمد لله رب العالمين ﴾

To my love and my support, to the one I love more than this world, to the most precious to my heart
(my father).

To my heaven, my candle in my dark nights, to the one who accompanied me with her prayers every step of the way
(my mother).

To those who were the light when the paths became dark my sisters
(Aicha, Nesrin, Khadidja, Bouthaina).

To my only moon and my third eye, my brother
(Mohamed).
To my second mother, to the one who loved me unconditionally, to my beloved
(my aunt)
. To the one who I found in my moments of weakness, my aunt
(Fatima).
To my beloved grandmother Aicha, and to those whom God took before this
(my aunt Bakhta, my grandmother Khadidja).
To the messages of God's kindness to me
(kaouthar,aicha,safaa,hadjer)

Finally, to my dear me, only you know what I have been through.
Thank you.

Houda

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Safaâ & Houda.

Abstract

This current study aimed to evaluate the ability of two soil fungal isolates, individually or in combination, to promote the growth of *Arabidopsis thaliana* col 0 and their potential to mitigate salt stress. The two fungi were isolated from two different soils, and were identified, microscopically, as belonging to the genus *Aspergillus*. The study of their salinity tolerance indicated that both fungi were halotolerant, with a wide range of NaCl tolerance, ranging from 0 to 20% (m/v). However, they exhibited an optimal growth at 5% (m/v) of NaCl. Four experimental treatments were applied for *A. thaliana* plants: a control group, without fungi, a group treated with *Aspergillus* sp.1 and *Aspergillus* sp.2, under three NaCl concentrations (0, 75, and 100 mM), and a group treated with a mixture of both, at 0 and 75mM of NaCl. The following growth parameters were measured: primary root length, number of secondary roots and leaves, fresh weight, and chlorophyll "a", "b", and total chlorophyll concentrations. The results were analysed using Welsh's ANOVA (with two factors) and the Games-Howell test for post hoc comparisons. The results showed that all fungal treatments led to significant improvements in growth parameters ($p < 0.05$) compared to the control group. The effect of *Aspergillus* sp.1 was more pronounced than the other treatments, especially at a concentration of 75 mM, while the fungal mixture showed no synergistic effect. These results indicate that *Aspergillus* sp.1 has great potential, not only to promote the growth of *Arabidopsis thaliana,* but also to mitigate the negative effects of salt stress, making it a promising candidate as an inoculant to improve sustainable agricultural systems and mitigate the negative effects of salinity without causing any negative environmental impact.

Keywords: *Arabidopsis thaliana* col 0; *Aspergillus*; PGPF; salt stress; root Architecture; Chlorophyll content.

Résumé

Cette étude avait pour but d'évaluer la capacité de deux isolats fongiques du sol, individuellement ou en association, à favoriser la croissance d'*Arabidopsis thaliana* col 0 et leur potentiel à atténuer le stress salin. Les deux champignons ont été isolés de deux sols différents et identifiés, au microscope, comme appartenant au genre *Aspergillus.* L'étude de leur tolérance à la salinité a montré qu'ils étaient halotolérants, avec une large plage de tolérance au NaCl, allant de 0 à 20 % (m/v). Cependant, leur croissance optimale était obtenue à 5 % (m/v) de NaCl. Quatre traitements expérimentaux ont été appliqués aux plants d'*A. thaliana* : un groupe témoin, sans champignon, un groupe traité avec *Aspergillus sp.1* et *Aspergillus sp.2,* à trois concentrations de NaCl (0, 75 et 100 mM), et un groupe traité avec un mélange des deux, à 0 et 75 mM de NaCl. Les paramètres de croissance suivants ont été mesurés : longueur des racines primaires, nombre de racines secondaires et de feuilles, poids frais, et concentrations de chlorophylle « a », « b » et totale. Les résultats ont été analysés à l'aide de Welsh ANOVA (à deux facteurs) et du test de Games-Howell pour les comparaisons a posteriori. Les résultats ont montré que tous les traitements fongiques ont entraîné des améliorations significatives des paramètres de croissance ($p < 0,05$) par rapport au groupe témoin. L'effet d'*Aspergillus sp.1* était plus marqué que celui des autres traitements, notamment à une concentration de 75 mM, tandis que le mélange fongique n'a montré aucun effet synergique. Ces résultats indiquent qu'*Aspergillus sp.1* présente un fort potentiel, non seulement pour favoriser la croissance d'*Arabidopsis thaliana,* mais aussi pour atténuer les effets négatifs du stress salin, ce qui en fait un candidat prometteur comme inoculant pour améliorer les systèmes agricoles durables et atténuer les effets négatifs de la salinité sans impact environnemental négatif.

Mots Clé: *Arabidopsis thaliana* col 0; *Aspergillus*; PGPF; stress salin; architecture racinaire; teneur en chlorophylle.

الملخص :

هدفت هذه الدراسة إلى تقييم قدرة عزلتين من فطريات التربة، بشكل فردي أو مجتمعتين، على تعزيز نمو نبات *Arabidopsis thaliana col 0* وإمكاناتهما في تخفيف الإجهاد الملحي. تم عزل الفطرين من نوعين مختلفين من التربة، وتم تحديدهما مجهريًا على أنهما ينتميان إلى جنس *Aspergillus*. أشارت دراسة تحملهما للملوحة إلى أن كلا الفطرين يتحملان الملوحة، مع نطاق واسع من تحمل كلوريد الصوديوم، يتراوح من 0 إلى 20٪ (م/حجم). ومع ذلك، فقد أظهرا نموًا مثاليًا عند 5٪ (م/حجم) من كلوريد الصوديوم. تم تطبيق أربع معالجات تجريبية على نباتات *A. thaliana*: مجموعة ضابطة، بدون فطريات، ومجموعة عولجت بفطري *Aspergillus sp.1* و *Aspergillus sp.2*، تحت ثلاثة تركيزات من كلوريد الصوديوم (0 و75 و100 ملي مولار)، ومجموعة عولجت بمزيج من كليهما، عند 0 و75 ملي مولار من كلوريد الصوديوم. تم قياس معايير النمو التالية: طول الجذر الأساسي، وعدد الجذور الثانوية والأوراق ، الوزن الطازج، تركيز الكلوروفيل "أ" و"ب"، و تركيز الكلوروفيل الكلي. حُلّلت النتائج باستخدام تحليل التباين Welch (بعاملين) واختبار Games-Howell للمقارنات اللاحقة. أظهرت النتائج أن جميع معاملات الفطريات أدت إلى تحسينات ملحوظة في معايير النمو ($p < 0.05$) مقارنةً بالمجموعة الضابطة. كان تأثير Aspergillus sp.1 أكثر وضوحًا من المعاملات الأخرى، وخاصةً عند تركيز 75 ملي مولار، بينما لم يُظهر خليط الفطريات أي تأثير تآزري. تشير هذه النتائج إلى أن Aspergillus sp.1 يتمتع بإمكانيات كبيرة، ليس فقط لتعزيز نمو نبات *Arabidopsis thaliana*، ولكن أيضًا للتخفيف من الآثار السلبية للإجهاد الملحي، مما يجعله مرشحًا واعدًا كملقح لتحسين النظم الزراعية المستدامة والتخفيف من الآثار السلبية للملوحة دون التسبب في أي تأثير بيئي سلبي.

الكلمات المفتاحية: *Arabidopsis thaliana* col 0 ، فطر *Aspergillus*، PGPF، الإجهاد الملحي، بنية الجذر، محتوى الكلوروفيل.

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Glossary

A microbial consortium refers to a community of two or more microbial species coexisting and interacting in a shared environment, often cooperating metabolically and functionally to perform complex tasks that individual species cannot efficiently accomplish alone. Such consortia exhibit metabolic complementarity and robustness, enabling improved resilience and function in processes like nutrient cycling, pollutant degradation, and plant growth promotion (Li *et al*., 2022).

Chelators: Chelators are organic or inorganic compounds that bind metal ions tightly, forming stable, water-soluble complexes known as chelates. This process facilitates the increased availability and uptake of essential micronutrients like iron, zinc, and magnesium by plants, enhancing nutrition and growth. Chelators also help prevent micronutrients from forming insoluble compounds unavailable to plants and can reduce plant pathogen growth by limiting available iron (Manik *et al*., 2023)

Phytohormones: Phytohormones are naturally occurring organic compounds that regulate various aspects of plant growth, development, and stress responses. These include auxins (such as indole-3-acetic acid), gibberellins, cytokinins, abscisic acid, and others, which modulate processes like cell division, elongation, seed germination, and systemic resistance to pathogens (EL Sabagh *et al*., 2022).

Antibiotics: In the context of plant-microbe interactions, antibiotics are biologically active compounds produced by microorganisms, including fungi and bacteria, that inhibit the growth of plant pathogens. These antimicrobial agents contribute both to biocontrol by suppressing harmful microbes and to promoting plant health (Roca & Matilla, 2023).

Topography is a fundamental geospatial feature that significantly influences a wide range of surface processes. It refers to the composition and physical properties of the Earth's surface, including elevation, slope, angle, and the arrangement of natural and human features.

Topography is a critical factor in the context of soil science and hydrology, controlling water flow, sediment distribution, and the spatial variation of soil properties across the landscape. Changes in topography create local climates and determine patterns of soil moisture, erosion, and deposition, which in turn influence soil composition (soil formation), nutrient availability, and plant distribution (Metternicht & Zinck, 2003).

Introduction

The food and agricultural organization of the United Nations estimate that human population growth is expected to rise by 34% by the year 2050 (Henchion *et al*., 2021; Giller *et al*., 2021). Consequently, food consumption will increase, especially for crops like rice and wheat, which are the primary food source for humans and animals worldwide (FAO 2025) According to the United Nations World Food Program (WFP) and the Climate Change Monitoring Program, long-term conflicts, economic shocks, and extreme weather conditions pose obstacles to achieving food security, rendering it unattainable worldwide. The increasing frequency of climate-related disasters, for instance droughts and floods, makes food production even more volatile.

Ensuring food securing for an ever-growing population is a complex challenge and difficult to resolve due to several factors:

- Diminishing tillable land area (Fan *et al*., 2020)
- Unstable environment.
- Unfavourable environmental conditions include Biotic and abiotic stresses (Tarroum *et al*., 2022).

Global warming increases soil evapotranspiration by raising air and soil temperatures, which increases the loss of water vapor from the soil and plant surfaces. This evaporation leads to a decrease in soil moisture and an increase in the concentration of dissolved salts in the soil water near the surface, raising soil salinity levels. Changes in rainfall patterns, especially the decrease and irregular distribution, exacerbate salinity by reducing salt leaching and promoting its accumulation in the root zone, negatively affecting crop growth and productivity (Corwin, 2020; Ullah *et al*., 2021).

Moreover, extreme temperature fluctuations disrupt plant metabolic and physiological functions, leading to reduced productivity (Bita & Gerats, 2013)

Biotic stress from pathogens and pests poses additional threats by interfering with nutrient transport and causing diseases that decrease crop yields (Pandey *et al*, 2017).

One way to increase crop yields and enhance their resilience has ever been the use of chemical fertilizers. This practice is no longer sustainable owing to the increasing costs of these products, their scarcity, their negative impact on the soil and marine ecosystems and to

their residual presence in the produced foods. Subsequently, the demand for ecofriendly biofertilizers, as a sustainable alternative, is increasing (Fan *et al*.,2020).

