University of Cyprus
Department of Biological Sciences

The Effect of Beneficial Microbes and Attapulgite on Growth and Productivity of *Lavandula angustifolia* and *Thymus vulgaris* under Drought Stress

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A Research-Based Master's Thesis
for the
Degree of Magister Scientiae in Biodiversity and Ecology

May 27, 2024

ABSTRACT

Climate change and desertification have become topics of broad interest, as their consequences are currently being experienced, and future projections investigate their prolonged effects. Desertification is primarily caused by anthropogenic activities, especially unsustainable agriculture practices that degrade soil's biological activity and its resilience mechanisms in combating climate change.

In the Mediterranean region, desertification is more intense. Decreased precipitation rates and increased frequency of heat waves and summer heat further affect agricultural productivity which is intrinsically low.

Actions for agriculture adaptation to dry conditions must be taken to mitigate those effects and achieve production and food sufficiency. Agents such as attapulgite soil and plant growth-promoting microorganisms gain more and more attention, as they assist plant growth and resilience.

In this experiment, the attapulgite soil-enhancing agent AGLEV® SI 200 (A) and the microbial plant-grown enhancing agent Micosat F-Olivo (M) were used in the cultivation of the Mediterranean species *Lavandula anguistifolia* in Vavla, Larnaca, and *Thymus vulgaris* in Oreites, Paphos, both in Cyprus. Their drought vulnerability was used to indicate the efficacy of AGLEV® SI 200 (A) and Micosat F-Olivo (M). Four different treatments were implemented during the *Lavandula anguistifolia* and *Thymus vulgaris* planting process: 1) The control treatment, 2) The A treatment, 3) The M treatment, and 4) The combination of A and M (AM treatment).

The results showed that the AM treatment significantly increased the base shrub volume of *Lavandula anguistifolia* in Vavla. In Oreites, the AM treatment showed an increase in *Thymus vulgaris* shrub biomass compared to the rest of the treatments, though not statistically significant. The A and M treatments also showed increased shrub volume and increased stem height for M treatment in Vavla, however they were not found to be significant.

In conclusion, the significant increase in base shrub volume in Vavla indicated that the combination of A and M positively affected plant growth. Soil and plant growth-enhancing agents used in agriculture are gaining more recognition for their potential benefits for plant growth. Their promising synergy should be further examined in other plant species and for longer testing periods and various pedoclimatic conditions to fully discover their potential.

ACKNOWLEDGEMENTS

Another learning journey has come to an end. It was filled with ups and downs, but with the valuable assistance and guidance of my research supervisor Dr. Demetrios Sarris, it is now accomplished. I would like to thank my colleague Kostas Karanicolas, as we shared our concerns and knowledge until the very end, and I wish him the best of luck. I would also like to thank my family, especially my mother, who is always there for me. Lastly, I would like to thank Marios, my significant other, for all his support and dedication.

COMPOSITION OF THE EXAMINATION COMMITTEE

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SEMINAR ANNOUNCEMENT

University of Cyprus
Department of Biological Sciences

Master Research Dissertation in Biodiversity and Ecology (BIO 831/601)

Student Presentation

Monday, 27 May 2024 at 13:30
Building CTF 01, Room 001, Panepistimioupoli Campus

This seminar is open to the public

Maria Konstantinou

Thesis Supervisor: Prof. Spyros Sfenthourakis

"The Effect of Beneficial Microbes and Attapulgite on Growth and Productivity of *Lavandula angustifolia* and *Thymus vulgaris* under Drought Stress"

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1. INTRODUCTION

1.1 Climate Change and Desertification

In contemporary societies, **climate change** and **desertification** have become topics of broad interest and intense study by the scientific community. Their consequences are currently being experienced, and future projections investigate their prolonged effects on food security, biodiversity, ecosystem health, and overall, the future of humanity. **Climate change** is characterized by a significant increase in average global temperature (Figure 1) and observation of extreme climatic events including precipitation, droughts (Figure 2), floods (Figure 3), winds, etc. (Pal et al. 2023; Zittis et al. 2022). Besides natural causes, climate change is closely related to anthropogenic factors, specifically the continuous emission of greenhouse gases since the Industrial Revolution (Figure 4) (Zittis et al. 2022). Greenhouse gases can absorb the heat that radiates from the Earth's surface in their molecules, which helps to maintain warm temperatures and support life on the planet. However, when there are excessive concentrations of greenhouse gases in the atmosphere, a warming effect is caused, leading to a rapid increase in average temperature (Kweku et al. 2018). This can eventually result in dire consequences for nature, the survival of living organisms, and the well-being of humanity by extension.

Figure 1: Global surface temperature change relative to 1850-1900. Source: Fyfe et al. 2021

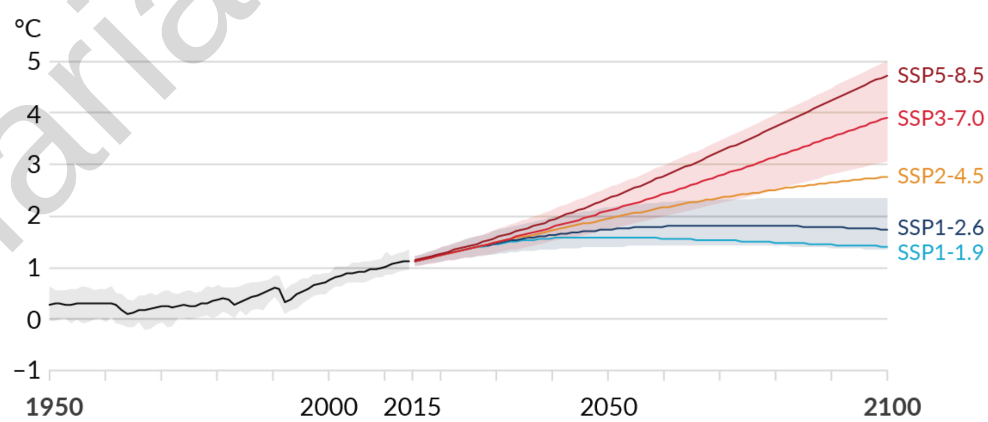


Figure 2: Drought and water shortage
Source: https://shorturl.at/ORSW0

Figure 3: Flood-caused disaster
Source: https://shorturl.at/aqsR3

Desertification is defined as land degradation and declined biological activity in a region. It mainly affects lands receiving minimal precipitation including drylands, semi-arid, and arid areas (Alliouche, Kouba 2023; Pal et al. 2023). Desertification is a complex issue, caused by natural factors such as climate change, low or erratic rainfall, and long-lasting droughts (Pal et al. 2023). Natural causes act synergistically with human activities such as anthropogenic climate change, deforestation (Figure 5), forest fires (Figure 6), overgrazing, and unsustainable land use practices including intensive farming and agriculture, as well as extensive use of chemicals like fertilizers and pesticides (Figure 7) (Burrell et al. 2020; Zittis et al. 2022; Pal et al. 2023; Kumar et al. 2023). As a result, soil erosion is observed, which prevents soil from holding water and organic / nutrient-rich materials, thus life and plant growth cannot be supported on it (Pal et al. 2023). Degraded soil is less stable, easily mobilized, and susceptible to floods, so runoff is also observed (Figure 8), which causes damage to agriculture, inhabited areas, and infrastructure (Rubio et al. 2024).

Figure 5: Deforestation
Source: https://shorturl.at/jkQXY

Figure 6: Forest fires
Source: https://shorturl.at/gjT14

Figure 7: Use of chemicals
Source: https://shorturl.at/aGNO9

Figure 8: Soil erosion and runoff
Source: https://shorturl.at/sxLM8

While natural causes such as climate variability and drought play a role in desertification, it is generally acknowledged that human activities are the dominant cause of dryland degradation (Burrell et al. 2020). This may be attributed to the limited water resources available in drylands, resulting in their adaptation to climatic fluctuations rather than human intervention (European committee of the regions, 2011). Generally, the soil possesses inherent natural mechanisms to cope with varying conditions. Nonetheless, anthropogenic activities can significantly impair these mechanisms, leading to their failure to mitigate the effects of severe environmental events, such as extreme natural phenomena (Kumar et al. 2023; Rubio et al. 2024).

The relationship between **climate change** and **desertification** is complex and interdependent, as both processes are mutually reinforcing. Desertification, primarily caused by anthropogenic activities, is exacerbated by climatic fluctuations when the natural resilience mechanisms of the earth are weakened. In turn, climate change is intensified by soil degradation and surface moisture loss due to excessive evapotranspiration, which leads to the dissipation of less solar energy. As a result, soil warming and altered atmospheric composition are observed, which affect climate regulation (European committee of the regions, 2011; Rubio et al. 2024).

1.2 Consequences and Future Projections

Climate change and desertification share numerous consequences of socio-economic and environmental nature, as both phenomena severely impact the well-being of living organisms and ecosystems (Alliouche, Kouba 2023; Rubio et al. 2024). The most significant consequences include biodiversity loss and environmental degradation, economic instability, poverty, displacement, food shortage, and degradation of ecosystem services.

1.2.1 Biodiversity Loss and Environmental Degradation

Living organisms have the remarkable ability to adapt to changes in their environment. Some of them are adapted to extreme climatic conditions that define their environment. However, with the rapid increase in temperature and frequent occurrences of extreme climatic events such as droughts and storms, many species cannot adapt fast enough to withstand these changes (Wingfield et al. 2011). Consequently, some species suffer habitat destruction, while others even face the threat of extinction. Habitats and all the environmental components are threatened by climatic change and desertification. The soil for example, as a fundamental component of the environment that supports and nurtures life, plays a crucial role in the survival of many species. Nevertheless, the deterioration of land quality, erosion, and overall environmental degradation will adversely affect all species that depend on it (Jhariya et al. 2023). Especially the survival of vegetation is highly dependent on the availability of fertile soil and water. Without these resources, the presence and thus the capacity of vegetation to sequester carbon dioxide into the humus is hindered, leading to its release into the atmosphere (Rubio et al. 2024). The accumulation of carbon dioxide, the most prevalent greenhouse gas, intensifies the global climate crisis. The depletion of atmospheric oxygen, which is vital for the survival of living organisms, is a direct consequence of this process. Therefore, it’s crucial to maintain all the environmental components such as soil quality and water resources, so habitats and biodiversity can thrive, and the adverse effects of climate change and desertification can be mitigated.

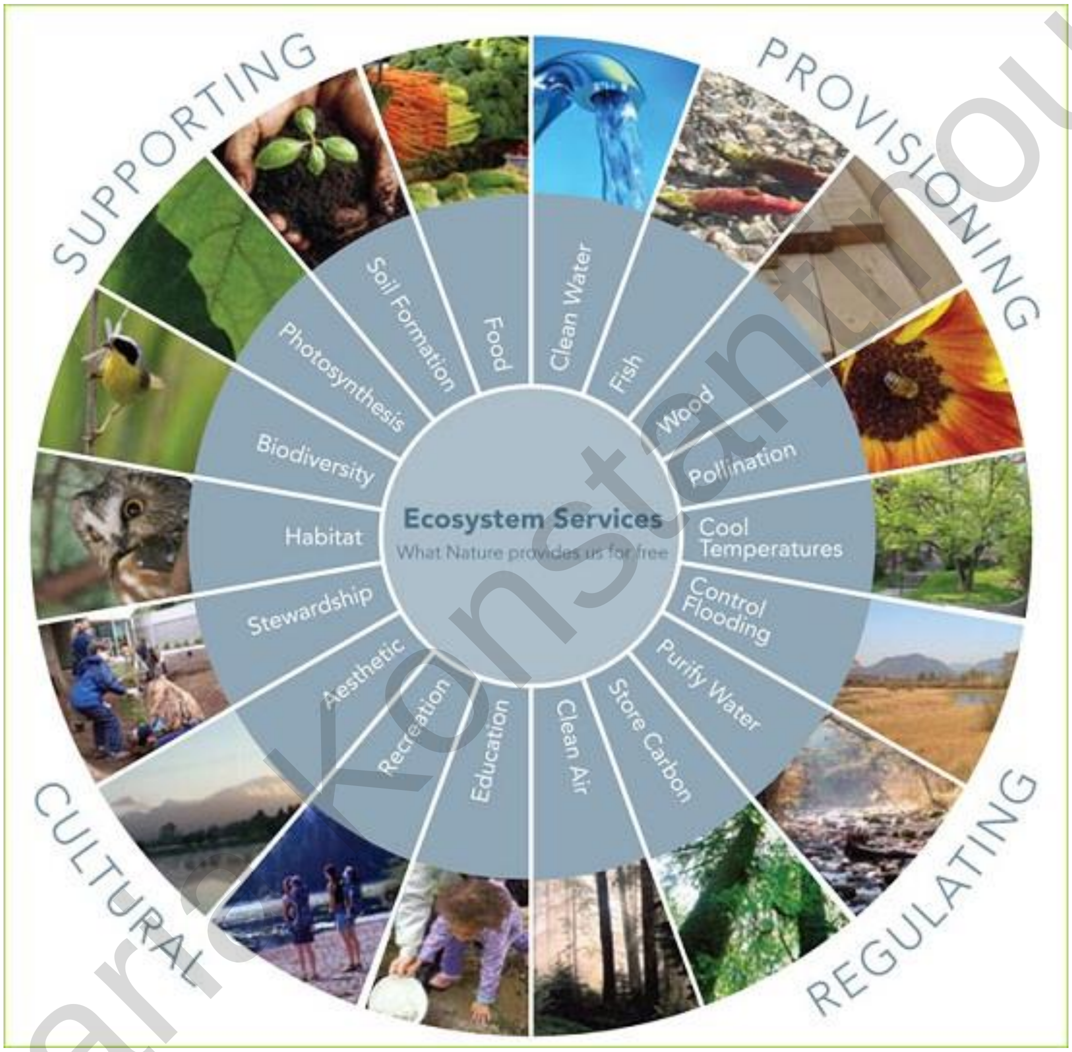
1.2.2 Food shortage, poverty, displacement, and socioeconomic issues

Approximately 41% of the earth's land surface is covered by drylands, which includes 45% of the worldwide agricultural land (Burrell et al. 2020). Such regions are home to billions of people who live mostly in undeveloped countries and are already affected by the consequences of climate change and desertification, with hunger, poverty, and displacement being the most prevalent ones (Pal et al. 2023). Displacement resulting from extreme climatic events and biblical-scale disasters has become more frequent. The United Nations Environmental Program has reported that desertification has already impacted 14 million square miles of land, with an additional 20,000 square miles rapidly approaching the limits of desertification every year (Pal et al. 2023). Land degradation severely impacts agriculture and farming, rendering the land unsuitable for cultivation. This, in turn, exacerbates issues related to food scarcity and poverty, particularly in areas with rapidly expanding populations (Pal et al. 2023; Abd-Elmabod et al. 2020). From an economic perspective, the prices will be affected by changed food production and consumption, creating an imbalance in the supply and demand equilibrium. This situation will eventually harm the economy (Abeysekara et al. 2023).