The use of biofertilizers dates back to the late 19th century with the commercial introduction of *Rhizobium* cultures. Despite their advantages over chemical fertilizers, their adoption has grown slowly due to challenges in formulation, shelf life, and field performance. Over the past decade, advances in microbial consortia and modern biotechnology have significantly expanded research and development in biofertilizers, with increasing studies and commercial applications worldwide (Aloo, 2022; Zhao *et al*., 2024)

Plant growth-promoting microorganisms (PGPMs), including fungi, offer multiple benefits for plants. For instance, improving their nutrient uptake, enhancing their protection against pathogens, inducing the plants' systemic resistance, and enhancing soil structure. Therefore, PGPM are regarded as biofertilizers, an eco-friendly alternative to conventional chemical methods for increasing crop productivity (Dhawi, 2023).

While the potential of plant growth promoting fungi (PGPF) is recognized globally, there is a lack of research evaluating isolated fungi from Algerian soil adapted to local climatic conditions.

The present study investigates the growth promoting potential, of two soil fungal strain isolated from Algerian soil, on the plant model *Arabidopsis Thaliana* Col- 0, by exploring the following aspects under different salinity conditions:

- The impact of the fungi on the primary root length, number of secondary roots and leaves and the fresh weight of the plant.
- The photosynthetic ability of the plant.

Literature Review

I. Soil salinization

One of the most serious environmental and agricultural challenges worldwide is soil salinization. The accumulation of dissolved salts, particularly sodium (Na^+) and chloride (Cl^-), in the rhizosphere degrades soil fertility and threatens food security (Rengasamy, 2006). According to the Food and Agriculture Organization (FAO), the area affected by salts worldwide is estimated at more than 833 million hectares, negatively impacting crop productivity and the well-being of rural communities.

The effects of soil salinization extend to the physical and chemical properties of the soil, not merely the productive aspect. It leads to the deterioration of soil structure and reduced permeability, in addition to inhibiting beneficial microbial activity (Rengasamy, 2006). Therefore, understanding the underlying causes of this phenomenon is a fundamental step in developing effective strategies to mitigate its severity and rehabilitate these lands.

Natural and human factors combine to cause soil salinization. Naturally, the geological parent rocks (the original soil material) are rich in minerals and salts which are released during rock weathering. This is particularly true in arid and semi-arid climates, where evapotranspiration rates exceed precipitation rates. The lack of precipitation limits salt leaching and leads to their concentration in the rhizosphere of the plants (FAO).

The rise of the saline groundwater level near the soil surface is another mechanism of soil salinization. This phenomenon induces salts ascension to the rhizosphere via capillary action therefore concentrating them in this area after water evaporation. This is particularly common in coastal areas, where seawater can infiltrate into groundwater reservoirs (Qadir *et al*., 2014). Topography also plays a pivotal role in soil salinization. Salts accumulate in low-lying areas (such as plains and valleys) that receive surface runoff (Metternicht and Zinck, 2003).

Several anthropogenic factors contribute to soil salinization. For instance, inefficient irrigation is the leading cause of soil salinity, and is the largest contributor to accelerating salinization worldwide. It includes over-irrigation, the use of poor-quality irrigation water and inadequate drainage systems. These practices raise the groundwater table and lead to soil salinization (FAO, 2021). Furthermore, the removal of vegetation due to urban sprawl or overgrazing contributes to raising the groundwater table and reducing transpiration,

facilitating salt accumulation (Nosetto *et al*., 2012). Moreover, excessive groundwater pumping, especially in coastal areas, also leads to the penetration of a saltwater wedge into freshwater aquifers, contaminating them (Werner *et al*., 2013).

II. Plant Growth Promoting Microbes

Microbes may affect plants growth and well-being in a beneficial, a neutral, or a detrimental way. Microorganisms can enhance the plant response to stressful conditions in several ways, including regulating their hormones. The benefits of these interactions can be exploited in agriculture and are considered as an achievable path towards agricultural sustainability (Laishram *et al*., 2024). The use of these microorganisms has proven an eco-friendly alternative to the application of pesticides and chemical fertilizers in agriculture (Jana *et al*, 2024).

Plant Growth Promoting Microbes (PGPMs) are microorganisms that positively impact the growth, development and survival of plants (Nsofor and Isaiah.,2021). PGPMs, found in the rhizosphere or associated with plant tissues, can promote plant growth through several mechanisms (Roberta *et al*., 2017). These mechanisms include the degradation and mineralization of organic compounds, solubilization of inorganic compounds and release of biologically active compounds like chelators, phytohormones, and antibiotics (Nsofor and Isaiah, 2021).

PGPMs include a variety of microorganisms, most notably Plant Growth-Promoting Rhizobacteria (PGPR) and Plant Growth-Promoting Fungi (PGPF). Some Protists, for instance *Amoabae*, have been reported as PGPMs (Nsofor and Isaiah., 2021).

1. Plant Growth Promoting Rhizobacteria

Plant growth promoting Rhizobacteria (PGPR), a diverse group of soil bacteria, including species of *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus*, and *Serratia* (Jitendra *et al*., 2017), are key components of soil plant systems and are among the most studied PGPMs (Jeyanthi and Kanimozhi, 2018; Stephane *et al*., 2005).

2. Plant Growth Promoting Fungi

Plant growth-promoting fungi (PGPF) are soilborne (Sudisha and Mostafa, 2019), non-pathogenic, saprophytic (Naziya *et al*., 2019; Naznin *et al*., 2014; Tohamy *et al*., 2021), filamentous fungi with the ability of significantly enhancing the growth of plants (Naznin *et al*., 2014). Species of the genera *Trichoderma*, *Fusarium*, *Penicillium* and *Phoma* have been reported to be beneficial to several crop plants.

For instance*, Trichoderma spp*. has been reported to promote the growth of tomatoes, rice, wheat and barley. Furthermore, their biocontrol ability involves inhibiting the growth of fungal pathogens such *as Rhizoctonia solani* and *Botrytis cinerea* via production of antifungal compounds and induction of systemic resistance in plants (Harman *et al*., 2004).

Penicillium spp. have been associated with growth promotion in tomatoes and rice by promoting their growth and offering protection against diseases, through phytohormone production, nutrient solubilization, and by providing protection against diseases via antifungal metabolite production and induction of systemic resistance (Adedayo & Babalola, 2023)

Fusarium species like *F. oxysporum* cause wilt diseases, however, related antagonistic species help protect against the deleterious effects of this plant infection, These biocontrol fungi include species of *Trichoderma* (e.g., *T. harzianum, T. viride), Gliocladium*, and non-pathogenic strains *of Fusarium oxysporum* itself, which inhibit pathogen growth through mechanisms such as competition for space and nutrients, production of antifungal metabolites, secretion of lytic enzymes, and induction of systemic resistance in host plants (Fravel *et al*., 2003).

Phoma species aid in suppressing soil-borne pathogens through multiple mechanisms,like production of antifungal metabolites, competition for nutrients and space in the rhizosphere, as well as the induction of systemic resistance in host plants. For exampel, *Phoma glomerata* produces epoxydon, a compound with inhibitory effects on pathogens responsible for clubroot disease in cruciferous plants, effectively reducing disease severity and promoting plant health (Deb *et al*., 2020).

III. Mechanisms for plant growth promotion by fungi

1. Phytohormone Production

Phytohormones perform an important function in controlling the growth of plants and their defence mechanisms (Adedayo *et al*., 2022). Indole acetic acid is a prominent auxin hormone that promotes the growth of crop plants' shoots and roots.

PGPFs, including *Aspergillus*, *Mortierella*, *Talaromyces*, *Fusarium*, *Penicillium*, and *Trichoderma*, isolated from the rhizosphere of tomato plant (*Solanum lycopersicum L*.) have been shown to produce IAA, thus contributing to their growth (Syamsia *et al*., 2021).

Several fungal species produce phytohormones such as auxins (IAA), gibberellins (GA), cytokinins (CKs), abscisic acid (ABA) and other phytohormones, thus, influencing plant growth and development. These fungal-produced hormones regulate seed germination, root and shoot development, and can modulate plant responses to biotic and abiotic stresses (Chanclud & Morel, 2016).

Studies show that fungal IAA can promote plant growth and induce systemic resistance, while directly reducing pathogen virulence by affecting spore germination, mycelial growth, and protein synthesis. For instance, the production of indole-3-acetic acid (IAA), by *Penicillium janczewskii*, inhibited the growth of the phytopathogen *Rhizoctonia solani* by disrupting its cellular functions. Furthermore, the fungus decreased the pathogens' mycelial dry weight and inhibited its proliferation, therefore, suppressing stem rot disease in the studied plants (Toghueo & Boyom, 2020)

2. Nutrient Mineralization

Nutrient Mineralizationis a biological process in which microorganisms decompose organic matter, releasing nutrients in inorganic forms accessible for plant uptake (Pantigoso & Newton, 2022).

PGPFs have been shown to stimulate enzymes produced by the plants. Key one involved are phosphatases and phytases, which catalyze the hydrolysis of organic phosphorus compounds such as phosphomonoesters into inorganic phosphate and alcohol. The released phosphate enhances phosphorus availability for plants and contributes to soil

phosphorus cycling (Pang *et al*., 2024). These enzymes originate from both the plants themselves and plant growth-promoting fungi (PGPFs).

- PGPF-mediated mineralization of soil nutrients plays a crucial role in enhancing plant growth (Adedayo & Babalola, 2023).
- Potassium (K) solubilization is predominantly performed by fungi such as *Aspergillus* niger and bacteria including Bacillus subtilis, Bacillus megaterium, and various Rhizobium species. These microorganisms release organic acids that convert insoluble potassium compounds into forms bioavailable to plants (Olaniyan *et al*., 2022).
- Zinc (Zn) solubilization is attributed to mineral-solubilizing bacteria, which facilitate zinc uptake necessary for various enzymatic functions essential to plant growth (Adedayo & Babalola, 2023).
- Iron (Fe) solubilization involves microbial production of siderophores, iron-chelating compounds that bind ferric ions (Fe3+), thereby improving iron acquisition by plants, which is vital for metabolic and photosynthetic processes (Adedayo & Babalola, 2023).

3. PGPFs as Biological Control Organisms

Plant growth-promoting fungi exhibit significant potential as biological control agents through antagonism, a process by which they prevent the growth of phytopathogens and spoilage organisms in plants. These fungi, often interacting synergistically with other microorganisms, deploy multiple mechanisms to exert biocontrol effects, including:

- Production of hydrolytic enzymes (chitinases, glucanases, proteases) and antifungal metabolites that directly degrade pathogen cell walls and inhibit their growth (Prapagdee *et al*., 2008).
- Induction of plant systemic resistance pathways that bolster the plant's innate immune system against pathogens (El-Saadony *et al*., 2022).
- shaping microbial community dynamics in the rhizosphere, which can suppress pathogen establishment (Andargie *et al*., 2023).
- Modifying fungal cell wall composition and regulating reactive oxygen species (ROS), which play roles in both pathogen defense and fungal colonization efficiency (Gow *et al*., 2017).

- Sequestration of effector proteins that facilitate fungal colonization and biocontrol efficacy (Snelders *et al*., 2020).

The PGPF biocontrol potential is attributed to the competition for nutrients that the organisms feed on, production of antimicrobial agents, and microbial predation. The process of Antagonism is also attributed to the stimulation of lytic enzymes such as chitinase, protease and glucanae (Adedayo *et al*., 2023), These enzymes are synthesized directly by the PGPFs (Adedayo & Babalola, 2023).

Examples of PGPF biocontrol mechanisms:

- Competition for nutrients: Nonpathogenic strains of *Fusarium oxysporum* isolated from rhizosphere soil of tomato (Solanum lycopersicum), chickpea (Cicer arietinum), banana (Musa spp.), and melon (Cucumis melo) (Iida *et al*., 2022). restrict pathogenic *Fusarium* growth by competing for nutrients and space, especially in plant roots (Adedayo & Babalola, 2023).
- Production of antimicrobial agents: *Gliocladium virens,* a plant growth-promoting fungus (PGPF), produces the antibiotic gliovirin, which inhibits the growth of the pathogen Pythium ultimum (Adedayo & Babalola, 2023) produces the antibiotic Gliovirin, which inhibits the growth of the pathogen *Pythium ultimum*. Furthermore, *Aspergillus niger* secretes hydrogen cyanide (HCN) and ammonia that enhance plant growth and inhibit pathogens pathogens including fungi and bacteria (Adedayo & Babalola, 2023).
- Lytic enzyme stimulation: *Trichoderma* spp. and other PGPF produce chitinase, protease, and glucanase enzymes that break fungal pathogen cell walls, as reported in multiple studies (Adedayo & Babalola, 2023; Yao *et al*., 2023).

IV. *Arabidopsis Thaliana* as a plant model

Arabidopsis thaliana was discovered by Johannes Thal, hence the name thaliana, in the sixteenth century. It is found in different habitats and has a broad geographical distribution, ranging from temperate Europe to high mountains in equatorial Africa (Laplaze *et al*., 2006)

Arabidopsis thaliana, also known as mouse cress (Leonelli., 2007), is a small herbaceous species (Xenie and David., 2007) belonging to the mustard family *Cruciferae*, also known as *Brassicaceae* (David, 1998; Elliot, 1987; Marcus *et al*., 2008; Leonelli, 2007).

The average length of *A. Thaliana* plants ranges from 10 to 15 cm (Leonelli, 2007). Nonetheless, some authors have reported lengths up to 20 cm (David,1998), 30 and 40 (Elliot, 1987).

The entire life cycle of *A. Thaliana*, from seed germination to maturation and formation of the first seeds is completed in 6 weeks (Woodward & Bartel, 2018) According to David, 1998; Elliot, 1987; Marcus et al., 2008; Leonelli, 2007; the cycle can extend to 8 weeks.

The genome of *Arabidopsis thaliana* was the first plant genome to be sequenced (Lionelli, 2007), at the end of 2000 (Arabidopsis Genome Initiative. 2000). The genome consists of five chromosomes, covering 115.4 Mb. It is the smallest known genome of a higher plant (Xenie and David, 2007). The reference ecotype for Arabidopsis thalian wild type genome is Columbia-0 (Col-0) (Michael *et al*., 2018).

The preferential use of *Arabidopsis* as a model for plant biology is due to several characteristics. It is small and easy to grow under laboratory conditions, with a short life cycle. Furthermore, it is very fertile (up to 100.000 seeds/plant) and self- or cross-pollination are easy (Laplaze *et al*.,2006). Moreover, the small genome of the plant facilitates the generation of mutant lines for the studies of several physiological processes. Hence, our choice of using this species for our study.

Methodology

In this study, we investigated the potential of two fungal isolates, Fu14 and Ch13 (previously isolated from soils located in the area of Laghouat) to promote the growth of *Arabidopsis thaliana* and alleviate salt stress. The study was conducted in a series of *in vitro* co-cultivation tests, involving the fungi individually and in combination, under salt stress conditions.

The experimental design included a complete factorial setup. Plant response was measured by assessing vegetative growth parameters (fresh weight, primary root length, number of secondary roots and leaves) and chlorophyll content. Statistical analyses were performed using Welch's ANOVA followed by Games-Howell post hoc tests to determine statistically significant differences between treatments.

I. Plant Material and growth conditions

Arabidopsis thaliana (wild type col 0) was used for the experiments of this study.

1. Surface sterilization of the *A. thaliana* seeds

Seeds of *A. thaliana* were surface sterilized as follows:

- Washing in an ethanol SDS (Sodium Dodecyl Sulfate) solution (0.02%, in 70% ethanol), with continuous mixing, for 12 minutes. The SDS solution is then discarded.
- The seeds are rinsed four times with an ethanol solution (70%). At each repetition, the ethanol solution is discarded.

After removing the ethanol, the seeds are left to dry completely under aseptic conditions.

2. Chilling and germination of the seeds

Twenty surface sterilized seeds were inoculated on 0,5 MS (Murashige and Skoog, including vitamins, Duchefa Biochimie) agar plates. The seeds are placed in two rows, distanced by 1 cm each, and at 1,5 cm distance from the edge of the Petri plate, thus allowing a good visualization of root growth.

To break dormancy and synchronize germination, the inoculated seeds were chilled at 4°C in the dark for 48 hours. At the end of the stratification period, the plates are

placed, at a 65° angle, in a phytotron to allow the germination and growth of the plants. The incubation lasted 7 d at 26°C under a 16 h light, 8 h dark (16L, 8D) cycle.

II. Fungal material and preparation of the spore suspension

Two fungal strains were assessed for the experiments of this study:

- Strain Fu14, isolated from a non-vegetated soil 5 km Northeast of the city of Laghouat.
- Strain Ch13, isolated from in hypersaline soil located at 70 km west of the city of Laghouat.

1. Summary identification of the Isolates

Each isolate is cultivated on PDA medium, subsequently, the macroscopic characteristics of the colony are described, according to the growth conditions, thus providing informations for the identification of the genus.

To perform the observation of the microscopic characteristics of the fungi, the slide culture technique was performed. Thereafter, the resulting mycelia and fungal fruiting bodies are observed, on the slides, using light microscopy.

2. Determination of the salinity optima for the studied fungi

To determine the optimal salinity for the studied fungi, the isolates were cultivated on PDA agar, supplemented with different NaCl concentrations (m/v): 0, 5%, 10%, 15% and 20 %. The tests were performed in triplicates.

The inoculated plates were incubated, at 35°C, for 7 days. At the end of the incubation period, the diameters of the colonies were measured.

3. Preparation of the spore suspension for the co-cultivation tests

Before the co-cultivation with *A. thaliana*, the fungi are grown on PDA agar, supplemented with 5% (w/v) NaCl, and incubated for 7 days at 35°C. The 7 days old fungal cultures are used for the preparation of a spore suspension, of 1×10^6 spores/ml. The spore's concentration is determined using a Malassez counting chamber.

III. Fungi- *Arabidopsis thaliana* Co-cultivation essays

1. Single fungus co-cultivation essay

The interaction of each fungal isolate with *A. thaliana* was evaluated on ½ MS with different NaCl concentrations:

- 0 mM
- 75 mM
- 100 mM

Prior to the co-cultivation essay, twenty seedlings, seven days old, were transferred from 0mM ½MS plate to ½ MS plates with the selected NaCl concentrations. The same seedling placement design, as previously described, is followed for these plates.

Twenty-five microliters of the preprepared fungal suspension are inoculated on the Petri plate, at 5 cm from the tip of the plant roots. The negative control is inoculated, in the same manner, with twenty-five µl of sterile physiological water. Each test is conducted in triplicate. The inoculated plates were incubated, at a 65° angle, for 15 days, in a climate chamber, at 26° under a 16 h light, 8 h dark (16L, 8D) cycle.

At the end of the incubation period the fresh weight of the plants was measured. Subsequently, the plants were flash-freezed in liquid nitrogen and stored at -20°C until the analysis of the chlorophyll content.

The length of the primary root and number of secondary roots were measured using the software ImageJ.

2. Dual co-cultivation essay

The potential for a synergistic activity for growth promotion on *A. thaliana* was evaluated on ½ MS agar medium, 0 and 75mM NaCl, where the two fungal isolates were inoculated simultaneously. The same seedlings placement design and growth conditions, as previously described were followed.

Twenty-five microliters of each fungal suspension were inoculated on the Petri plate simultaneously, at 5 cm from the tip of the plant roots. The negative control is inoculated, in the same manner, with twenty-five µl twice of sterile physiological water. Each test is

conducted in triplicate. The inoculated plates are incubated for 15 days, in a phytotron, at 26° under a 16 h light, 8 h dark (16L, 8D) cycle.

At the end of the incubation period the fresh weight of the plants was measured. Subsequently, the plants were flash-freezed in liquid nitrogen and stored at -20°C until the analysis of the Chlorophyll content.

The length of the primary root and number of secondary roots were measured using the software ImageJ.

IV. Chlorophyll content measurement

1. Chlorophyll extraction

The chlorophyll content was extracted and measured following a protocol adapted from Senthilkumar *et al*. (2021). Briefly, 0.1 g of the fresh aerial plant material was harvested and ground to a pulp in liquid nitrogen. The homogenate was then mixed with 2 ml of a cooled, freshly prepared 80% acetone solution, buffered to a pH of 7.5-8 (Chazaux *et al*., 2022). The resulting macerate was covered with aluminium foil and stored in the dark at 4°C for 24 hours to facilitate extraction. Following this maceration period, the mixture was centrifuged for 5 minutes, and the supernatant was collected. The pellet was re-extracted with fresh 2 ml volumes of 80% acetone, followed by centrifugation, until it became colorless. All supernatants were combined, and the final volume of the extract was adjusted to 10 ml using the 80% acetone solution. The absorbance of the extract was measured at 663.6 nm and 646.6 nm, corresponding to the maximum absorption of chlorophyll a and chlorophyll *, respectively, against a solvent blank (Chazaux *et al*., 2022).

2. Chlorophyll content determination

To determine the concentration of Chlorophyll a, Chlorophyll b and the total Chlorophyll content, expressed in µg/ml, in the acetone solution, the following equations, proposed by Chazaux *et al*, (2022), were followed:

- $[\text{Chl a}] = (12{,}18 \times A_{663{,}6\ \text{nm}} - 2{,}36 \times A_{646{,}6\ \text{nm}})$
- $[\text{Chl b}] = (20{,}19 \times A_{646{,}6\ \text{nm}} - 4{,}59 \times A_{663{,}6\ \text{nm}})$
- $[\text{Chl total}] = (17{,}83 \times A_{646{,}6\text{nm}} + 7{,}58 \times A_{663{,}6\ \text{nm}})$

For the determination of the chlorophyll content per fresh weight (FW) of plant material, the following equation is used:

Chlorophyll (µg/g FW) = [Chlorophyll]$_{(\text{in extract } \mu g/ml)}$ × Volume of extract $_{(ml)}$ / Fresh Weight of tissue (g)

V. Experimental Design, Treatments and Statistical analyses

A full factorial design was employed to investigate the main and interactive effects of fungal inoculation and salt stress. The experiment consisted of two factors: (1) fungal inoculation with four treatments [Control (not inoculated with the fungi), Fu14, Ch13, and a mixture of Fu14 and Ch13], and (2) salt concentration with three levels [0, 75, and 100 mM NaCl]. The fungal mixture treatment was excluded from the 100 mM concentration of NaCl**.** This resulted in a total of eleven distinct experimental treatments. Each treatment was replicated 3 times. All petri dishes were completely randomized within the phytotron to minimize the effect of any environmental gradients.

Data were analysed using ANOVA Welch test (with two factors) followed by Games-Howell test via SPSS software version 23. Each value presented is the mean of three replicates for all measured parameters. Different letters on bar charts are used to indicate means that differ significantly at $p < 0.05$.