1.2.3 Ecosystem services and human well-being

The transformation of undisturbed areas into deserted ones can potentially give rise to severe consequences for the stability of societies, as such condition also affects human well-being and ecosystem services (Alliouche, Kouba 2023; Jhariya et al. 2023). **Ecosystem services** refer to all the benefits nature provides and are divided into categories (Figure 9) (Balvanera et al. 2017). **Provisioning services** provide humans with the necessary resources for their survival and well-being and play a crucial role in maintaining the balance of the ecosystem. These include products and resources such as food, water, timber, fiber, and medicinal resources. **Regulating services** refer to the numerous benefits derived from the natural regulation of ecosystems. These benefits are essential for human well-being and include the pollination of crops, the purification of water and air, the sequestration of carbon, the decomposition of waste, the regulation of climate, and many other critical services. Ecosystems rely on **supporting services** such as nutrient cycling and primary production to create a healthy environment for various organisms to thrive.

Figure 9: Ecosystem services and their significance for life. Source: https://shorturl.at/atGZ9



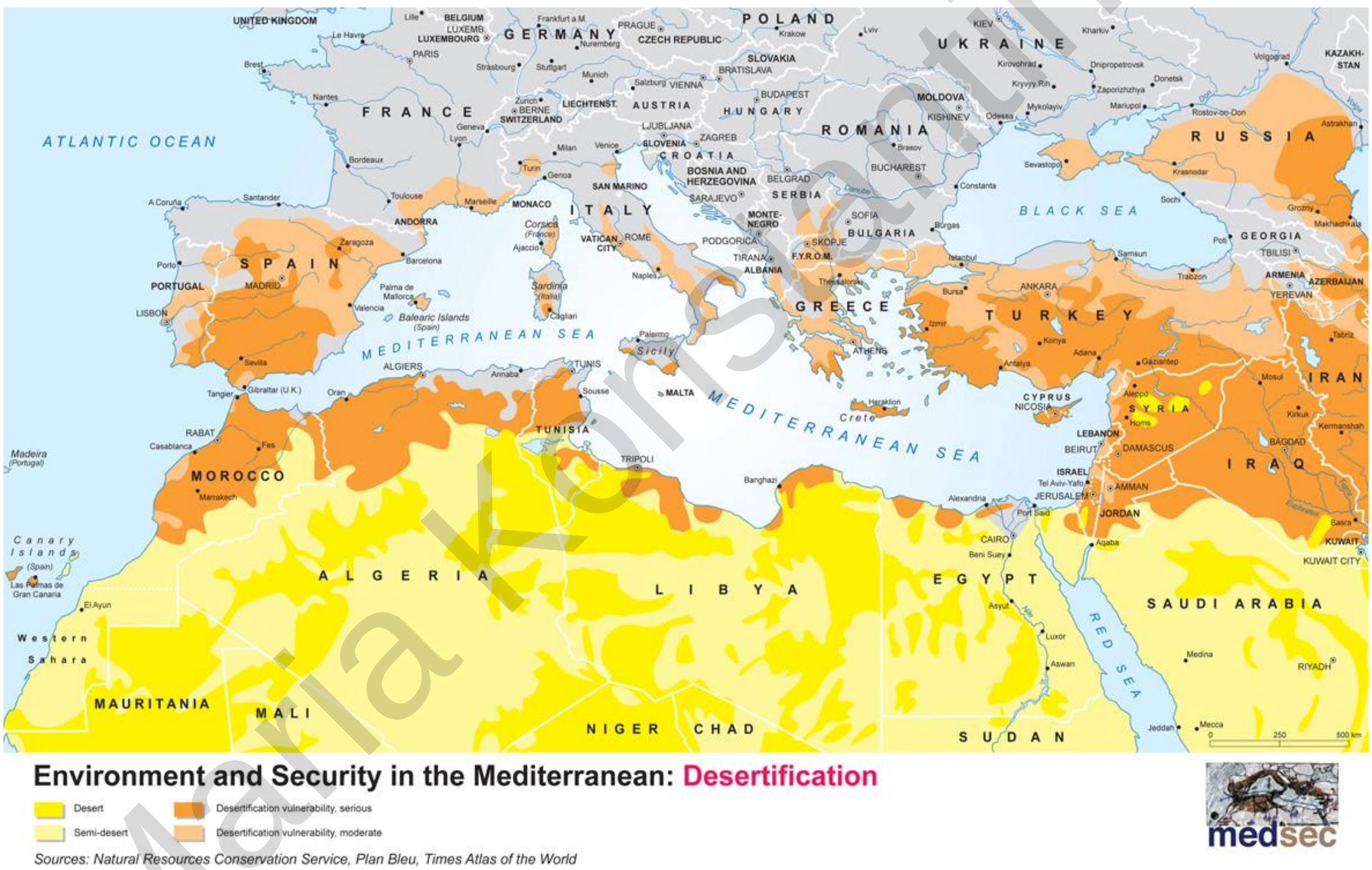
Cultural ecosystem services refer to the non-material benefits that humans derive from ecosystems. These benefits include recreational, aesthetic, and spiritual experiences and educational and inspirational opportunities. Examples of cultural ecosystem services include tourism, outdoor recreation, and cultural heritage sites (Balvanera et al. 2017). Ecosystem services are essential for human well-being and contribute significantly to the economy. Without them, survival and quality of life would be in jeopardy, and ecosystems could not function properly. With climate change, desertification, and environmental degradation in general, posing a serious threat to ecosystem services and human well-being, measures must be taken to ensure their protection.

1.3 Climate Change and Desertification in the Mediterranean

Desertification and climate change are increasingly severe issues in the Mediterranean region. Figure 10 shows a map of desertification vulnerability in the Mediterranean, projecting moderate and serious vulnerability, with the southeastern Mediterranean showing serious vulnerability. According to (Zittis et al. 2022), the Eastern Mediterranean and Middle East region is a climate change hotspot. Its warming is rapid and approximately two times faster than the global average. One of the main reasons for this observation is the excessive emission of greenhouse gases compared to other regions (Zittis et al. 2022). Future projections suggest that the observed warming trends will continue to intensify, as well as the current observation of extreme weather events, such as increased duration of heatwaves, dust storms, droughts, floods, etc. (Zittis et al. 2022; Muñoz-Rojas et al. 2017; Abd-Elmabod et al. 2020). As per the latest climate models, there is a projected decline in precipitation in the future. Meanwhile, the frequency of summer heat is expected to increase (Zittis et al. 2022; Muñoz-Rojas et al. 2017; Abd-Elmabod et al. 2020). (Pal et al. 2023) mentions that a rise of two degrees Celsius in the average temperature of the Mediterranean region will transform southern Spain into a desert. This increase in temperature, if global, will result in approximately 30% of the Earth's land turning arid. The impact of this transformation will be significant, particularly on the millions of people residing in underdeveloped and vulnerable regions.

With such conditions, many sectors will be impacted. Those include water source management, human health, ecosystem, agriculture and production, biodiversity, forest fires, etc. (Zittis et al. 2022; Rubio et al. 2024). The agricultural productivity in the Mediterranean region is intrinsically low, primarily because of the region's dependence on irrigation. Additionally, the low soil organic carbon content in this area is a significant factor in its vulnerability to environmental change and degradation (Muñoz-Rojas et al. 2017). According to prediction models, higher temperatures and decreased rainfall will lead to higher soil organic carbon decomposition rates (Muñoz-Rojas et al. 2017). Eventually, future agricultural production will decrease, as production depends on water supplies and soil quality (Abd-Elmabod et al. 2020). As food demand continues to rise, sustainable production methods and better regulation of ecosystem services are essential to address the challenge. Thus, scientific strategies that promote sustainability and ecosystem resilience are crucial to ensure the long-term viability of agricultural production (Abd-Elmabod et al. 2020).

Figure 10: Desertification Vulnerability in the Mediterranean. Source: https://shorturl.at/1vZZY



1.4 Mitigation of Climate Change and Desertification

The reversible nature of climate change and desertification is contingent upon the mitigation or cessation of anthropogenic interventions that contribute to the emergence of these conditions. Sustainable practices that minimize environmental impact are essential to achieve this goal. For example, emission increase results in higher temperatures and consequent climatic changes, which can negatively impact wetland ecosystems (Xu et al. 2024). Therefore, emission mitigation is crucial to sustain these fragile ecosystems. In addition, sustainable production methods and effective regulation of ecosystem services are essential to counter the negative impact of human activities and climate change on soil resources (Abd-Elmabod et al. 2020; Pal et al. 2023). The primary production sector has the potential to achieve sustainable growth by taking a scientific approach to increasing crop production. This can be accomplished by assessing the environmental constraints of a specific site, which can determine its suitability and capability for production. Environmental constraints evaluation involves analyzing the site's soil properties, management practices, topography, and climatic conditions. By utilizing the appropriate scientific techniques based on the assessment of environmental constraints, the primary production sector can enhance its productivity while minimizing its environmental impact (Abd-Elmabod et al. 2020). Lastly, raising awareness about climate change and desertification mitigation is crucial for protecting biodiversity and human well-being (Pal et al. 2023). By consciously reducing greenhouse gas emissions and preventing land degradation, we can safeguard ecosystem services and ensure long-term species survival. Promoting sustainable land use practices and conservation efforts can enhance ecosystem resilience and help restore degraded ecosystems.

1.5 Use of Microbes as natural plant growth-enhancing agents in agriculture

The soil constitutes one of the most important factors in mitigating the negative impacts of human mismanagement that causes land degradation, such as pollution with toxic heavy metals, oils, xenobiotics, and polycyclic aromatic hydrocarbons. Soil’s resilience mechanisms can be strengthened in organic matter-rich soils, with improved properties, and microbial communities (Kumar et al. 2023). Microorganisms have a key role in soil health, as they are responsible for 1) soil fertility, 2) promotion of plant growth, 3) improvement of soil properties such as hydraulic conductivity, 4) root improvement for better water absorption, and 5) soil restoration through nutrient fixation and biochemical cycles. An important example of nutrient fixation is nitrogen fixation, an essential component for soil fertility. Land degradation is observed mainly due to declining soil carbon and nitrogen stocks. Additionally, microbes are responsible for removing toxic elements through bioaccumulation, bioremediation, or transformation that reduces their effects (Kumar et al. 2023). Overall, microbes in agriculture are essential as an eco-friendly and cost-effective solution for soil restoration and enrichment with organic nutrients.

1.5.1 Use of Micosat F-Olivo

In the present study, **Micosat F-Olivo** (Figure 11) was used as a plant growth-enhancing agent with beneficial microorganisms [MICOSAT F- Olivo (viorylagro.gr)]. It consists of 1) the endo-mycorrhizal fungi *Glomus coronatum*, *Glomus intraradices*, and *Glomus viscosum*, 2) the biologically active bodies *Pseudomonas spp. Bacillus subtilis*, and *Streptomyces spp.*, 3) the saprophytic fungi *Trichoderma spp.*, *Trichoderma viride*, and *Trichoderma harzianum*, and 4) the yeast species *Picia pastoris*. According to the manufacturer, these factors can promote root system growth and improve nutrient and water absorption. Additionally, they assist beneficial microflora growth on the rhizosphere and inhibit the establishment of pathogenic microorganisms, rots, tumors, and nematodes. **Micosat F-Olivo** can make plants independent in nutrient and water intake and resistant to pathogens and water stress. It can even assist the plants in increasing their production and shifting their flowering earlier.