Results and Discussion

I. Summary study of the fungal isolates

1. Macroscopic and microscopic identification of the studied fungi

The figures,1 and 2, represent the colonies and the microscopic characteristics of the studied fungal isolates, Fu14 and CH13, respectively. The fungi were cultivated on PDA supplemented with 5% (m/v) NaCl, and incubated 7 days at 35°C.

The colony of the isolate Fu14 (fig. 1; photo on the left) reaches a diameter of 90mm after 7 days of incubation. It presents a powdery texture with dark green in color, with spots of yellow on the periphery of the colony.

The microscopic observation of this fungus displays septate hyphae and a fruiting body (fig. 1, photo on the right) typical of *Aspergillus* species. No sexual reproductive structures, were seen. Therefore, microscopically, this isolate belongs to the genus *Aspergillus*, and will be henceforth in the manuscript named as *Aspergillus* sp. 1.

The colony of the isolate Ch13 (fig. 2; left photo), after an incubation period of 7 days, on PDA (5% NaCl) reaches a 9 cm diameter. As displayed in the figure, it presents a powdery texture with an olive-green color, with white grayish powdery aspect on the periphery.

The microscopic structure of this fungal isolate (fig. 2; right photo) presents branched hyphae with septa and a fruiting body characteristic of the fungal genus *Aspergillus*. Hence, microscopically, this isolate belongs to the genus *Aspergillus*, and will be, hereafter, referred to in the manuscript, as *Aspergillus* sp. 2.

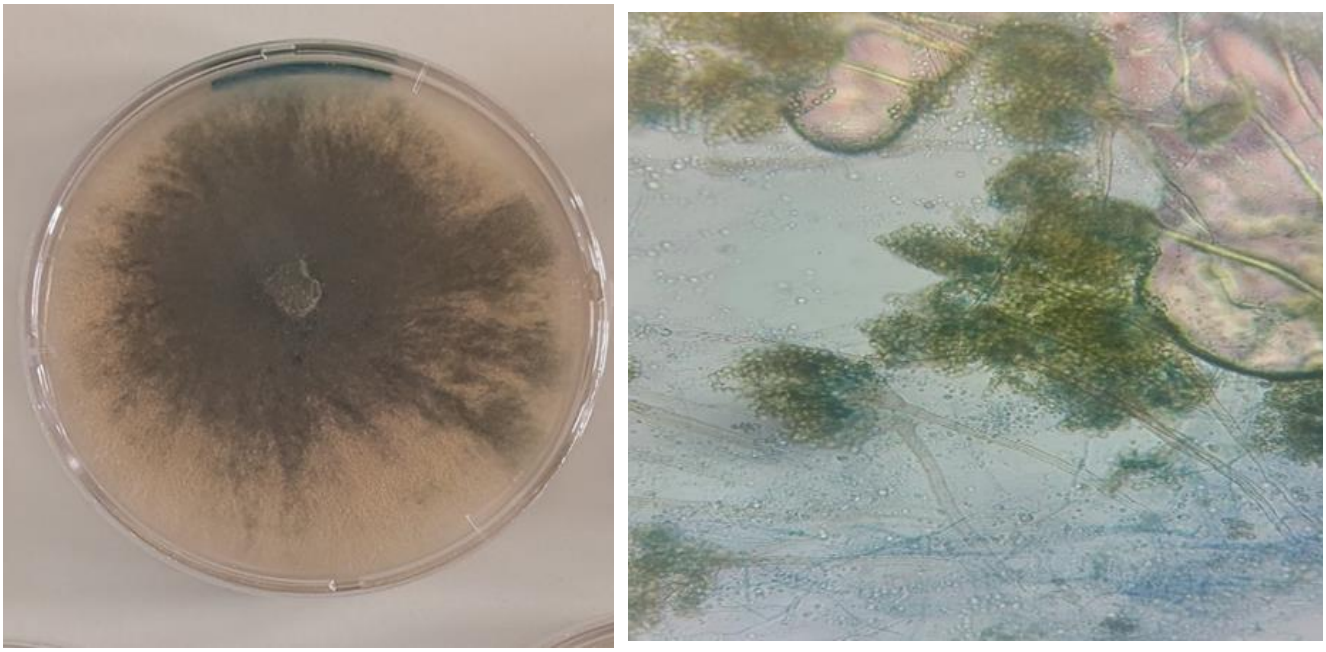


Figure 1: Macroscopic and microscopic characteristics of the fungal isolate Fu14 (personal photos)
On the left: Characteristics of the Colony. On the right: microscopic structure of the fungus.

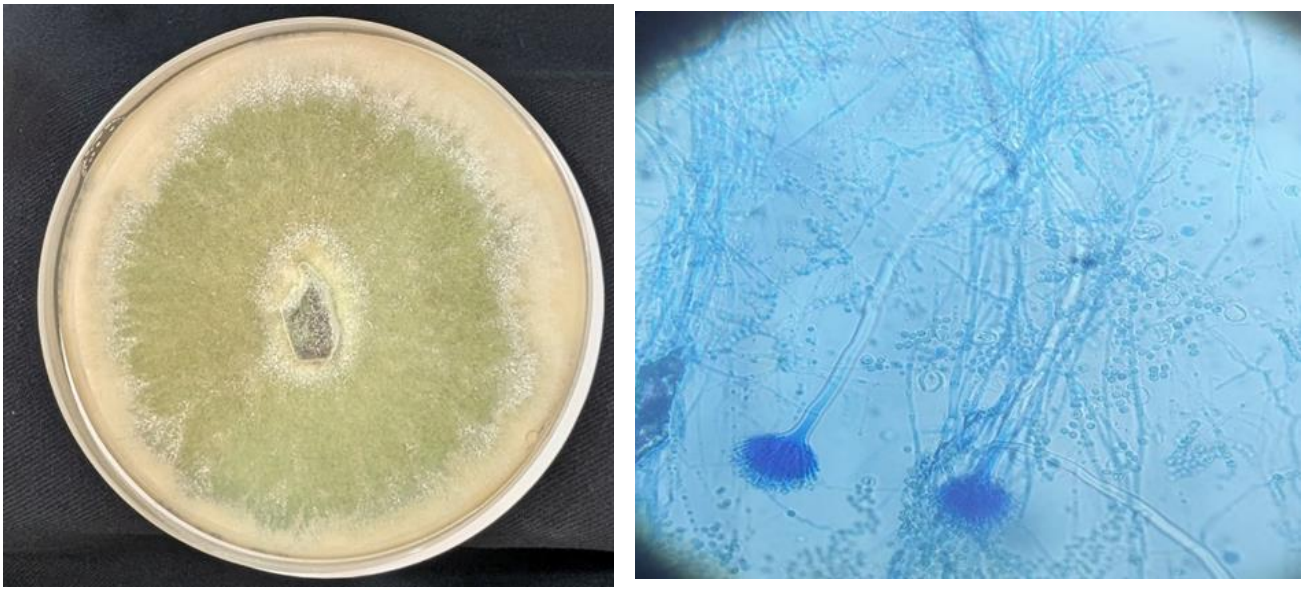


Figure 2: Macroscopic and microscopic characteristics of the fungal isolate CH13 (Personal photos)
On the left: Characteristics of the Colony. On the right: the microscopic structure of the fungus.

2. Determination of the salinity optima of the studied fungi

Figure 3 illustrates the effect of different NaCl concentrations on the growth of the studied fungi. The results are expressed by the average diameter of the fungal colony, generated from a triplicate testing, at each NaCl concentration.

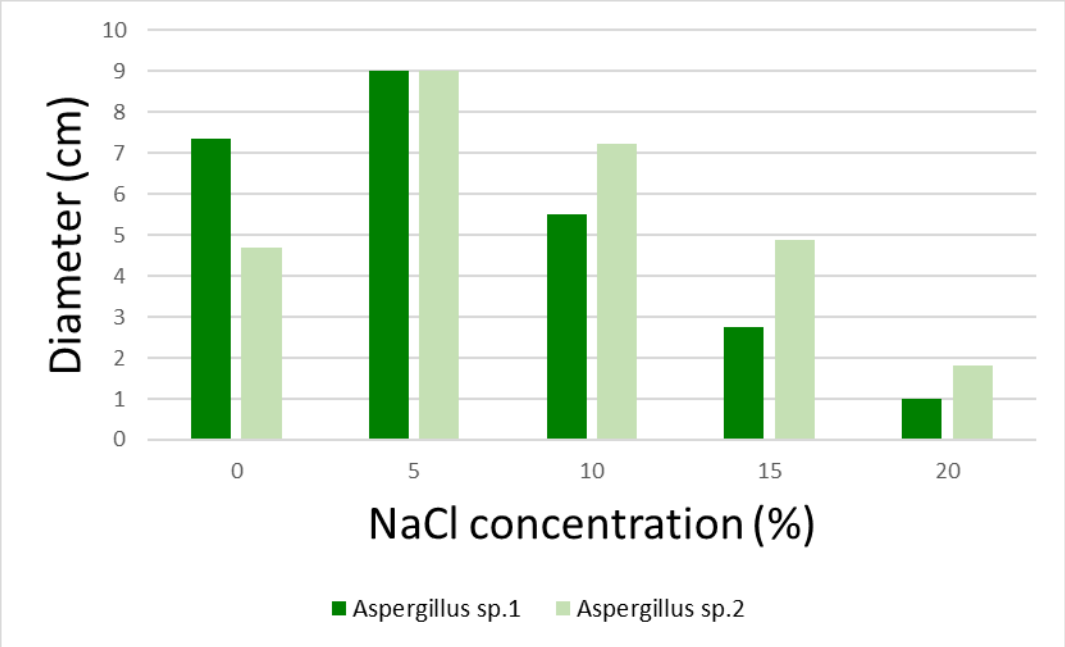


Figure 3 Growth diameter of *Aspergillus* sp. 1 and *Aspergillus* sp. 2 in different NaCl concentrations.

As displayed in the fig. 3, both fungal isolates grow, albeit not always optimally, at all tested NaCl concentrations. Both fungi grow optimally at (5%). However, their growth slightly decreases at (10%). For *Aspergillus* sp. 1, the growth is remarkably slowed beyond the concentration (15%). However, it the concentration (20%) that sharply reduces the growth of *Aspergillus* sp.2.

Based on these results, the studied fungi can be classified as halotolerant (Barnett & Hunter, 1998).

II. *A. thaliana* biometric results after the co-cultivation tests

1. Effect on Primary root length measurements

After a fifteen-day incubation period of *Arabidopsis thaliana* plants, co-cultivated with *Aspergillus sp.* 1, *Aspergillus sp*. 2 and a mixture of both, as described in the Methodology chapter, the primary root of each plant was measured, using the software ImageJ.