Figure 11: Micosat F-Olivo
Source: https://shorturl.at/fpzEY

1.6 Use of the soil enhancing Attapulgite Soil

Another important topic for soil health and mitigation of desertification effects is the existence of available water supplies and their management, especially regarding irrigation. In the Mediterranean region, water supply and availability for irrigation constitute major issues, as mentioned above. Attapulgite soil is a natural clay used in agriculture, and its use is considered an ecologically friendly solution for water absorption and reduction of heavy metal concentrations in the soil. Due to its chemical, physical, and colloidal properties, and crystal structure, it has excellent surface absorption properties for water, heavy metals, and ions (Yang et al. 2022a). As a result, it can increase the water-holding capacity of soil and improve its physical and chemical properties (Yang et al. 2022a, Dai et al. 2019). Additionally, attapulgite can contribute to the stocks of soil organic matter, nutrients, and soil fertility by promoting the variation of bacterial communities in the soil (Zhang et al. 2024). In general, the addition of attapulgite in small dosages can reduce overall evaporation, although in higher dosages can increase the risk of water loss, as it can cause an increase in the crack area and crack aperture of the soil (Yang et al. 2022a).

1.6.1 Use of AGLEV® SI 200

In the present study, **AGLEV® SI 200** was used (Figure 12). It is a 100% natural and environmentally friendly clay soil improver based on attapulgite [Geohellas | Soil Improvers]. According to the manufacturer, this product can improve soil aeration and fertility, decrease watering needs, and increase the strength and growth of plants due to the high absorbency of attapulgite for water and nutrients.

Figure 12: AGLEV® SI 200

Source: https://shorturl.at/syIK6

1.7 Use of Vegetation in Sustainable Agriculture

Vegetation is another effective and natural solution to prevent water erosion and increase CO_2 sequestration, soil sedimentation, and organic matter accumulation. It is an advantageous solution as it is low-maintenance and can adapt to environmental conditions. However, scientific research has shown that indigenous vegetation is critical in combating desertification. Indigenous vegetation has evolved to thrive in specific environmental, landscape, and climatic conditions of a region, making it better equipped to withstand fluctuations in the ecosystem (Hooke, Sandercock 2012). It can positively impact the environment by maintaining and increasing native biodiversity (Hooke, Sandercock 2012). Thus, using indigenous vegetation is a promising approach to combat desertification, and promote sustainable land management practices and native biodiversity. In our experiment, we cultivated two native Mediterranean species in Cyprus (Eastern Mediterranean region), belonging to the Lamiaceae family: *Thymus vulgaris* (Common thyme) and *Lavandula angustifolia* (English lavender). Native Mediterranean species can be cultivated for their numerous properties and uses in everyday life, and at the same time, they can assist in soil restoration and benefit native biodiversity.

1.8 Mediterranean Aromatic plants and their properties

1.8.1 Common thyme – *Thymus vulgaris*

Thymus vulgaris is a perennial evergreen shrub native to the Mediterranean region and commonly found in southern Europe. It is a highly aromatic plant, with grey-green leaves, a woody base, and clusters of purple or pink flowers (Figure 13, Figure 14). *Thymus vulgaris* is adapted to dry, sunny habitats with coarse, nutrient-poor soils (Galovičová et al. 2021, Hosseinzadeh et al. 2015). Despite its adaptations, it is semi-sensitive to drought, as it contains lower relative water content than *Thymus kotschyanus*, which is semi-tolerant to drought (Ashrafi et al. 2022). *Thymus vulgaris* has been used for centuries in traditional medicine of various civilizations, and it continues to be a subject of scientific interest due to its several health benefits. *Thymus vulgaris* essential oil is known to possess a wide range of properties such as anti-microbial, anti-inflammatory, anti-biofilm, antioxidant, anti-fungal, antiseptic, antispasmodic, antibacterial, antiviral, and insecticidal, which have been extensively documented in the scientific literature. These therapeutic properties can be attributed to the complex chemical composition of *Thymus vulgaris*, which contains several bioactive compounds such as **thymol**, **p-cymene**, **1,8-cineole**, **γ-terpinene**, **carvacrol**, **rosmarinic acid**, and **flavonoids** (Galovičová et al. 2021, Hosseinzadeh et al. 2015). Its relative species *Thymbra capitata*, native to Cyprus, has a similar composition and properties.

Figure 13: Illustration of *Thymus vulgaris*
Source: https://shorturl.at/pINQ8

Figure 14: Thymus vulgaris
Source: https://shorturl.at/fjA69

1.8.2 Mediterranean thyme - *Thymbra capitata*

Thymbra capitata is a perennial evergreen plant native to the Mediterranean region. It is known for its strong drought resistance and is widely used for its medicinal properties (Constantinou et al. 2023). This subshrub reaches up to 60cm in height, and its pink flowers are densely grouped in terminal, capituliform inflorescences (Figure 15, Figure 16). It can be found in scrubs or thyme bushes, stony–rocky sites, sandy soils in pine forests, limestone, or clay soils (Bellache et al. 2022). Thymbra capitata is renowned for its flavor as a spice and its medicinal properties related to inflammation, skin complications, and wound healing. It also projects anti-microbial, antioxidant, anti-radical, anti-biofilm, antispasmodic, antihypertensive, and anticarcinogenic properties (Alves-Silva et al. 2023; Hcini et al. 2022). Additionally, it is beneficial in treating respiratory and gastrointestinal tract disorders (Hcini et al. 2022). Generally, *Thymbra capitata* has been used since antiquity for its undeniable and valuable health benefits, many of which are attributed to the polyphenolic compounds present in this shrub (Hcini et al. 2022).

The essential oil and hydro-distillation residual water of *Thymbra capitata* both contain phenolic compounds, and research into their properties is endless. The essential oil mainly contains the **volatile molecule** of **carvacrol**, followed by **thymol**, **p-cymene**, and **γ-terpinene**. High levels of **carvacrol** can inhibit the action of collagenase, elastase, hyaluronidase, and tyrosinase and promote the expression of type I collagen. As a result, it prevents age-related oxidative damage, inhibits skin-aging effects, and offers protection against age-related melanogenesis dysregulation (Alves-Silva et al. 2023). Additionally, it showed phytotoxic effects regarding seed germination and seedling growth of various weeds, such as *E. bonariensis*, an invasive weed. Its herbicidal potential is attributed to the allelopathic effect of **carvacrol**, which interacts with cell membranes and induces alterations in the lipid bilayer (Bellache et al. 2022). Its hydro-distillation residual water is mainly characterized by **rosmarinic acid**, followed by **salvianolic acids J, B, E,** and **A**, and **flavonoids**. It presented potential anti-tumoral properties in recent studies, as it reduced cell migration and etoposide-induced senescence, while it promoted cellular senescence. It can also protect cells from UV radiation-induced aging (Alves-Silva et al. 2023). The potential of *Thymbra capitata* is endless, and many of its properties are being explored through ongoing research.

Figure 15: Illustration of *Thymbra capitata*
Source: https://shorturl.at/AORWX

Figure 16: Thymbra capitata
Source: https://shorturl.at/citMU

1.8.3 English lavender - *Lavandula angustifolia*

Lavandula angustifolia, commonly known as English lavender, is a perennial aromatic, evergreen, and medicinal shrub native to the Mediterranean region. English lavender can reach up to 1-2 meters in height, with leaves narrowly linear in shape (Soheili, Salami 2019, Smigielski et al. 2018). The tubular-shaped, purple-colored flowers are arranged in clusters of a whorl pattern along the inflorescence (Figure 17, Figure 18). It can be found in dry and rocky areas, pastures, or scrublands. *Lavandula angustifolia* exhibits a drought-tolerant strategy toward water stress, which is stronger than its relative species *L. x intermedia* (Saunier et al. 2022). Generally, lack of water during a drought negatively affects plant growth, but the plant's ability to adapt can help mitigate these effects (Du, Rennenberg 2018). However, according to the experiment of two water deprivation cycles on *Lavandula angustifolia* plants from Du, Rennenberg 2018, more significant decreases in the shoot/root ratio, and the total carbon and soluble protein contents were observed during the second cycle of water deprivation. The results indicated that frequent drought and watering cycles may affect the growth and production of *Lavandula angustifolia* plants.

Lavandula angustifolia has been used in the traditional medicine of different cultures, such as Persia, due to its many pharmaceutical properties (Firoozeei et al. 2021; Soheili, Salami 2019). In the literature, many beneficial effects of *Lavandula angustifolia* have been reported, such as sedative, anxiolytic, antispasmodic, antidepressant, neuroprotective, analgesic, antimicrobial, antioxidant, anti-inflammatory, and local anesthetic properties (López et al. 2017; Smigielski et al. 2018; Soheili, Salami 2019; Firoozeei et al. 2021). Even cytotoxic effects on lung cancer and inhibition of carcinogenic cell development are reported (Smigielski et al. 2018). Aromatherapy or phytotherapy with essential oil or aqueous extract is widely used as a therapeutic method for 1) prolonged sleep, 2) anxiety, stress, and depression relief, and 3) improvement in memory and learning (Soheili, Salami 2019; Smigielski et al. 2018; López et al. 2017). The main components of the essential oil of *Lavandula angustifolia* are **linalool** and **linalyl acetate** (López et al. 2017; Smigielski et al. 2018; Soheili, Salami 2019; Firoozeei et al. 2021). Olfactory receptors recognize **linalool** by its aromatic odor, which can modulate ion-channel receptors of the central nervous system (Smigielski et al. 2018). For example, the essential oil of *Lavandula angustifolia* is believed to have antidepressant effects due to its ability to modulate the NMDA receptor, inhibit the SERT, and protect SH-SY5Y cells from hydrogen peroxide-induced neurotoxicity. Its antidepressant action projects similarities to that of **benzodiazepines**, but without causing side effects such as sexual dysfunction, neuropsychiatric disorders, or sleep disturbances (López et al. 2017). Additionally, **linalool** can cause alterations in gene expression associated with neurotransmitter regulation, neuroplasticity in the hypothalamus, hippocampal neurogenesis, and improved dendritic branching (Firoozeei et al. 2021). The aqueous extract of lavender is composed mainly of **caffeic acid** and **luteolin-7-glycosid**, a flavonoid compound (Soheili, Salami 2019). The aqueous extract inhibits acetylcholinesterase (AChE) and amyloid beta (Aβ) activity, whose actions constitute the main factors in the pathogenesis of Alzheimer's disease plaque. In addition, they project radical scavenging activity (Soheili, Salami 2019).

Figure 17: Illustration of *Lavandula angustifolia*
Source: https://shorturl.at/irNOP

Figure 18: Lavandula angustifolia Source: https://shorturl.at/lmuxC

1.9 Objectives of this study

The present experiment aimed to compare the effect of AGLEV® SI 200 and Micosat F-Olivo on the viability and productivity of *Thymus vulgaris* and *Lavandula angustifolia*. The two species were planted in two experimental areas in Cyprus. Given that Cyprus is highly susceptible to desertification, which is expected to intensify due to climate change (Constantinou et al. 2021), the experiment was conducted to investigate the performance of these species under different treatments, that can potentially be used in agroecosystems to mitigate their degradation. One of the experimental areas was on a sun-exposed south-facing slope with drier soil, while the other was on the north aspect under partial shade with higher soil moisture. These two species were selected to assist in soil-enhancing and plant growth-promoting practices because their vulnerability in dry areas can indicate effectiveness regarding AGLEV® SI 200 and Micosat F-Olivo activity.

In total, 80 plants of *Thymus vulgaris* were planted in the drier site and 102 plants of *Lavandula angustifolia* were planted in the wetter site. During the planting process, 4 different treatments were implemented in both instances: the first being the control, with no soil addition of Micosat F-Olivo or AGLEV® SI 200; the second with the soil addition of AGLEV® SI 200; the third with the soil addition of Micosat F-Olivo; and the fourth with the soil addition of both AGLEV® SI 200 and Micosat F-Olivo. Our main goals were: 1) To observe the effect of the four implemented treatments on the productivity and surface soil moisture in both experimental areas, and 2) to find out which one of the four treatments better supported the survival and growth of the plants.

2. MATERIALS AND METHODS

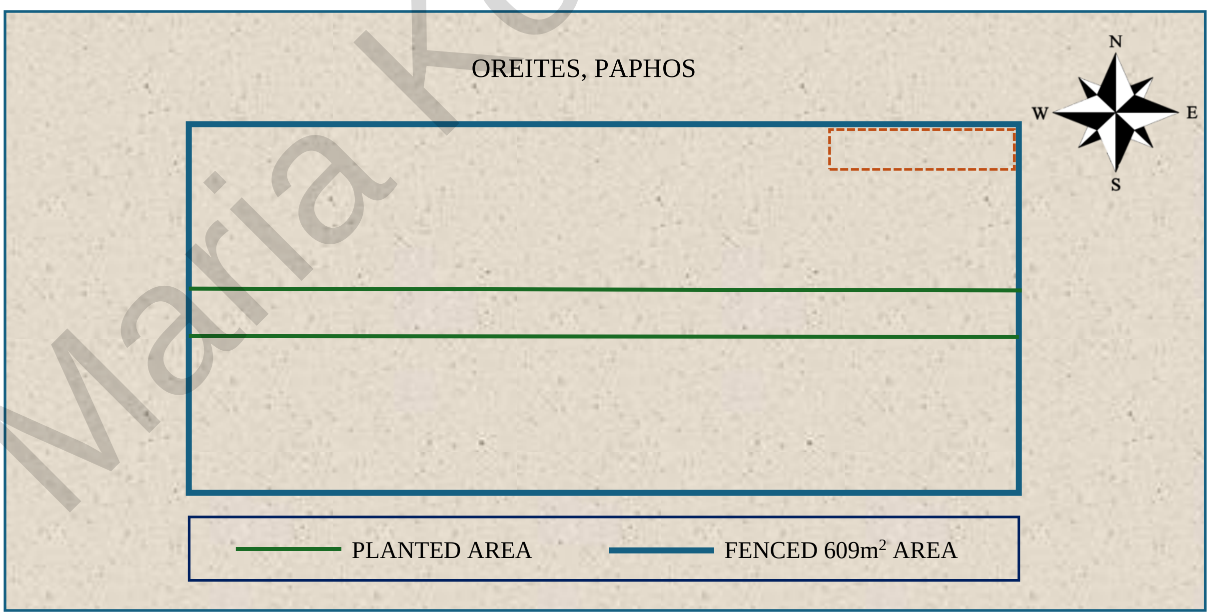
2.1 Experimental Sites Description and Soil Quality

For this experiment, two species of aromatic Mediterranean shrubs were planted in two different sites in Cyprus, to compare the growth and productivity of the plants in different soil conditions. *Thymus vulgaris* shrubs were planted within a 609m^2 (29m x 21m) fenced area in Oreites, Paphos, with an altitude of 390m, and with coordinates of **34°43'29.96"N** and **32°37'47.50"E** (Figure 19, Figure 20). This area presented a south aspect and a terrain slope of about 5% to the southeast, with sun exposure and drier soil. Within the fenced plot there were 16 carob trees for the configuration of the experimental surface. In total, 80 *Thymus vulgaris* shrubs were planted along 2 parallel lines in the center of the experimental area, with 40 shrubs planted in each line (Figure 21). The experiment in Oreites, Paphos was conducted by Manoli, 2022 and Nearchou, 2022 for their undergraduate theses.

Figure 19: Satellite image of the experimental area in Oreites, Paphos. Source: Google Earth, 2022.

Figure 20: The experimental surface
in Oreites, Paphos.
Field picture, January 2022.*Figure*

Figure 21: Illustration of the experimental area in Oreites, Paphos.



According to the study of Christofi, 2019, the soil of the experimental site in Oreites, Paphos was characterized by high $CaCO_3$ concentration values (39,51%) and low concentration values of organic C (2,02%), organic matter (7,41%), total N (0,68%), and N-NO_3 (0,08%) (Table 1, obtained from Christofi, 2019). Based on the concentrations of the chemical elements and compounds, the terrain of the experimental surface in Oreites can be characterized as calcareous, alkaline, with low fertility, and therefore poor in nutrients (Christofi, 2019).

Table 1: Concentration of elements and compounds and their Mean and Typical Error in Oreites, Paphos. Source: Christofi, 2019.

	$CaCO_3$ (%)	Organic C (%)	P (mg/kg)	Total N (%)	Inorganic N (mg/kg) and N-NO_3 (mg/kg)
F1a	13,22	5,08	5,57	0,07	1,15
F1b	49,74	1,53	6,89	0,08	0,00
F3a	45,84	1,02	11,69	0,08	0,80
F3b	44,86	1,85	5,85	0,09	0,00
F4a	41,44	1,11	5,55	0,08	0,00
F4b	41,96	1,55	8,89	0,10	2,10
Mean	**39,51**	**2,02**	**7,41**	**0,08**	**0,68**
Typical Error	**5,40**	**0,62**	**1,00**	**0,00**	**0,35**

Mediterranean shrubs of *Lavandula angustifolia* were planted by the writers of the present study, in an experimental plot in Vavla, Larnaca. The altitude of the experimental area is 455m and the coordinates are **34°50'33"N** and **33°16'08"E** (Figure 22 - Figure 24). In total, 102 shrubs of *Lavandula angustifolia* were planted for ornamental purposes and biodiversity promotion. The experimental area presented a north aspect and a terrain slope of 5% to the north, with partial shade and higher soil moisture. Within the experimental area, there were 4 flower beds, inside the 3 of which shrubs of *Lavandula angustifolia* were planted, whilst the last shrubs were planted around the perimeter of the 4th flower bed, as shown in Figure 25.

Figure 22: Satellite image of the experimental area in Vavla, Larnaca. Source: Google Earth, 2024.

Figure 23: Experimental surface in Vavla, Larnaca (F.B. 1). Field picture, May 2023.

Figure 24: Experimental surface in Vavla, Larnaca (F.B. 4). Field picture, May 2023.

Figure 25: Illustration of the experimental area in Vavla, Larnaca.

In April 2024, in Vavla, Larnaca, 4 soil horizon measurements were obtained within the experimental area, using a manual hand drill (Figure 26, Figure 27). One measurement was obtained for each flower bed, to assess the soil homogeneity regarding depth. In Table 2, all the details regarding the depth and exact location of the 4 measurements are shown. All 4 measurements showed homogeneity in a depth over 31cm, and the soil seemed to have a sandy loam texture, although a more detailed analysis regarding the soil's consistency is needed (Figure 28-Figure 32). Sandy loam soils have greater water-holding capacity, are richer in nutrients, and are better at draining and infiltrating water and air. Overall, the soil quality in Vavla seemed to be better than in Oreites.

Figure 26: Hand drill insertion in Vavla, Larnaca. Field picture, April 2024

Figure 27: Hand drill extraction in Vavla, Larnaca. Field picture, April 2024

Table 2: Depth and Location of the 4 soil horizon measurements in Vavla, Larnaca.

Flower bed	Depth (cm)	Location (Google Maps)
1	Over 31	8G6MR7V9+3J
2	35	8G6MR7V9+5M
3	Over 31	8G6MR7V9+4P
4	Over 31	8G6MR7V9+4J

Figure 28: Flower bed 1 measurement.
Field picture, April 2024

Figure 29: Flower bed 2 measurement.
Field picture, April 2024

Figure 30: Flower bed 3 measurement.
Field picture, April 2024

Figure 31: Flower bed 4 measurement.
Field picture, April 2024

Figure 32: Sandy loam
soil in Vavla, Larnaca.
Field picture, April 2024

2.2 Planting, Treatment Implementation, and Measurements in Oreites, Paphos

2.2.1 Planting of *Thymus vulgaris* and treatment implementation in Oreites, Paphos

On the 15th and 16th of January 2022 (Table 3), the planting process of 80 shrubs of *Thymus vulgaris* aged 6 months was completed in Oreites, Paphos, by Nearchou, 2002 and Manoli, 2022. The shrubs were planted in two parallel lines in the middle of the experimental area, with 40 shrubs in each line (Figure 21). The distance between the 2 parallel lines was 2,6m, the distance between the plants was 50cm, and the basins where the shrubs were planted had a depth of 9cm. During the planting process, 4 different treatments were implemented in each basin. The first was the control treatment (C), with no implementation of any soil-enhancing agent. All the plants were marked with pipes and additional colored markings according to their treatment. The control shrubs were marked with a pipe, without an additional marking. The second treatment was the addition of 10gr of AGLEV® SI 200 attapulgite (A), and these shrubs were marked with white markings on their pipe. The third treatment was 10gr of Micosat F-Olivo (M), marked with a green marking on the pipe. The last treatment was the implementation of 10gr of AGLEV® SI 200 attapulgite and 10gr of Micosat F-Olivo (AM), marked with blue marking on the pipe (*Table 4*). Both AGLEV® SI 200 and Micosat F-Olivo were in the form of grain and were added directly to the basin before the plant was placed in and covered with soil (Figure 33, Figure 34). During the planting process, any other plants on the ground near or in the basins were removed to avoid competition between the plants. The planting process started from the east going to the west, following the above order of treatment. Each of the four treatments was implemented in 19 repetitions, and ultimately the planting process ended with 20 plants corresponding to each treatment, with 80 plants in total.

Table 3: Timescale of planting and measurements regarding *Thymus vulgaris* in Oreites, Paphos.

Date	Action	Conducted by
15-16/01/2022	Planting and treatment implementation	Manoli, 2022 and Nearchou, 2022
16/01/2022	Height and diameter measurements	Manoli, 2022 and Nearchou, 2022
10/04/2022	Height, diameter, and soil moisture measurements	Manoli, 2022 and Nearchou, 2022
13/07/2022	Biomass count	Konstantinou, 2024

Table 4: Thymus vulgaris quantity, treatment, and marking in Oreites, Paphos.

Treatment	Implementation	Quantity	Marking
C	None	20	None
A	10gr AGLEV® SI 200	20	White
M	10gr Micosat F-Olivo	20	Green
AM	10gr AGLEV® SI 200 + 10gr Micosat F-Olivo	20	Blue
Total	-	80	-

2.2.2 Height and Diameter Measurements of *Thymus vulgaris* in Oreites, Paphos

Right after the planting process was completed on January 16th, 2022 (Table 3), height and diameter measurements were obtained for each thyme shrub, using a tape measure. These measurements were considered as the initial count of productivity, which would later be

Figure 33: Treatment implementation
Field picture January 2022

Figure 34: Planting Process
Field picture January 2022

compared to future measurements. Regarding height measurements, the measuring tape was placed perpendicular to the ground, and the measurement was taken from the base of the shoot at the soil surface to the tallest thyme shoot. During this measurement, the shoots were held together to show the actual height of the shrub (Figure 35). Regarding the diameter measurement, horizontal and vertical measurements (cross-measurements) were taken on the surface of the plant's crown (Figure 36, Figure 37). The shape of the shrubs resembled an ellipsoid, and from these measurements, the volume of each ellipsoid/shrub was calculated and used for further statistical analysis.

Figure 35: Height measurement
Field picture January 2022

Figure 36: Horizontal measurement
Field picture January 2022

Figure 37: Vertical measurement Field picture January 2022

On April 10th, 2022 (Table 3), the same height and diameter measurements were obtained from the shrubs. Before them, a thyme survival observation was carried out, as a period of cold and rain preceded, to ensure that all the shrubs were alive. Vegetation around the shrubs was removed to facilitate the height and diameter measurements. The methodology followed for these measurements was the same as implemented in January. Likewise, the volume of each shrub was calculated and used later for statistical analysis.

2.2.3 Soil Moisture Measurement of *Thymus vulgaris* in Oreites, Paphos

On April 10th, 2022 (Table 3), moisture measurements were conducted under the crown of each shrub. The portable soil moisture sensor SM 150T [SM150T Soil Moisture Sensor - Soil Water content - Soil Humidity Sensor (delta-t.co.uk)] was used for this measurement (Figure 38). The device's electrodes were completely inserted in the soil, and the volumetric water content was displayed on the screen, with a **± 3%** accuracy (Figure 39). Two soil moisture measurements were obtained from under the crown of each shrub, one from the north side and another from the south side of the shrub. The mean of the two measurements was used for further statistical analysis.

Figure 38: SM 150T soil moisture sensor
Source: https://shorturl.at/hkGNO

Figure 39: Soil Moisture Measurement under *Thymus vulgaris* crown
Field picture April 2022

2.2.4 *Thymus vulgaris* Biomass Measurement

For this study, additional measurements of *Thymus vulgaris* biomass were conducted, on July 13th, 202, as it was observed that all the shrubs were deceased. The shrubs were cut from the base of the shoot and taken to the laboratory to measure each biomass on a high-accuracy weight scale. Before the shrubs were cut, some of them were accidentally injured by a lawnmower. The injured ones were marked in case they had to be removed from the statistical analysis. The biomass was used for further statistical analysis.

2.2.5 Precipitation Data for Oreites, Paphos

The precipitation factor was critical for the experiment, as the shrubs weren't watered. Precipitation data were obtained for Paphos Airport, the nearest station from the experimental area of Oreites. Even though Oreites village is situated at an altitude 400m higher than the airport and it was expected that the precipitation rates would be higher, Paphos Airport station showed a general image regarding the precipitation levels in Oreites. All the data belonged to the records of the Department of Meteorology of Cyprus, 2024.

Table 5 shows the monthly precipitation data from October 2021, the initial month of the hydrological year, to July 2022, when the last measurement, the biomass count, was obtained. As shown in Table 5, during December 2021 and January 2022 precipitation levels exceeded the normal of the month. During February 2022, the precipitation almost approached the normal levels of the month, while as the spring and summer months passed the precipitation levels didn't reach the normal levels of the month, except June 2022 that the levels of precipitation exceeded the normal of the month. By the time of the biomass measurements in July 2022, all the shrubs were dry.

Table 6 shows the daily precipitation record for March and April 2022, which were considered critical for soil moisture and thus the productivity of *Thymus vulgaris* for the shrub's height and cross measurements. The last natural watering the shrubs received was on March 22, 23, and 24 with a total of 20.9 mm. That is, 17 days before the soil moisture measurements of April 10th, 2022, which could have been slightly higher due to the recent rainfall.

Table 5: Monthly Precipitation Data from October 2021 – July 2022 in Oreites, Paphos.