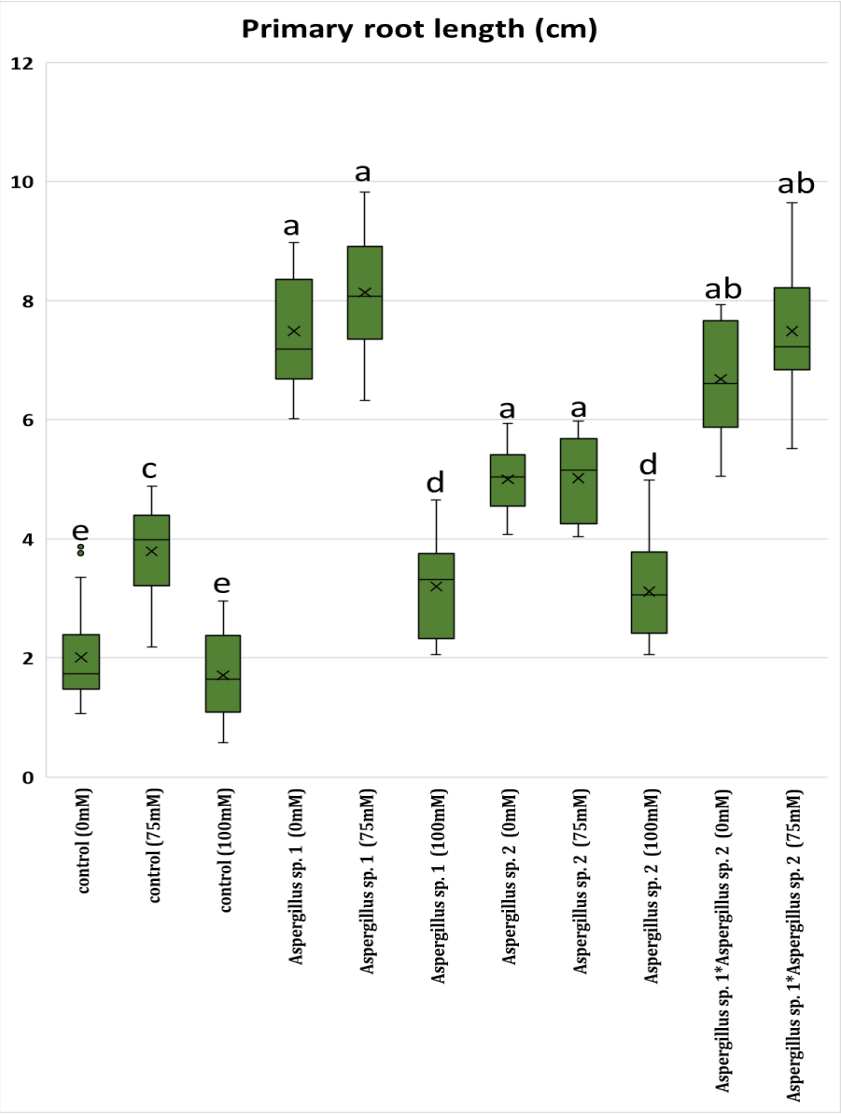


Figure 4 Distribution of primary root lengths across the study groups (control*, Aspergillus sp.1, Aspergillus sp.2, Aspergillus sp.1* Aspergillus sp.2)* at different NaCl concentrations (0 mM; 75mM; 100mM)

The distribution and homogeneity of variance of the data regarding the primary root length of the plants were examined before conducting statistical analysis. The results concluded that data does not follow a normal distribution. Therefore, due to the violation of the normality assumption, a Welsh ANOVA analysis, with two factor was performed in SPSS. The outcome of the test confirmed the presence of statistically significant differences in the primary root lengths between the studied groups, control group, group treated with Aspergillus sp.1, group treated with Aspergillus sp.2 and the group treated with the combination of the two fungus Aspergillus sp.1 and Aspergillus sp.2 at the different NaCl concentration (0,75,100 mM), with a confidence interval of 95%. The figure 4 displays the different groups.

The Games-Howell test was used to identify where the significant differences lie among the groups:

- There is a statistically significant difference (sig. < 0.05) between both the 0 mM and 75 mM concentrations of the control group. While the 100 mM group is statistically similar to the 0 mM concentration.
- All fungal treatments, individually or in combination, led to a statistically significant increase in the length of the primary root compared to the control group at the same NaCl Concentration (all p-values = .000). For the *Aspergillus* sp.1-treated group, at all tested NaCl concentrations, the root length was greater than the control group (fig. 4). For instance, at a concentration of 75 mM, the root length was 4.34 cm (53.38%) greater than the roots of the control group at the same NaCl concentration.

  Aspergillus sp. 2 also exhibited a positive effect on the length of the primary root compared to the control group at all concentrations. At 0 mM, the primary roots of this group were 1.22 cm (24.4%) longer than the control group at 0 mM. At 75 mM, the primary root length increased by 1.22 cm (24.3%) compared to the control group at the same concentration.

  However, the performance of *Aspergillus* sp.1 was significantly better than that of *Aspergillus* sp.2. For example, at 0 mM (sig.= .000), the difference between them was 2.49 cm (33.22%).

- The groups treated with both *Aspergillus* sp.1 and *Aspergillus* sp.2, at the same time, showed statistically significant differences compared to the control group, at all tested NaCl concentrations (sig. = 0.000). At 0 mM, root length increased by 4.67 cm (75.70%) compared to the control group. The performance of the mixture was statistically similar to that of *Aspergillus* sp.1 alone; for instance, at 75 mM, the sig. = 1.000, the treatments were not statistically different.

Several studies confirm the ability of PGPF to promote plant growth and mitigate salt stress, especially those of the genus *Aspergillus* (Hung & Rutgers, 2016), and this is consistent with our study. According to the results presented in Figure 4, we conclude that all groups treated with *Aspergillus* fungi, either individually or in combination, significantly increased primary root length compared to the control group at all tested NaCl concentrations. In a study by Ismail *et al*. (2020), it was found that *Aspergillus Niger* produces two phytohormones, auxin (IAA) and gibberellic acid (GA). These hormones increase cell division and elongation, which increases root length and biomass in wheat after treatment with this fungus. These phytohormones elongate the primary root by influencing plant gene expression and cell division in the root elongation zone, which explains the significant increase in primary root length of *Arabidopsis thaliana* plants (Contreras-Cornejio *et al*., 2019). The physiological response of increased primary root length observed in plants treated with fungi, either alone or in combination, suggests that the mode of action of *Aspergillus* sp.1 and *Aspergillus* sp.2 may be similar to that of *Aspergillus Niger* described in this study, by producing growth-promoting plant hormones such as auxins (IAA) and gibberellins (GA).

As illustrated in Figure (4), *Aspergillus* sp.1 outperformed *Aspergillus* sp.2, this observation can be explained by several mechanisms; *Aspergillus* sp.1 could be a more effective activator of plant defence and adaptation mechanisms, as explained by Hashem *et al*. (2019). Furthermore, it could be due to the ability of this species to produce higher concentrations of quality growth-promoting compounds.

The results of our study showed no statistical difference between the performance of the fungal mixture and the performance of *Aspergillus* sp.1 alone. This indicates the

potential absence of a synergistic effect between *Aspergillus* sp.1 and *Aspergillus* sp.2, and that *Aspergillus* sp.1 was the dominant factor while *Aspergillus* sp.2 did not provide any measurable benefit. This is what several studies have found. Due to competition for colonization sites or nutrients between microorganisms, the mixture of strains may not achieve better results than the effective strain alone (Barea *et al*., 2017).

2. Effect of the fungal treatments on the number of lateral roots

The numbers of lateral roots, for each plant of A. thaliana was counted after the cocultivation period with the fungal treatments.

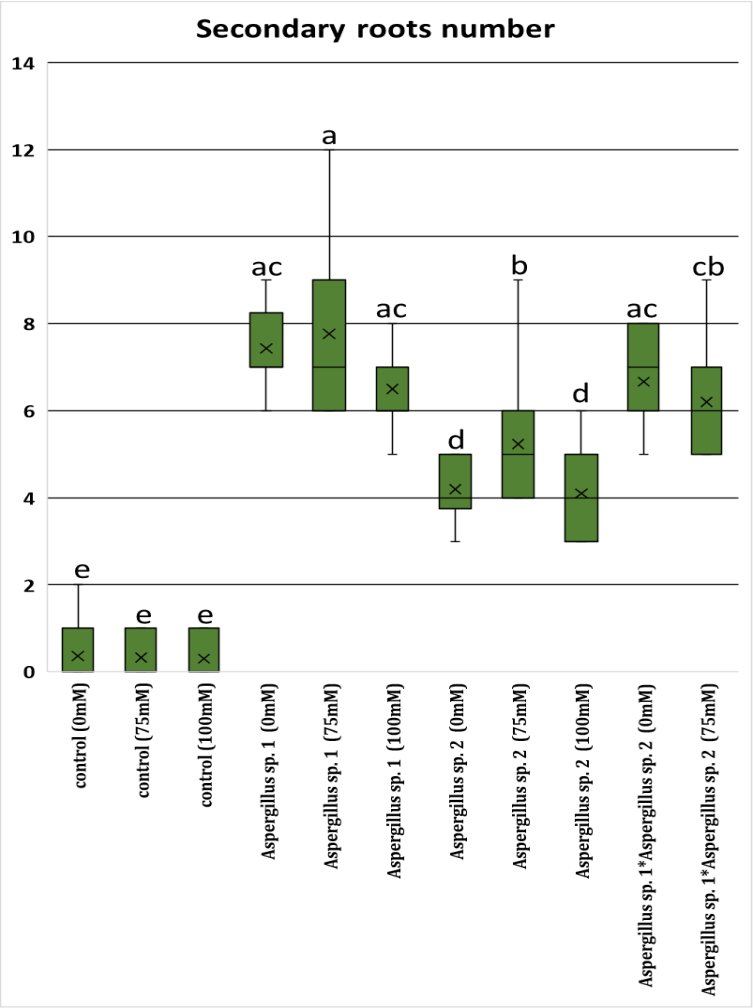


Figure 5 Distribution of secondary roots count values across the study groups (control, *Aspergillus sp.1, Aspergillus sp.2, Aspergillus sp.1* Aspergillus sp.2)* at different NaCl concentrations (0 mM; 75mM; 100mM)

Due to the non-normality of the data and the heterogeneity of variance, a Welsh ANOVA analysis, with two factors, was used for the analysis of the generated data of the number of lateral roots, with a confidence interval of 95%. The test yielded a high statistically significant difference between the means of the number of secondary roots of the groups (fig. 5).

The Games-Howell test was performed for post hoc pairwise comparisons, and the most significant differences are as follows:

There were no statistically significant differences in the number of secondary roots between the different salinity levels (0.75, 100 mM) for the control group. This indicates that salt stress, whether medium (75 mM) or high (100 mM), did not negatively affect the number of secondary roots, in contrast to its clear effect on the length of the primary root.

All fungal treatments showed a statistically significant increase ($p < .001$) in the number of secondary roots compared to the control group at all salinity levels.

At 0 mM concentration, the number of roots increased by 7.07 lateral roots (95.15%) in the *Aspergillus* sp.1 treated group and by 3.83 lateral roots (91.19%) in the *Aspergillus* sp.2 group, compared to the control group. At 75 mM concentration, *Aspergillus* sp.1 positively affected the number of secondary roots, increasing by 7.43 secondary roots (95.62%), thus maintaining its statistically significant superiority over *Aspergillus* sp.2 in most cases, both under normal conditions and under salt stress. For example, at 0 mM NaCl concentration, the difference between *Aspergillus* sp.1 treated group and *Aspergillus* sp.2 treated group was 3.23 lateral roots corresponding to a percentage increase of 43.47% ($P < .001$).

The treated plants with *Aspergillus* sp.1 * *Aspergillus* sp.2 mixture showed a significant increase in the number of lateral roots compared to the control group, at all tested NaCl concentrations (fig. 5). The mean difference of secondary roots between these two groups being 6.300 (94.45%) and 5.867 (94.45%) at 0 mM and 75 mM, respectively. However, this treatment did not show a statistically significant superiority over the single use of *Aspergillus* sp. 1.