Month	Normal rainfall of the month (1961-90) (mm)	Quantity for the last 24 hours (mm)	Total amount from the 1st of the month (mm)	Comparison with the normal rainfall of the moth (%)
Oct 2021	31,0	0,0	0,4	1,00%
Nov 2021	52,0	0,5	12,0	23,00%
Dec 2021	98,0	10,9	179,2	183,00%
Jun 2022	94,0	3,7	139,4	148,00%
Feb 2022	69,0	2,5	55,0	80,00%
Mar 2022	49,0	0,0	42,9	88,00%
Apr 2022	24,0	0,0	0,0	0,00%
May 2022	10,0	0,0	0,4	4,00%
Jun 2022	0,7	0,0	5,4	7,71%
Jul 2022	0,2	0,0	0,0	0,00%

Table 6: Daily Precipitation Data (mm) for March and April 2022 in Oreites, Paphos.

Day	March 2022	April 2022
1	2,6	0,0
2	2,9	0,0
3	0,5	0,0
4	0,3	0,0
5	0,0	0,0
6	0,0	0,0
7	0,0	0,0
8	0,1	0,0
9	5,5	0,0
10	5,1	0,0
11	2,0	0,0
12	0,2	0,0
13	2,2	0,0
14	0,0	0,0
15	0,0	0,0
16	0,0	0,0
17	0,3	0,0
18	0,3	0,0
19	0,0	0,0
20	0,0	0,0
21	0,0	0,0
22	5,7	0,0
23	10,6	0,0
24	4,6	0,0
25	0,0	0,0
26	0,0	0,0
27	0,0	0,0
28	0,0	0,0
29	0,0	0,0
30	0,0	0,0
31	0,0	-
Total	42,9	0,0
Normal Precipitation	49	24

2.3 Planting, Treatment Implementation, and Measurements in Vavla, Larnaca

2.3.1. Planting of *Lavandula angustifolia* and treatment implementation in Vavla, Larnaca

The planting process of 102 shrubs of *Lavandula angustifolia* aged 2 years in Vavla, Larnaca was completed in 4 phases, the first being on November 24th, 2022, the second being on December 3rd, 2022, the third being on December 31st, 2022, and the fourth being on January 21st, 2023 (Table 8). The shrubs were planted in 3 flower beds and around the perimeter of a fourth flower bed, as shown in Figure 25. The depth of the basins where the shrubs were planted was uniform, approximately 20cm.

During the planting process, 4 different treatments were implemented in each basin. The first was the control treatment, with no implementation of any soil-enhancing agent. All the treated plants were marked with colored markings according to their treatment. The control shrubs had no marking. The second treatment was the addition of 25gr of AGLEV® SI 200 attapulgite, and these shrubs had red markings. The third treatment was 15gr of Micosat F-Olivo, marked with white marking. The last treatment was the implementation of 10gr of AGLEV® SI 200 attapulgite and 15gr of Micosat F-Olivo, marked with white and red markings (Table 7). As in Oreites, Paphos, the grain form of AGLEV® SI 200 and Micosat F-Olivo were added directly to the basin before the plant was placed in and covered with soil (Figure 40, Figure 41).

During the planting process, any other plants near or in the basins were removed to avoid competition between the plants. After every planting phase, the shrubs were watered with 5L of water each. In total, 28 shrubs were planted in Flower Bed 1 (F.B. 1), 30 shrubs were planted in Flower Bed 2 (F.B. 2), 18 shrubs were planted in Flower Bed 3 (F.B. 3), and 26 shrubs were planted around Flower Bed 4 (F.B. 4) (Table 7). The shrubs planted in Flower Bed 1 were initially pruned right after they were planted, except shrubs 016 (C), 022 (M), 027 (M), and 028 (AM). Also, shrubs 097 (C), 100 (M), 101 (A), and 102 (AM) around Flower Bed 4 were wind-damaged before planting. In total, 28 out of 102 shrubs were pruned or wind-damaged initially.

Figure 40: Treatment implementation
Field picture December 2022

Figure 41: Planting Process
Field picture December 2022

Table 7: Lavandula angustifolia treatment and implementation, quantity, and marking in every F.B., Vavla, Larnaca.

Treatment	Implementation	F.B. 1	F.B. 2	F.B. 3	F.B. 4	Total	Marking
C	None	7	8	5	5	25	None
A	25gr AGLEV® SI 200	7	8	3	8	26	Red
M	15gr Micosat F-Olivo	7	7	5	6	25	White
AM	10gr AGLEV® SI 200 + 15gr Micosat F-Olivo	7	7	5	7	26	Red, White
Total	-	28	30	18	26	102	-

2.3.2 Height and Shrub Measurements for *Lavandula angustifolia*

Measurements for *Lavandula angustifolia* were held six times as shown in Table 8. The obtained measurements were about the maximum height of the plant, and the measurements of the shrub-like structure formed at the base of the shoots and was observed to grow (Figure 42). For the maximum height measurements, all the shoots were held together to show the actual height of the shrub. A wooden ruler was used for this measurement, placed on a fixed reference point on the ground (Figure 43), and measured the height to the last node of the tallest shoot, likewise with the height measurements of Thymus vulgaris shrubs in Oreites, Paphos (Figure 35). The maximum height would be used for further statistical analysis. After the first phase of measurements, it was observed that a shrub-like structure was formed at the base of the shoot and was growing between the next measurement phases, so shrub measurements were obtained from phase 2 onwards (Table 8).

Figure 42: Lavandula angustifolia main shoot (2) and its base shrub (1).

Field picture April 2024

Table 8: Timescale of planting and measurements regarding *Lavandula angustifolia* in Vavla, Larnaca.

Date	Phase	Height Measurements	Shrub Measurements	Individual Actions
24/11, 03/12, 31/12/2022 21/01/2023	-	-	-	Planting and treatment implementation
21/01, 03/02, 10/02, 10/03/2023	1st	X	-	-
11/04, 13/04/2023	-	-	-	Quadrat measurements
03/05/2023	2nd	X	X	Weeding
01/06/2023	3rd	X	X	-
07/07/2023	4th	X	X	-
04/08/2023	5th	X	X	-
19/10/2023	-	-	-	Soil moisture measurement
19/10, 20/10, 24/10, 25/10/2023	6th	X	X	-

Figure 43: Fixed reference point for *Lavandula angustifolia* height measurements.

Field picture April 2024

From these base shrubs, measurements of height from the fixed reference point were obtained, as well as the crown's length and width (cross-measurements) (Figure 44-Figure 46). From these dimensions and given that the shape of the shrubs resembled an ellipsoid, the volume of each ellipsoid/shrub was calculated and used for further statistical analysis.

Figure 44: Horizontal Measurement.
Field picture, April 2024

Figure 45: Vertical Measurement.
Field picture, April 2024

Figure 46: Height Measurement.
Field picture, April 2024

2.3.3 Quadrat Measurements for Wild Vegetation Count of *Lavandula angustifolia* Shrubs

In spring 2023, wild vegetation was observed in the experimental area due to the season, and quadrat measurements were conducted for wild vegetation identification purposes. On April 11th and 13th 2023, wild vegetation count was obtained for 2 plants of every treatment, for each F.B. In total 8 quadrat measurements were obtained from every F.B. (Table 9). Figures 47- 49 show an example of the quadrat measurement. The species were identified at the level of Family, and the coverage rate of each one was calculated on the spot. Their soil-beneficial properties were then searched in the bibliography. After the wild vegetation count and during the 2nd phase of measurements, all Flower Beds were weeded to avoid competition with the lavender shrubs (Figures 50-51).

Table 9: All 32 Quadrat measurements, 8 from each F.B., 8 from each treatment.

Quadrat	Flower Bed	Experiment	Treatment
Q1	1	4	C
Q2	1	23	C
Q3	1	8	A
Q4	1	6	M
Q5	1	22	M
Q6	1	26	A
Q7	1	15	AM
Q8	1	24	AM
Q9	2	29	C
Q10	2	57	A
Q11	2	39	M
Q12	2	41	AM
Q13	2	46	M
Q14	2	50	A
Q15	2	52	AM
Q16	2	56	C
Q17	3	60	M
Q18	3	62	C
Q19	3	63	AM
Q20	3	67	M
Q21	3	68	A
Q22	3	70	A
Q23	3	74	AM
Q24	3	76	C
Q25	4	77	A
Q26	4	81	C
Q27	4	84	M
Q28	4	86	AM
Q29	4	89	C
Q30	4	92	M
Q31	4	99	A
Q32	4	102	AM

Figure 47: Quadrat placement.
Field picture, April 2023ENTRANCE

Figure 48: Apiaceae sp.
Field picture, April 2023

Figure 49: Poaceae sp. and *Fabaceae* sp.
Field picture, April 2023

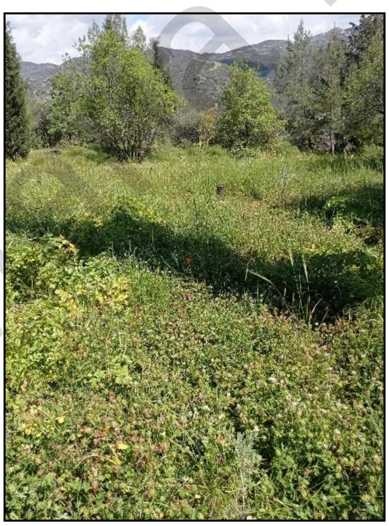


Figure 50: Before weeding.
Field picture, May 2023

Figure 51: After weeding.
Field picture, May 2023

2.3.4 Soil Moisture Measurement of *Lavandula angustifolia*

On October 19th, 2023, soil moisture measurements were obtained using the ML3 ThetaProbe Soil Moisture Sensor [ML3 ThetaProbe Soil Moisture Sensor - Soil Moisture Measurement - Soil Moisture Meter (delta-t.co.uk)] (Figure 52). The device's electrodes were completely inserted in the soil, and the volumetric water content was displayed on the screen, with a ± **1%** accuracy (Figure 53). Three measurements were obtained from 3 different points under the shrub's crown, and the means for each shrub were used for further statistical analysis.

Figure 52: ML3 ThetaProbe Soil Moisture Sensor.
Source: https://shorturl.at/hxCJU

Figure 53: Soil Moisture Measurement. Field picture, October 2023

2.3.5 Precipitation Data for Vavla, Larnaca

The precipitation factor was critical for the growth of the plants, so precipitation data should be obtained from the Department of Meteorology of Cyprus for stations near Vavla. The closest stations were in Kornos, with an altitude of 300m, and in Kellaki, with an altitude of 600m. Vavla is situated between them at an altitude of 455m, so the precipitation values were expected to be between the levels of Kornos and Kellaki. All the data belonged to the records of the Department of Meteorology of Cyprus, 2024.

Table 10 and Table 11 show the monthly precipitation data for Kornos and Kellaki, respectively. The tables show data from October 2022, the initial month of the hydrological year, to October 2023, when the 6th and last measurement was completed. Overall, Vavla is situated between two sites that receive adequate precipitation levels, which are favorable for the growth and development of *Lavandula angustifolia* plants.

Table 10: Monthly Precipitation Data from October 2022 – October 2023 in Kornos.

Month	Normal rainfall of the month (1961-90)	Quantity for the last 24 hours (mm)	Total amount from the 1st of the month	Comparison with the normal rainfall of the moth
Oct 2022	35.7	2.2	107.1	300%
Nov 2022	45.9	0,0	25.3	55%
Dec 2022	93.2	0,0	17.4	19%
Jan 2023	87.7	0,0	63,0	72%
Feb 2023	72.8	0,0	36.1	50%
Mar 2023	57.9	0,0	39.5	68%
Apr 2023	26.9	0,0	61.9	230%
May 2023	24.9	0,0	62,0	249%
Jun 2023	10.3	0,0	22.9	222%
Jul 2023	3.3	0,0	0,0	0%
Aug 2023	1.3	0,0	48.5	3731%
Sept 2023	5.7	0,0	3,0	53%
Oct 2023	35.7	0,0	38.6	108%

Table 11: Monthly Precipitation Data from October 2022 – October 2023 in Kellaki.

Month	**Normal rainfall of the month (1961-90)**	**Quantity for the last 24 hours (mm)**	**Total amount from the 1st of the month**	**Comparison with the normal rainfall of the moth**
Oct 2022	41,0	0,0	109.4	267%
Nov 2022	58.5	3.3	42.2	72%
Dec 2022	117.3	0,0	45.5	39%
Jan 2023	115.8	0,0	91.1	79%
Feb 2023	96.9	0,0	54,0	56%
Mar 2023	66.4	0,0	69.6	105%
Apr 2023	33.3	0.8	35.2	106%
May 2023	24.6	0,0	17.7	72%
Jun 2023	5.9	0,0	0,0	0%
Jul 2023	1.4	0,0	9,0	643%
Aug 2023	2.6	0,0	0,0	0%
Sept 2023	7.7	0,0	43.4	564%
Oct 2023	41,0	0.4	19,0	46%

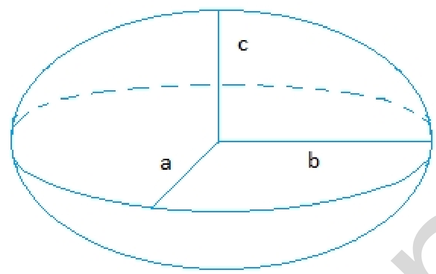
2.4 Data Processing and Statistical Analysis

2.4.1. Data Processing

All data processing was performed in Microsoft Excel. Regarding height and cross measurements of *Thymus vulgaris* shrubs in Oreites, and height and cross measurements of base shrubs of *Lavandula angustifolia* in Vavla, the shrub volume was calculated using the three dimensions collected, to transform the three dimensions into one for convenience in statistical analysis. The shrubs resembled the shape of a vertical ellipsoid, so the ellipsoid volume formula was used to calculate the shrub volume (Figure 54). The shrub volume comparison in Vavla was conducted between Flower Beds and between treatments, while in Oreites between treatments only.

Figure 54: Volume of Ellipsoid Formula. Source: https://shorturl.at/aBD04

$$V = \frac{4}{3} \times \pi \times a \times b \times c$$



The growth percentage was then calculated using the initial and final volume. In Vavla, the initial volume was 0, so the final volume was equal to the growth percentage. The formula for percentage change was used to calculate the shrubs' growth percentage in Oreites (Figure 55).

Figure 55: Percentage Change Formula. Source: https://shorturl.at/dgl28

$$\frac{\text{Final value - Initial value}}{\text{Initial value}} \times 100$$

The same formula was used for calculating the growth percentage for the tallest shoot's height for *Lavandula angustifolia* in Vavla, and likewise, the initial and final maximum heights were used.

Subsequently, all the growth percentages of shrub measurements of *Thymus vulgaris*, the base shrub measurements of *Lavandula angustifolia*, and the maximum height of *Lavandula angustifolia* were categorized by treatment (C, A, M, AM) in each experimental area. For each treatment, the mean and the standard deviation were calculated and used for z-score calculation (Figure 56).

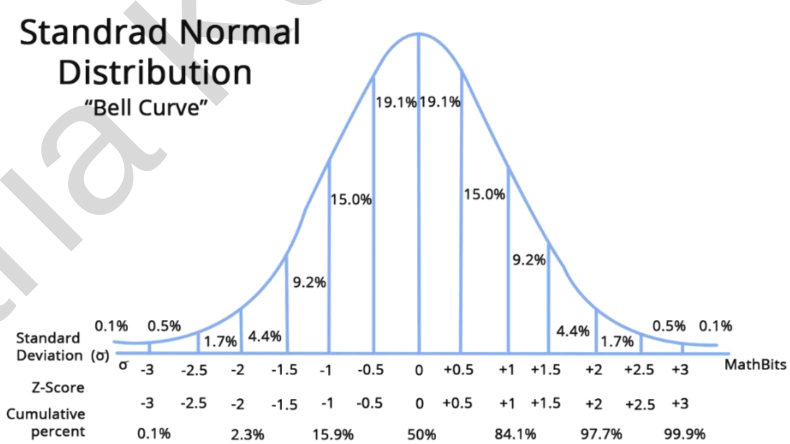
Figure 56: Z – Score Formula. Source: https://shorturl.at/UsDKf

$$z = \frac{x - \mu}{\sigma}$$

$\mu =$ Mean
$\sigma =$ Standard Deviation

Z-scores greater than 2 and lower than -2 were marked, as there was a possibility of rejecting them from further statistical analysis since those outliers influence the results and deviate from the rest of them. At the end of this procedure, those results had a normal distribution (Figure 57).

Figure 57: Standard Normal Distribution based on z-score. Source: https://shorturl.at/luAFV



Regarding soil moisture measurements in each experimental area, all values were divided by treatment and the mean values were calculated for each treatment. The means would be used for further statistical analysis. The same procedure was followed for the biomass count of *Thymus* *vulgaris* in Oreites and quadrat measurements in the Flower Beds of the Vavla experimental area.

2.4.2. Statistical Analysis

For the statistical analysis, SPSS software was used. Initially, the data was inserted into the software and a Kolmogorov-Smirnov test would be performed to check whether the data followed a normal distribution. If the data followed a normal distribution, the parametric method of ANOVA was performed, which examines the means of the experiment's measurements. If not, the non-parametric Kruskal-Wallis test was conducted, examining the measured values' medians. Additionally, post-hoc tests were performed to identify which measurements differed from the rest, such as Tukey HSD and Bonferroni. The results in figure form were obtained and used in the result section.

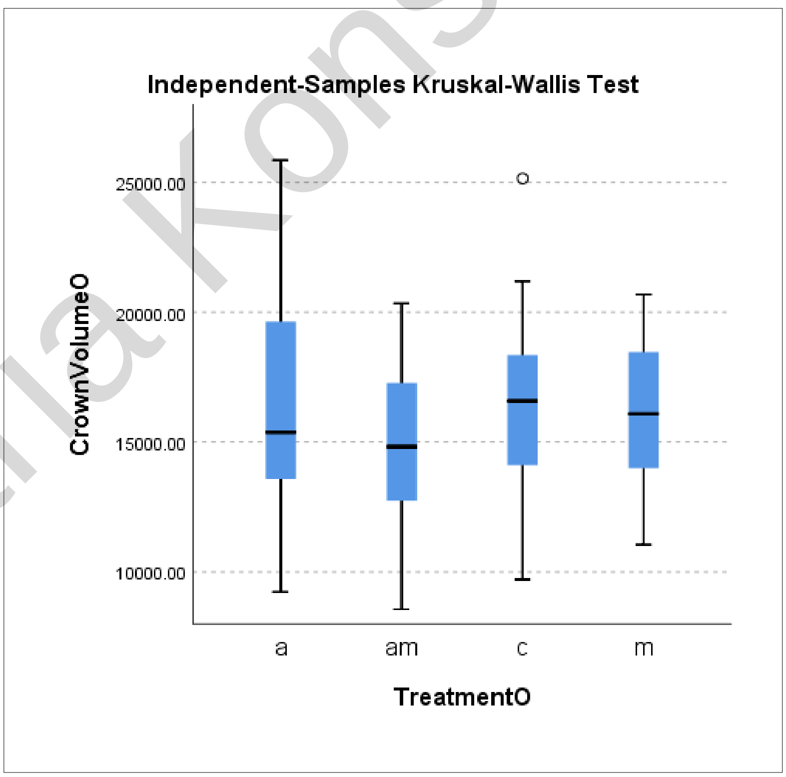
3. RESULTS

3.1 Oreites Results

3.1.1 *Treatment* and *Shrub Volume* Comparison in Oreites

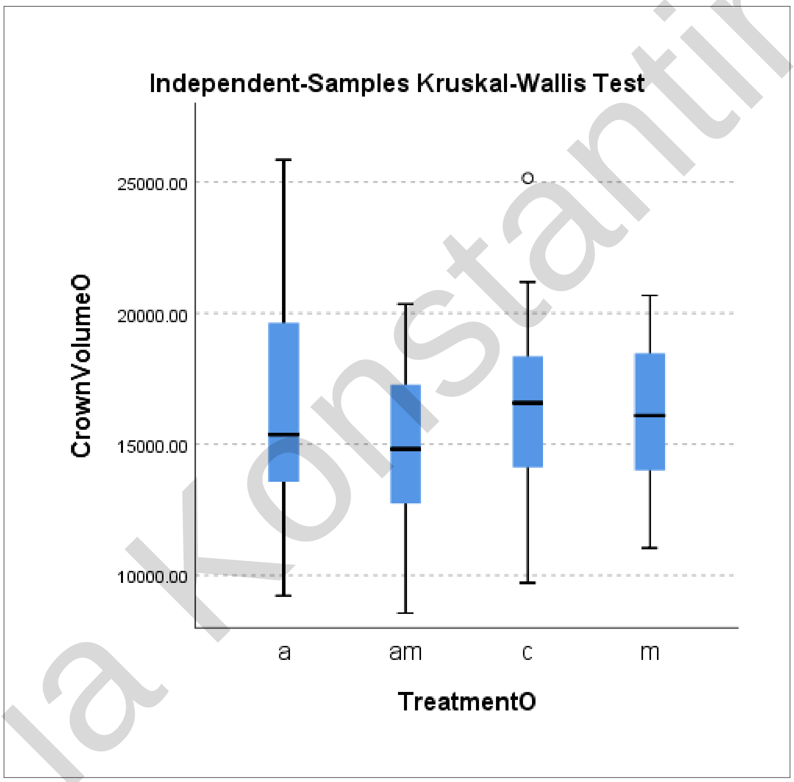
The comparison of the final shrub volume between treatments in Oreites is shown in *Figure 058*. All the experiments were included in the present analysis (n=80). The values followed a non-normal distribution (sig. > 0,05), so the non-parametric Kruskal-Wallis test was performed. Figure 58 shows no significant difference between treatments regarding the final shrub volume (p>0,05). The C treatment had the highest median, followed by the M, A, and AM treatments. Treatment A had the greatest volume range, followed by AM and C with similar ranges, and the M treatment.

Figure 58: Treatment and *Shrub Volume* Comparison in Oreites (n=80).



Subsequently, the same comparison was performed, but 2 outlier values were excluded, as their z-score was greater than 2 (n=78). The values followed a non-normal distribution (sig. > 0,05), so the non-parametric Kruskal-Wallis test was performed. As in Figure 58, Figure 59 shows no significant difference between treatments regarding the final shrub volume (p>0,05). The C treatment had the highest median, followed by the M, A, and AM treatments. Treatment A had the greatest volume range, followed by AM and C with similar ranges, and the M treatment.

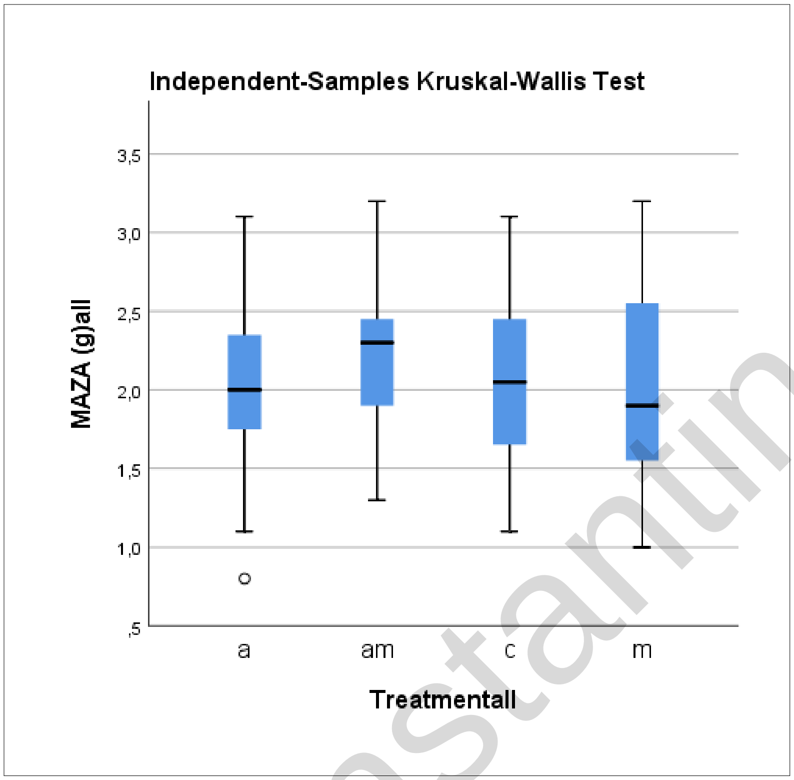
Figure 59: Treatment and *Shrub Volume* Comparison in Oreites (n=78).



3.1.2 *Treatment* and *Shrub Biomass* Comparison in Oreites

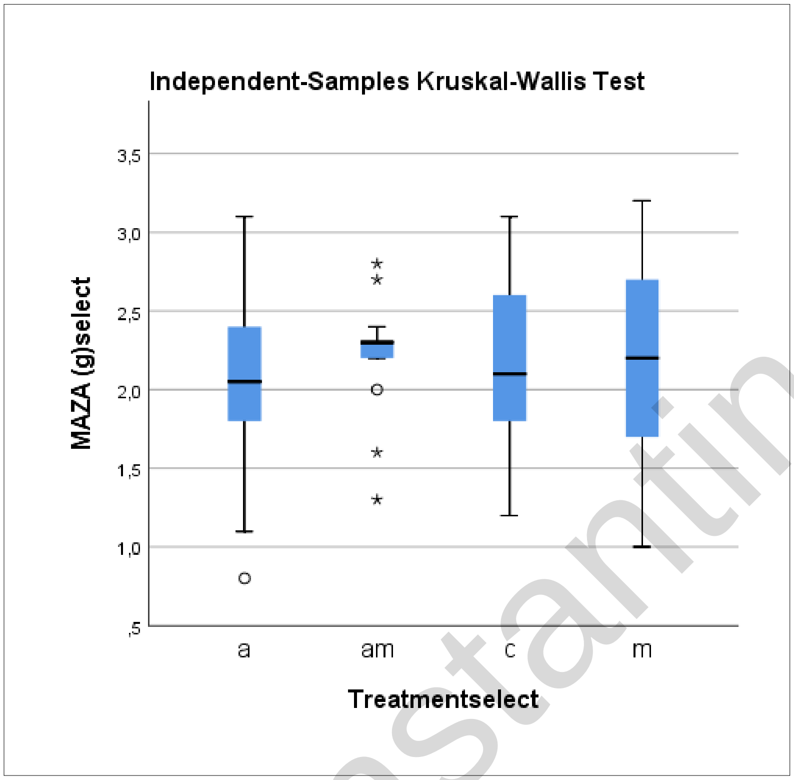
The comparison of shrub biomass between treatments in Oreites is shown in Figure 60. All 80 experiments were included in the present analysis. The values followed a non-normal distribution (sig. > 0,05), so the non-parametric Kruskal-Wallis test was performed. *Figure 060* shows no significant difference between treatments regarding shrub biomass (p>0,05). The AM treatment had the highest median, followed by the C, A, and M treatments.