These results indicate that even in the absence of fungi, root branching (lateral root formation) resisted salt stress more than root elongation. This is consistent with the hypothesis proposed by Munns & Tester. (2008) that each process is regulated somewhat independently. By disrupting membrane functions and hormonal balance, salt stress disrupts Meristematic cell elongation (Munns & Tester, 2008), while the sites of secondary root formation (lateral root primordia) have already formed (Pérez-Torres *et al*., 2008).

If we return to the figure 5, we notice that all groups treated with fungi exhibited an increase in the number of secondary roots, compared to the control groups. This result leads to believe the *Aspergillus* sp.1 and *Aspergillus* sp.2 are strong stimulants for root growth and development as well. Contreras-Cornejo *et al*. (2016) attributed this effect to the hormone auxin produced by this type of fungi, as it plays an important role in activating a series of reactions in the peripheral cells of the pericycle, which leads to the development of a new secondary root (Lavenus *et al*., 2013). Our results are consistent with a study conducted by Abdelaziz *et al*. (2017) where the authors reported an increase in the number of secondary roots in *Arabidopsis thaliana* plants treated with *Piriformospora Indica*, a plant root endophytic fungus, at 100 mM NaCl. This increase is one of the mechanisms by which the plant copes with salt stress, as it expands the surface area for the absorption of various ions such as potassium and calcium.

The results in Figure (5) demonstrate that *Aspergillus* sp.1 outperforms *Aspergillus* sp.2 in stimulating secondary root formation. According to Rouphael *et al*., (2017), this indicates that *Aspergillus* sp.1 produces higher quantities of potent growth-stimulating compounds, such as IAA and gibberellic acid. Furthermore, it may have a greater root colonization capacity than *Aspergillus* sp.2 (Hashem *et al*., 2019). The results in Figure (5) indicate that the fungal mixture was not statistically superior to the group treated with *Aspergillus* sp.1 alone. This could mean that a synergistic effect between the two fungi is absent.

3. Effect of the studied fungi on the number of leaves

At the end of the two weeks cocultivation period, the number of leaves for each plant was counted.

Due to the violation of the assumption of normality and homogeneity of variances, a Welsh ANOVA test, with two factors, was performed for obtained data, with a confidence interval of 95%. The average leaf counts of the various groups showed high statistically significant differences, suggesting that the treatments had a significant impact on leaf count, as displayed in figure 6.

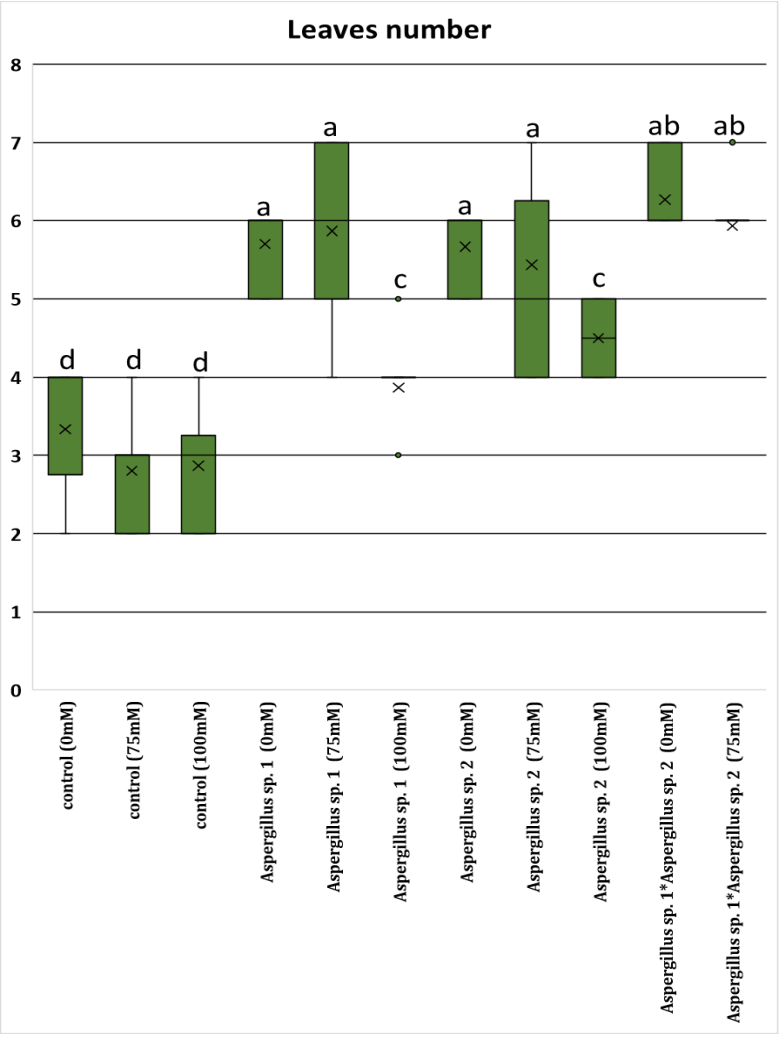


Figure 6 Distribution of leaves number across the study groups (control, *Aspergillus* sp.1, *Aspergillus* sp.2, *Aspergillus* sp.1* *Aspergillus* sp.2) at different NaCl concentrations (0 mM; 75mM; 100mM)

The results of pairwise comparisons, using the Games-Howell test, showed the following:

- There were no statistically significant differences in the control groups at the different tested NaCl concentrations (fig. 6). This means that salt stress did not cause any statistically significant change in leaves number in the absence of fungi.
- According to the results displayed in fig. 6, the tested Fungi have exhibited a beneficial role for *Arabidopsis thaliana* plants. All fungal treatments, whether alone or in combination, led to a significant increase in leaves number ($P < 0.001$) compared to the corresponding control group at the same NaCl concentration.
- The *Aspergillus* sp. 1 and *Aspergillus* sp. 2 treatments were statistically similar in their effect on leaf number (fig. 6). For example, at 0 mM NaCl concentration, the mean increase in leaf number in the *Aspergillus* sp.1 group was estimated at 2.367 leaves (41.52%), in the *Aspergillus* sp.2 group at 2.333 leaves (41.14%), compared to the control group at the same NaCl concentration ($P < 0.001$).
- The increase in leaf numbers was most evident in the combined treatment groups (*Aspergillus* sp.1* *Aspergillus* sp.2), with the results showing that the differences between this group and the control group were as follows: 2.933 leaves (46.77%) and 3.133 (52.83%) at both concentrations of 0 mM and 75 mM, respectively. and the individual fungal groups were positive and statistically significant in most comparisons ($P < 0.001$). This indicates that there is no synergistic effect between the two fungi on the leaves number increase of *Arabidopsis thaliana* plants, however all groups treated with the fungi (single or combined) continued to show a significant increase in leaves number under high salt stress (100 mM) compared to the control group, indicating that these fungi confer tolerance to salt stress to *Arabidopsis thaliana* plants.

As with the number of secondary roots, there were no statistical differences in the number of leaves in the control group at various salt concentrations (0, 75, and 100 mM), this suggests that the number of leaves was not affected by the increase in salt. This indicates that *Arabidopsis* possesses mechanisms that enable it to maintain vegetative

development under certain levels of stress, as discussed by Julkowska and Testerink (2015).

The studied fungi, *Aspergillus* sp. 1 and *Aspergillus* sp. 2, proved their benefits as a significant increase in the number of *Arabidopsis thaliana* leaves was observed in all groups treated with the two fungi (Figure 6), even under high levels of NaCl. This indicates the ability of these fungi to enhance the plant's physiological fitness, allowing it to grow better and withstand stress.

Our results are consistent with the study conducted by Pandya and Saraf (2010), in which they investigated the effect of fungal isolates from different genera (*Aspergillus*, *Penicillium*, and *Fusarium*) on chickpeas at 2% NaCl (340 mM). They noticed that all isolates significantly increased the number of leaves compared to the control group (untreated with any fungi). Two species of *Aspergillus* in this study also showed an increase of 45.09% in the number of leaves. The authors theorized that these improvements may be linked to direct mechanisms such as phosphate solubilization and the production of siderophores produced by some *Aspergillus* species. These mechanisms improve nutrient availability for plants under stress.

Another study showed that *Aspergillus niger* and *Aspergillus parasiticus* enhanced plant growth parameters, namely, leaf number in green beans (Mung Bean) by producing siderophores that convert (Fe^{3+}) to (Fe^{2+}) for easy absorption by the plant, which improves the nutritional efficiency of the plant and has a positive impact on all growth traits (Patel *et al*., 2017).

Aspergillus fungi are also known to produce plant hormones that lead to increased leaf number by stimulating cell differentiation in the apical meristem and lateral buds (Khan *et al*., 2011). Fungi also provide the nutritional support necessary for the development of new leaves by facilitating nutrient uptake in the soil under normal or salt-stress conditions (Hashem *et al*., 2019). To maintain the integrity and activity of meristematic cells, fungi also contribute to mitigating oxidative stress caused by salt ions in salt stress conditions (Egamberdieva *et al*., 2017).

In our study, the plants treated with the fungal mixture yielded the highest values in leaf number compared to the control group, but its effect remained statistically similar to the group treated with *Aspergillus* sp.1 alone. This indicates that the effect between the two fungi was additive and not synergistic, some studies have suggested that this may be due to colonization sites and resources in the rhizosphere preventing interaction between them due to competition for them (Barea *et al*., 2017; Latz *et al*., 2018).

4. Effect of the fungi on the Fresh weight of the plants

At the end of the two weeks cocultivation period, the fresh weight for the individual plants was measured. Since the normality and homogeneity of variances assumptions of the data were not met, The Welsh's ANOVA test, with two factors, was performed for obtained data, with a confidence interval of 95%.

Welch's ANOVA test revealed high significant differences between group means (p<0.001). The results are presented in figure 7. These results confirm the significant influence of the studied factors on the fresh weight variable of *Arabidopsis thaliana* plants.

Pairwise comparison analysis, using the Games-Howell test, revealed the following results:

- There were no statistically significant differences ($P > 0.05$) between for the control group at the tested NaCl concentrations (fig. 7). This result suggests that salt (NaCl) stress did not cause any statistically significant change in the fresh weight of *A. thaliana* col-0 in the absence of fungi.
- All fungal treatments, whether alone or in combination, led to a significant increase in fresh weight ($P < 0.001$) compared to the corresponding control group at the same NaCl concentration (fig. 7). This indicates that fungi played a beneficial role for *Arabidopsis thaliana* plants.
- The performance of *Aspergillus* sp.1 and *Aspergillus* sp.2 strains was statistically similar at most salt concentrations (fig. 7). The *Aspergillus* sp.1 group treated at 100 mM NaCl showed a slight superiority over *Aspergillus* sp2, with a difference in fresh weight estimated at 1.45 mg (21.42%).

- Co-inoculation of the two fungi (*Aspergillus sp*.1* *Aspergillus sp*.2) did not result in any statistically significant increase compared to the single inoculation of either *Aspergillus* sp.1 or *Aspergillus* sp.2. This indicates the absence of a synergistic effect between the two fungi on the fresh weight of the plants. However, all groups treated with the fungi (either alone or in combination) exhibited a significant increase in fresh weight under the highest tested NaCl concentration (100 mM), compared to the control group (Fig.7). This indicates that these fungi confer tolerance to salt stress to *Arabidopsis thaliana* plants.

Figure 7 illustrates the absence of statistical differences between the results of the control groups at all tested NaCl concentrations, it suggests that salinity did not affect the fresh weight in this group. This result could be due to the mechanisms that *Arabidopsis* possesses, which enable it to maintain its growth development under certain levels of stress (Julkowska & Testerink, 2015) by producing antioxidants or accumulating osmotically compatible substances such as proline, which facilitates water absorption and maintains the main determinant of fresh weight, cell turgor. These mechanisms can compensate for the osmotic effect of salinity and thus the plant maintains its fresh weight (Hasegawa *et al*., 2000; Ashraf and Harris, 2004).

All fungal treatments showed a significant increase in the fresh weight of the plants. This result confirms that *Aspergillus* sp. 1 and *Aspergillus* sp. 2 fungi stimulate *Arabidopsis thaliana* growth and improve its tolerance to salt stress, hence the increase in fresh weight.

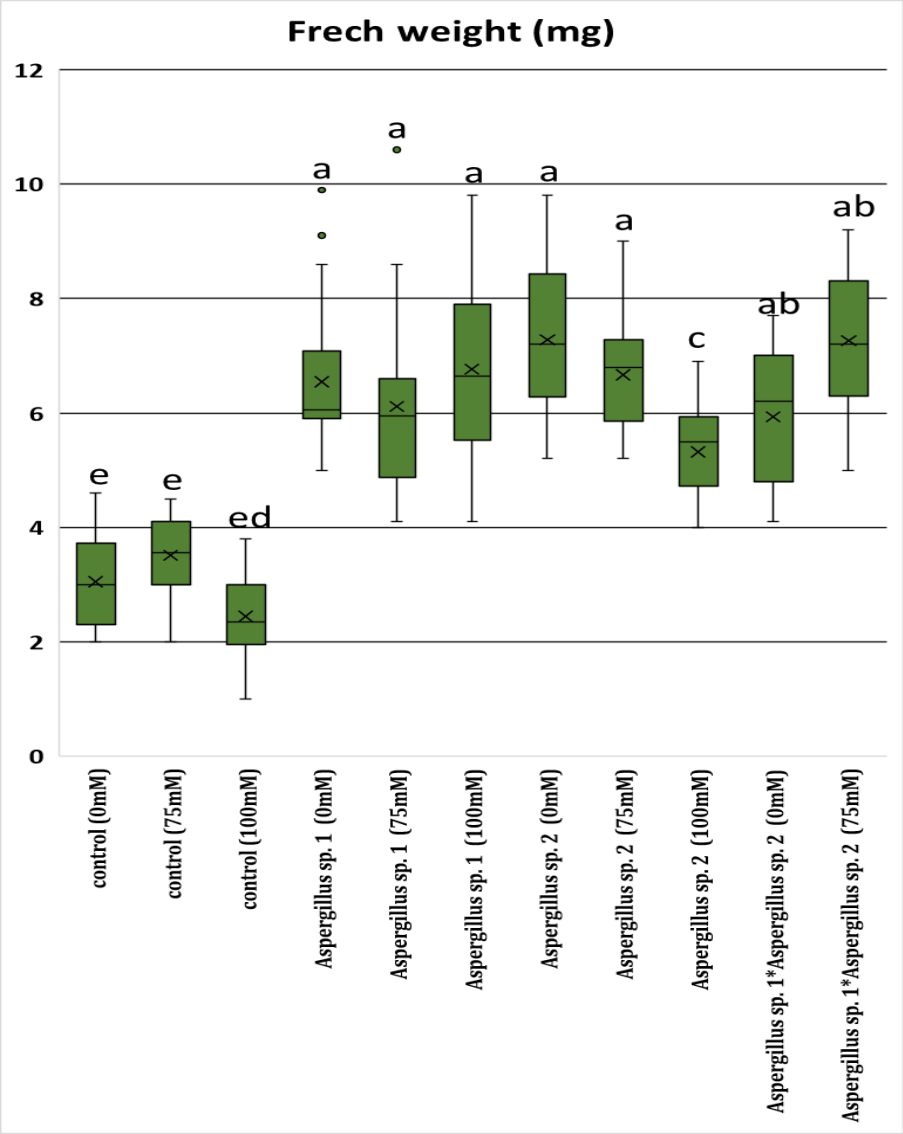


Figure 7 Distribution of fresh weight values across the study groups (control, *Aspergillus* sp.1, *Aspergillus* sp.2, *Aspergillus* sp.1* *Aspergillus* sp.2) at different NaCl concentrations (0 mM; 75mM; 100mM)

In a study conducted by Pandya & Saraf (2010), the authors found that fungal isolates from the genus *Aspergillus* led to a significant increase in fresh weight at a concentration of 340 mM of NaCl compared to the control group. They linked these improvements to the fungus's ability to solubilize phosphate and produce siderophores. Patel *et al*. (2017) confirmed that these improvements are indeed related to these mechanisms. They found that *Aspergillus Niger* and *Aspergillus parasiticus* enhanced mung bean growth parameters, for instance in fresh weight, by producing these siderophores. The fungi in our study could use the same mechanisms to enhance the fresh weight of the plants.

Figure 7 shows the statistical similarity between the mixed-fungal treatment group and the single-fungal treatment. This indicates the absence of a synergistic effect between the two strains on the fresh weight.

When discussing the synergistic effect between two fungi, opinions differ, as some studies contradict our results. For instance, Camprubi *et al*. (1993) established a synergistic effect on the growth of *Citrus geranium* through the interaction of the mycorrhizal fungus *Glomus intraradices* and the saprophytic fungi *Trichoderma aureoviride*. Similarly, Haggag and Abd-Ellatif (2001) and Calvet *et al.* (1993) showed that co-inoculation of the mycorrhizal fungus *Glomus mosseae* and a pathogen-antagonist *Trichoderma aureoviride* had a synergistic effect on the growth of geranium and marigold plants.

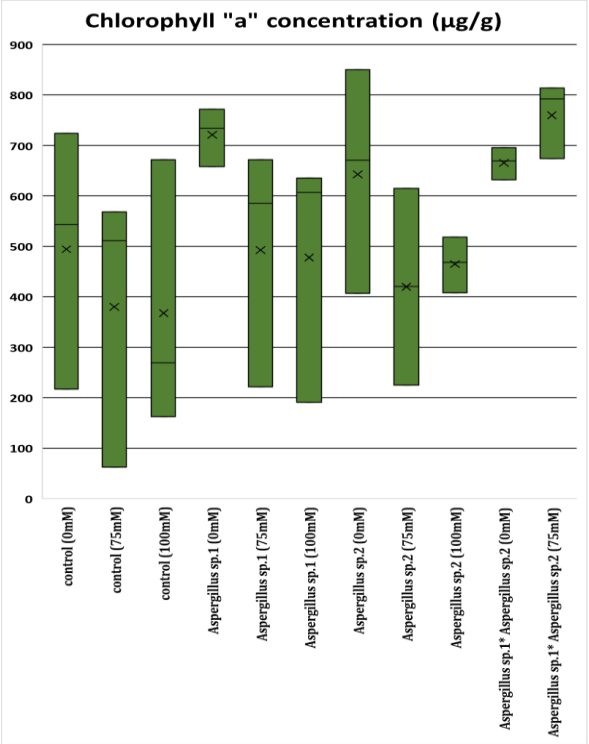
III. Determination of the chlorophyll content of the plants

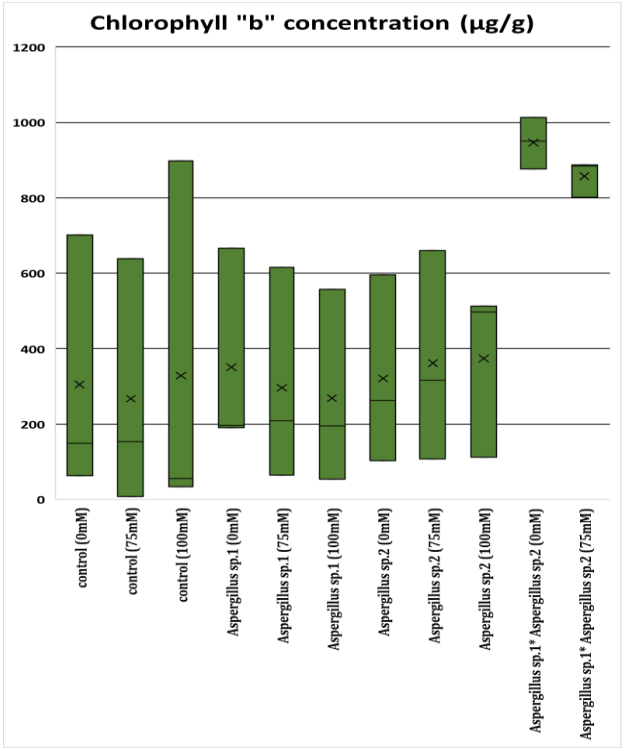
1. Analysis of the Chlorophyll a Content

At the end of the two weeks cocultivation period, the chlorophyll a content of the plants was measured (Fig. 8). The normality assumption of the chlorophyll a concentration data was met but not the homogeneity of variances, therefore, the Welch's ANOVA test, with two factors, was used for the statistical analysis. the analysis revealed statistically significant differences between the average chlorophyll a concentration ($P = 0.042$).

The Games-Howell test was used for post-hoc comparisons to identify groups that differed from each other. The results of this test showed no statistically significant differences ($P > 0.05$).

The results of the Games-Howell test are inconsistent with the results of the Welch ANOVA test. This is likely due to the small sample size (triplicate testing, n =3) in each group, which limits the statistical power to detect differences between particular pairs, i.e., the differences could be too small to be identified by the test.





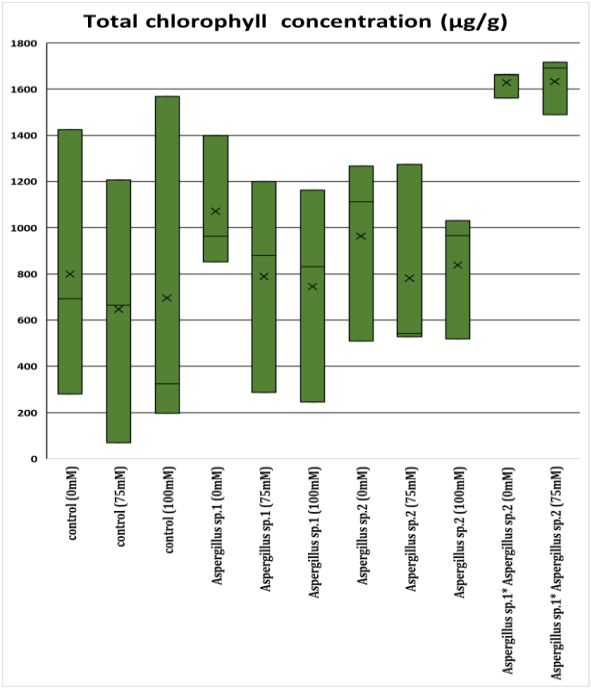


Figure 8 Distribution of chlorophyll "a", "b" and total chlorophyll concentration values across the study groups (control, *Aspergillus sp.1, Aspergillus sp.2, Aspergillus sp.1* Aspergillus sp.2)* at different NaCl concentrations (0 mM; 75mM; 100mM)

2. Analysis of the Chlorophyll b Content

At the end of the two weeks cocultivation period, the chlorophyll b content of the plants was measured (Fig. 8). Since the normality and homogeneity of the variances of the data were violated, the Welch's ANOVA test was used, with a confidence interval of 95%. The results showed that there are statistically significant differences between the average chlorophyll b concentrations of the different groups.

To determine these differences, the Games-Howell test was used, and its results showed that all comparisons between groups did not show statistically significant differences, given that all Sig. values > 0.05.