Figure 60: Treatment and *Shrub Biomass* Comparison in Oreites (n=80).



Subsequently, the same comparison was performed, with 22 values excluded as 22 shrubs had undergone a mechanical disruption and lost some of their biomass (n=58). The values followed a non-normal distribution (sig. > 0,05), so the non-parametric Kruskal-Wallis test was performed. Figure 61 shows no significant difference between treatments regarding shrub biomass (p>0,05). Without the 22 disturbed experiments, the AM treatment had a higher median, followed by the M, C, and A treatments. The AM treatment had the smallest biomass range.

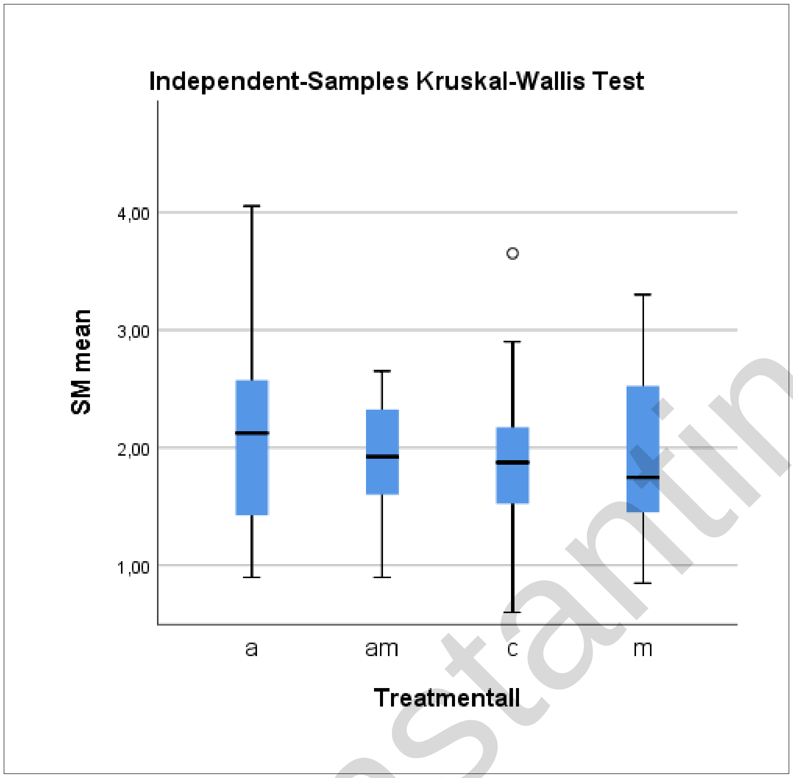
Figure 61: Treatment and *Shrub Biomass* Comparison in Oreites (n=58).



3.1.3 *Treatment* and *Soil Moisture* Comparison in Oreites

The comparison of soil moisture between treatments in Oreites is shown in Figure 62. All 80 experiments were included in the present analysis (n=80). The values followed a non-normal distribution (sig. > 0,05), so the non-parametric Kruskal-Wallis test was performed. Figure 62 shows no significant difference between treatments regarding soil moisture (p>0,05). The A treatment had a higher median, followed by the AM, C, and M treatments. The A treatment also had the greatest soil moisture range, followed by C and M with similar ranges, and the AM treatment.

Figure 62: Treatment and *Average Soil Moisture* comparison in Oreites (n=80).

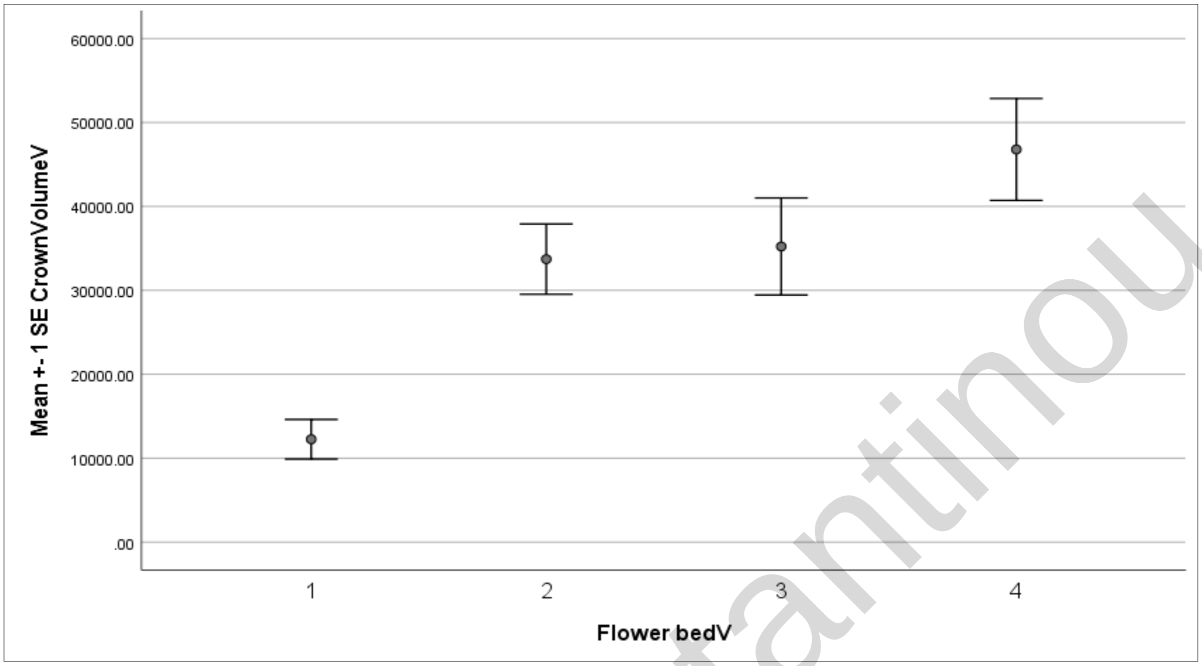


3.2 Vavla Results

3.2.1 *Flower Bed* and *Shrub Volume* Comparison in Vavla

The comparison of shrub volume between Flower Beds in Vavla is shown in Figure 63. Three experiments were excluded (031, 063, and 064) since they were deceased before shrub development (n=99). The data followed a normal distribution (sig.<0,05) and ANOVA was used for the statistical analysis. In Figure 63, Flower Bed 1 exhibited significantly lower development regarding shrub volume (p<0,05). It also showed the smallest value range.

Figure 63: Flower Bed and *Shrub Volume* Comparison in Vavla (n=99).



3.2.2 *Treatment* and *Shrub Volume* Comparison in Vavla

The comparison of shrub volume between treatments in Vavla is shown in Figure 64. The same three experiments as in Figure 63 were excluded, and all the experiments from Flower Bed 1 (n=71). The data followed a normal distribution (sig.<0,05) and ANOVA was used for the statistical analysis. Figure 64 shows that plants with the AM treatment had significantly greater mean volume than the C treatment (p<0,05). Plants with the A treatment, followed by the M treatment, displayed an increase in the mean volume, but it wasn't significant.

The same comparison as in Figure 64 was performed, but another 4 values were excluded, as their z-scores were greater than 2 (n=67). The data followed a normal distribution (sig.<0,05) and ANOVA was used for the statistical analysis. Figure 65 shows that plants with the AM treatment had a significantly greater mean volume than the C treatment (p<0,05). Plants with the A treatment, followed by the M treatment, displayed an increase in the mean volume, but it wasn't significant.

Figure 64: Treatment and *Shrub Volume* Comparison in Vavla (n=71).

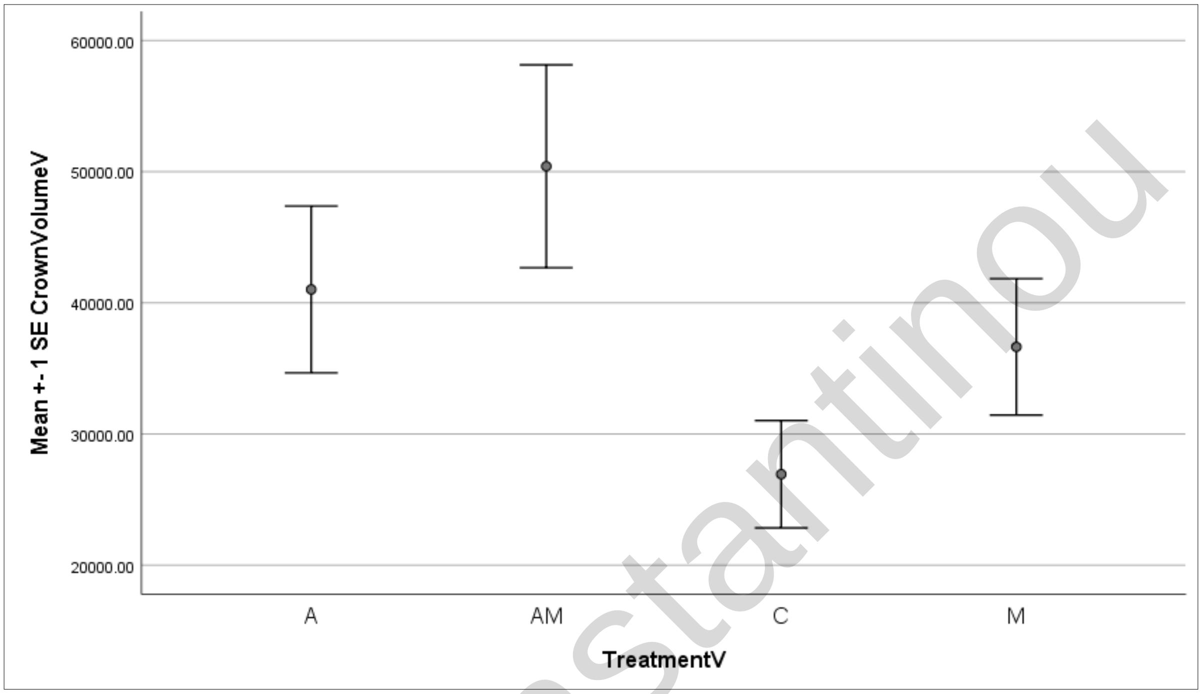
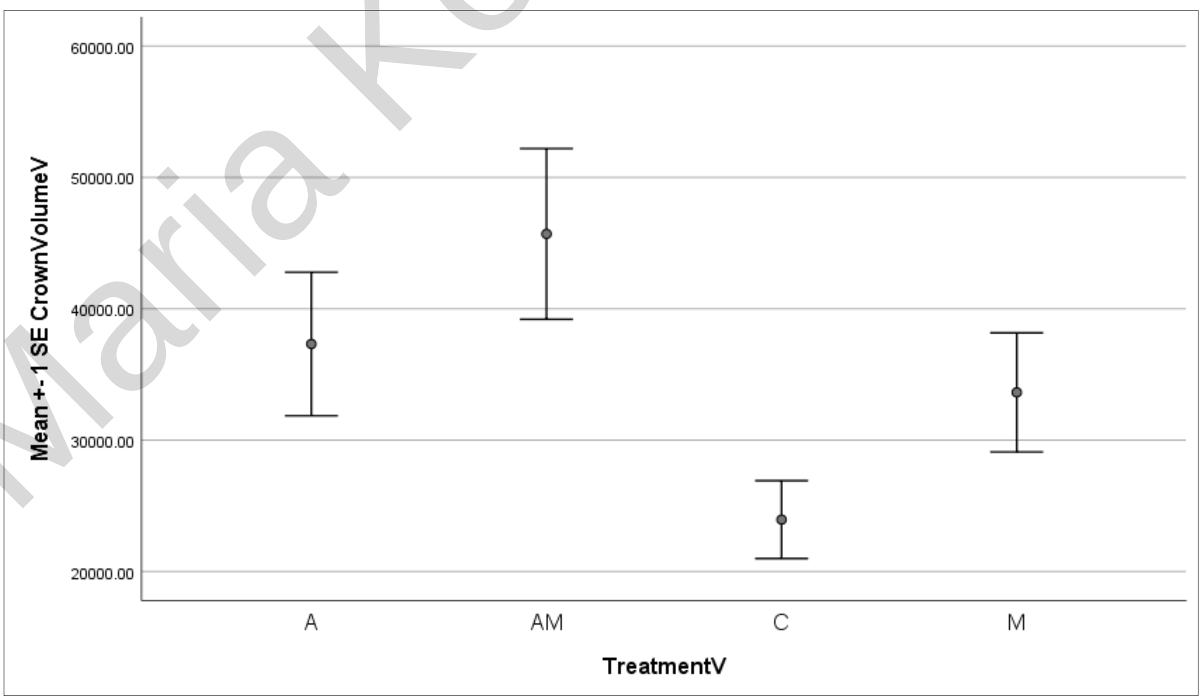