3. Analysis of the total Chlorophyll concentration

After the end of the cocultivation period, the total chlorophyll concentration of the plants was measured (Fig. 8). The normality and homogeneity of the variance of the data were not met; hence, the Welch's ANOVA test was used, with a confidence interval of 95%. The outcome of the analysis showed the presence of statistically significant differences between the averages of total chlorophyll concentration between groups (p = 0.015).

To determine the source of these significant differences, the Games-Howell test was used, the results of which showed that no statistically significant individual differences were found between any of the pairs of groups.

The statistical results indicate a significant overall effect of the interaction between salt (NaCl) stress and the fungi used, *Aspergillus* sp.1 and *Aspergillus* sp.2, on the concentrations of chlorophyll "a", "b", and total chlorophyll in *Arabidopsis thaliana*, although it is difficult to determine significant differences between groups in pairwise comparisons.

Salt stress affects plant growth and productivity, resulting in a decrease in photosynthetic efficiency, i.e., a decrease in total chlorophyll concentration (Ali *et al*., 2004), principal molecules involved in the photosynthetic process (Khaleghi *et al*., 2012).

The results presented in Figure 8 demonstrate that the studied *Aspergillus* fungi, either individually or in combination, played a role in mitigating salt stress. Although the differences were not statistically significant, they helped maintain high concentrations of chlorophyll "a", chlorophyll "b", and total chlorophyll under varying salinity concentrations (0, 75, and 100 mM). The mechanisms that fungi rely on in this situation may include:

- Production of plant hormones such as auxin or gibberellins, which stimulate cell division and differentiation, potentially enhancing chlorophyll synthesis.
- Modulation of stress hormone levels (abscisic acid) and growth hormones.
- Some fungi, including some Aspergilli, facilitate the absorption of essential nutrients that are important for chlorophyll synthesis.
- Stimulating the activities of antioxidant enzymes in plants, such as catalase and peroxidase, which help remove toxic reactive oxygen species resulting from salt stress, thus protecting chloroplasts from damage (Hossain *et al*., 2017).

Several studies have shown that some well-known fungal genera, such as *Aspergillus*, can have positive effects on plant photosynthetic efficiency (Hossain *et al*., 2017). In a study conducted by Li *et al*. (2017), perennial rye (*Lolium Perenne*) was inoculated with the fungus *Aspergillus aculeatus* under different salt concentrations (0, 200, and 400 mM). They found that the fungus improved the photosynthetic efficiency of the plants, reduced the activity of antioxidant enzymes, and mitigated salinity-induced lipid peroxidation compared to plants not inoculated with the fungus.

Dargiri *et al* (2025) also found in a study that inoculating wheat seeds (*Tricticum aestivun* L.) with *Penicillium chroysogenum* under 150 mM NaCl, preserved photosynthetic pigments and reduced oxidative damage compared to uninoculated plants.

Conclusion

This study sought to provide important insights into the potential ability of two *Aspergillus* isolates, *Aspergillus* sp.1 and *Aspergillus* sp.2, individually and in combination, to promote the growth of *Arabidopsis thaliana* and mitigate salt stress.

The results showed that both fungi significantly improved vegetative and root growth parameters (length of primary root, number of secondary roots and leaves, fresh weight) under normal conditions (without salt stress) and under salt stress (75 and 100 mM of NaCl). This indicates the role of these fungi as growth promoters.

The fungi also contributed to maintaining the total chlorophyll concentration of the plant, indicating that these fungi have the potential to protect the photosynthetic system of *Arabidopsis thaliana* from damage caused by salt stress.

Furthermore, the results showed that the effectiveness of alleviating salt stress depended on the fungal species and salt concentration. Welsh's ANOVA analysis and the Games-Howell test indicated that *Aspergillus* sp.1 was most effective at 75 mM of NaCl. This is due to *Aspergillus* sp.1 being halotolerant, achieving optimal growth at very high salt concentrations (5-10% sodium chloride). *Aspergillus* sp.2 was less tolerant, but did not demonstrate the same efficiency as *Aspergillus* sp.1, which was a more effective partner for the plant under stress.

The combination of the two fungi did not offer any additional advantage. This indicates the absence of a synergistic effect and potential competition within the rhizosphere. The performance of the combination was similar to - or slightly lower, than that of *Aspergillus* sp.1. This could be due to the fact that *Aspergillus* sp.1 is the primary factor responsible for the observed positive effect. Based on these results, it is recommended to focus on *Aspergillus* sp.1 as a promising candidate for developing a biofertilizer for economically important crops in saline environments. For future research, we recommend the following:

- Identifying the species of the studied fungal isolates.
- Exploring the precise molecular and biochemical mechanisms (such as hormone production, ionic balance, etc.) used by *Aspergillus* sp.1 to promote growth under saline conditions.

- Testing the effect of this fungus under semi-natural conditions, i.e., using non-sterile soil to better simulate the natural rhizosphere environment, as a crucial step toward field application to economically important crops.

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Abstract

This current study aimed to evaluate the ability of two soil fungal isolates, individually or in combination, to promote the growth of *Arabidopsis thaliana* col 0 and their potential to mitigate salt stress. The two fungi were isolated from two different soils, and were identified, microscopically, as belonging to the genus *Aspergillus*. The study of their salinity tolerance indicated that both fungi were halotolerant, with a wide range of NaCl tolerance, ranging from 0 to 20% (m/v). However, they exhibited an optimal growth at 5% (m/v) of NaCl. Four experimental treatments were applied for *A. thaliana* plants: a control group, without fungi, a group treated with *Aspergillus* sp.1 and *Aspergillus* sp.2, under three NaCl concentrations (0, 75, and 100 mM), and a group treated with a mixture of both, at 0 and 75mM of NaCl. The following growth parameters were measured: primary root length, number of secondary roots and leaves, fresh weight, and chlorophyll "a", "b", and total chlorophyll concentrations. The results were analysed using Welsh's ANOVA (with two factors) and the Games-Howell test for post hoc comparisons. The results showed that all fungal treatments led to significant improvements in growth parameters ($p < 0.05$) compared to the control group. The effect of *Aspergillus* sp.1 was more pronounced than the other treatments, especially at a concentration of 75 mM, while the fungal mixture showed no synergistic effect. These results indicate that *Aspergillus* sp.1 has great potential, not only to promote the growth of *Arabidopsis thaliana,* but also to mitigate the negative effects of salt stress, making it a promising candidate as an inoculant to improve sustainable agricultural systems and mitigate the negative effects of salinity without causing any negative environmental impact.

Keywords: *Arabidopsis thaliana* col 0; *Aspergillus*; PGPF; salt stress; root Architecture; Chlorophyll content.

Résumé

Cette étude avait pour but d'évaluer la capacité de deux isolats fongiques du sol, individuellement ou en association, à favoriser la croissance d'*Arabidopsis thaliana* col 0 et leur potentiel à atténuer le stress salin. Les deux champignons ont été isolés de deux sols différents et identifiés, au microscope, comme appartenant au genre *Aspergillus.* L'étude de leur tolérance à la salinité a montré qu'ils étaient halotolérants, avec une large plage de tolérance au NaCl, allant de 0 à 20 % (m/v). Cependant, leur croissance optimale était obtenue à 5 % (m/v) de NaCl. Quatre traitements expérimentaux ont été appliqués aux plants d'*A. thaliana* : un groupe témoin, sans champignon, un groupe traité avec *Aspergillus sp.1* et *Aspergillus sp.2,* à trois concentrations de NaCl (0, 75 et 100 mM), et un groupe traité avec un mélange des deux, à 0 et 75 mM de NaCl. Les paramètres de croissance suivants ont été mesurés : longueur des racines primaires, nombre de racines secondaires et de feuilles, poids frais, et concentrations de chlorophylle « a », « b » et totale. Les résultats ont été analysés à l'aide de Welsh ANOVA (à deux facteurs) et du test de Games-Howell pour les comparaisons a posteriori. Les résultats ont montré que tous les traitements fongiques ont entraîné des améliorations significatives des paramètres de croissance ($p < 0,05$) par rapport au groupe témoin. L'effet d'*Aspergillus sp.1* était plus marqué que celui des autres traitements, notamment à une concentration de 75 mM, tandis que le mélange fongique n'a montré aucun effet synergique. Ces résultats indiquent qu'*Aspergillus sp.1* présente un fort potentiel, non seulement pour favoriser la croissance d'*Arabidopsis thaliana,* mais aussi pour atténuer les effets négatifs du stress salin, ce qui en fait un candidat prometteur comme inoculant pour améliorer les systèmes agricoles durables et atténuer les effets négatifs de la salinité sans impact environnemental négatif.

Mots Clé: *Arabidopsis thaliana* col 0; *Aspergillus*; PGPF; stress salin; architecture racinaire; teneur en chlorophylle.

ملخص

هدفت هذه الدراسة إلى تقييم قدرة عزلتين من فطريات التربة، بشكل فردي أو مجتمعتين، على تعزيز نمو نبات *Arabidopsis thaliana* col 0 وإمكاناتهما في تخفيف الإجهاد الملحي. تم عزل الفطرين من نوعين مختلفين من التربة، وتم تحديدهما مجهريًا على أنهما ينتميان إلى جنس *Aspergillus*. أشارت دراسة تحملهما للملوحة إلى أن كلا الفطرين يتحملان الملوحة، مع نطاق واسع من تحمل كلوريد الصوديوم، يتراوح من 0 إلى 20٪ (م/حجم). ومع ذلك، فقد أظهرا نموًا مثاليًا عند 5٪ (م/حجم) من كلوريد الصوديوم. تم تطبيق أربع معالجات تجريبية على نباتات *A. thaliana*: مجموعة ضابطة، بدون فطريات، ومجموعة عولجت بفطري *Aspergillus sp.1* و *Aspergillus sp.2*، تحت ثلاثة تركيزات من كلوريد الصوديوم (0 و75 و100 ملي مولار)، ومجموعة عولجت بمزيج من كليهما، عند 0 و75 ملي مولار من كلوريد الصوديوم. تم قياس معايير النمو التالية: طول الجذر الأساسي، وعدد الجذور الثانوية والأوراق ، الوزن الطازج، تركيز الكلوروفيل "أ" و"ب"، وتركيز الكلوروفيل الكلي. حُلّلت النتائج باستخدام تحليل التباين Welch (بعاملين) واختبار Games-Howell للمقارنات اللاحقة. أظهرت النتائج أن جميع معاملات الفطريات أدت إلى تحسينات ملحوظة في معايير النمو ($p < 0.05$) مقارنةً بالمجموعة الضابطة. كان تأثير Aspergillus sp.1 أكثر وضوحًا من المعاملات الأخرى، وخاصةً عند تركيز 75 ملي مولار، بينما لم يُظهر خليط الفطريات أي تأثير تآزري. تشير هذه النتائج إلى أن Aspergillus sp.1 يتمتع بإمكانيات كبيرة، ليس فقط لتعزيز نمو نبات *Arabidopsis thaliana*، ولكن أيضًا للتخفيف من الآثار السلبية للإجهاد الملحي، مما يجعله مرشحًا واعدًا كملقح لتحسين النظم الزراعية المستدامة والتخفيف من الآثار السلبية للملوحة دون التسبب في أي تأثير بيئي سلبي.

الكلمات المفتاحية: *Arabidopsis thaliana* col 0 ، فطر *Aspergillus*، PGPF، الإجهاد الملحي، بنية الجذر، محتوى الكلوروفيل.