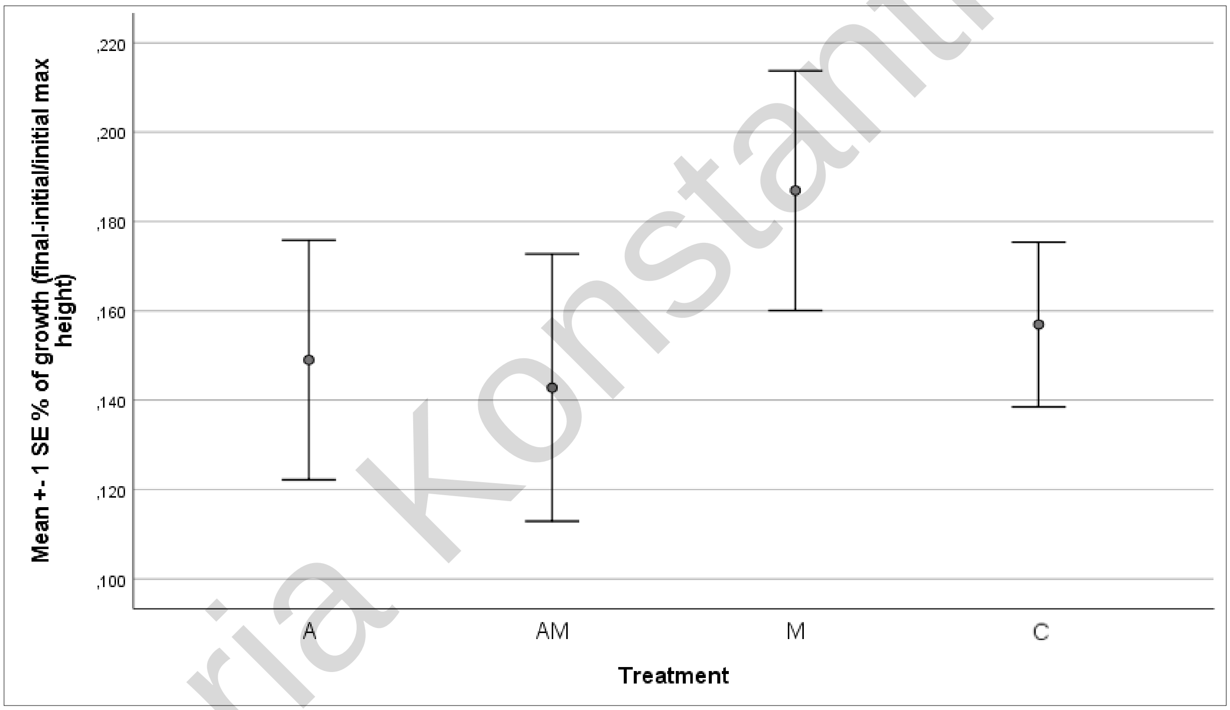
Figure 65: Treatment and *Shrub Volume* Comparison in Vavla (n=67).



3.2.3 *Treatment* and *Maximum Height Growth Rate* Comparison in Vavla

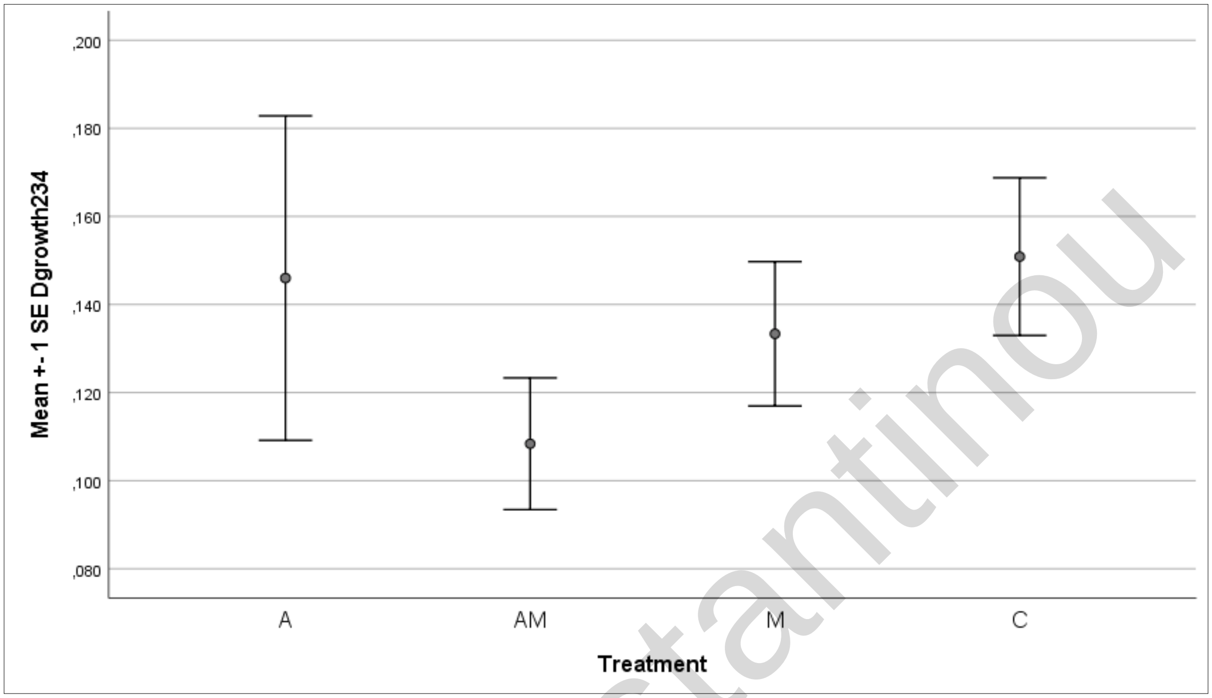
The comparison of the maximum height growth rate between treatments in Vavla is shown in Figure 66. All the selected data presented an initial and a final maximum height, with the latter exceeding the former (n=68). The data followed a normal distribution (sig.<0,05) and ANOVA was used for the statistical analysis. The results in Figure 66 did not show statistical significance between treatments and maximum height growth rate. The M treatment showed an increase in the mean maximum height growth rate, but it wasn't significant.

Figure 66: Treatment and *Maximum Height Growth Rate* Comparison in Vavla (n=68).



The same comparison as in Figure 66 was performed, but the measurements of maximum height from the Flower Bed 1 were excluded (n=47). The data followed a normal distribution (sig.<0,05) and ANOVA was used for the statistical analysis. The results in Figure 67 did not show statistical significance between treatment and maximum height growth rate. The plants with the C treatment showed a greater rate of maximum height growth, followed by the A, M, and AM treatments. The A treatment had the greatest value range.

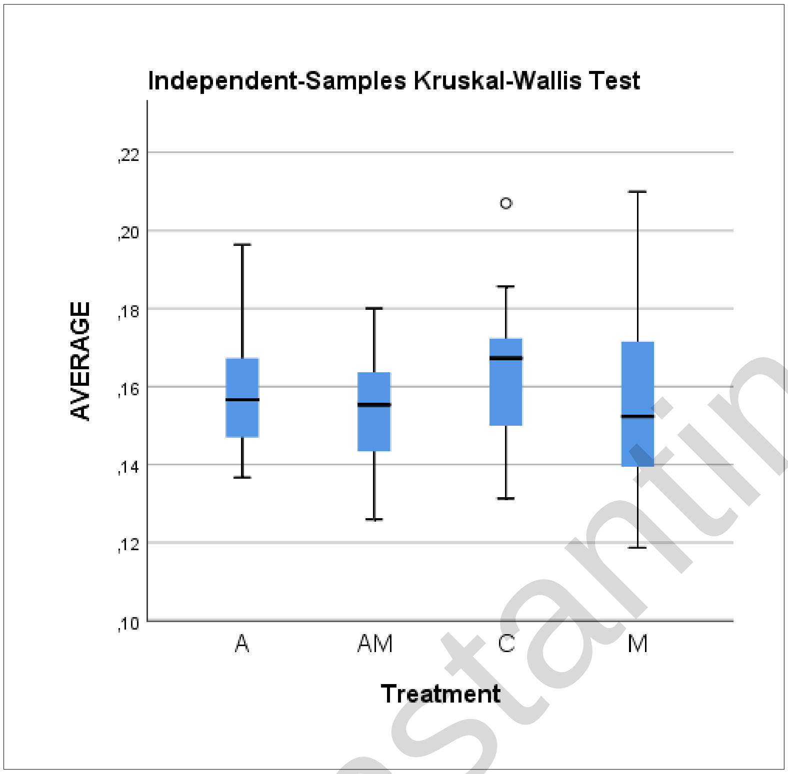
Figure 67: Treatment and *Maximum Height Growth Rate* Comparison in Vavla (n=47).



3.2.4 *Treatment* and *Soil Moisture* Comparison in Vavla

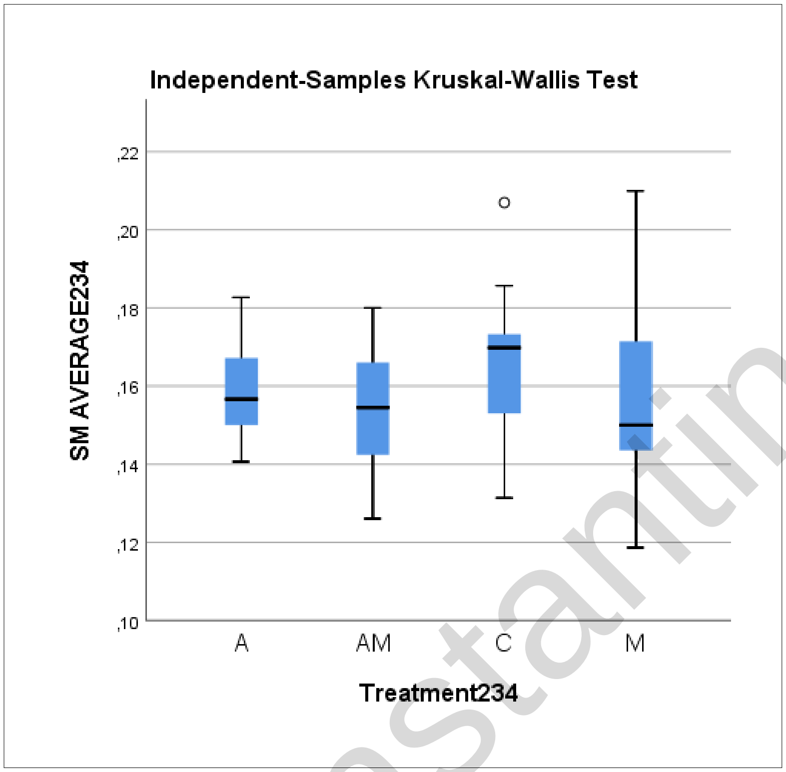
The comparison of soil moisture between treatments is shown in Figure 68 Six experiments were excluded (024, 025, 026, 031, 063, and 064) as they were deceased before the conduction of soil moisture measurements (n=96). The data followed a non-normal distribution (sig.>0,05) and the non-parametric test Kruskal-Wallis was used for the statistical analysis. The results in Figure 68 did not show statistical significance between treatments and average soil moisture (p>0.05). The C treatment showed a greater median of average soil moisture, followed by the A, AM, and M treatments. The M treatment showed the greatest value range compared to other treatments.

Figure 68: Treatment and *Average Soil Moisture* Comparison in Vavla (n=96).



The results in Figure 69 show the results of the same comparison as Figure 68, with the only difference being that the average soil moisture measurements from Flower Bed 1 were excluded (n=71). The data followed a non-normal distribution (sig.>0,05) and the non-parametric test Kruskal-Wallis was used for the statistical analysis. The results in Figure 69 did not show statistical significance between treatment and average soil moisture (p>0,05). The C treatment showed a greater median of average soil moisture, followed by the A, AM, and M treatments. Although the M treatment had the smallest mean value, it exhibited the greatest value range compared to other treatments.

Figure 69: Treatment and *Average Soil Moisture* Comparison in Vavla (n=71).



3.2.5 *Treatment* and *Wild Vegetation* in Vavla

Table 12 shows the results of the mean coverage of wild vegetation species for every treatment. Overall, the greatest mean range of coverage within Vavla's experimental area belonged to the Poaceae family (35,16% to 52,34%), followed by the Fabaceae family (17,97% to 34,38%), bare soil or other species (14,45% to 17,97%), and the Apiaceae family (9,77% to 17,19%).

Table 12: Treatment and Mean Coverage of wild vegetation, Vavla (n=32)

Treatment	N	A	M	AM
Apiaceae Mean Coverage	17,19%	9,77%	13,28%	12,11%
Poaceae Mean Coverage	35,16%	40,63%	52,34%	39,84%
Fabaceae Mean Coverage	29,69%	34,38%	17,97%	33,59%
Bare Soil/Other Species Mean Coverage	17,97%	15,23%	16,41%	14,45%

4. DISCUSSION

The present work aimed to use soil and plant growth-enhancing agents as a potential approach to adapt agriculture in Cyprus under climate change and desertification conditions. The attapulgite agent *AGLEV® SI 200* decreases the plant's water needs, due to attapulgite's structure and water-holding ability. *Micosat F-Olivo* is a mixture of soil-beneficial microbe strains, including *Glomus, Picia, Trichoderma, Streptomyces*, *Bacillus*, and *Pseudomonas*. These microbial strains can make the plant resistant to pathogens, and independent in nutrient and water intake. As a result, more optimal conditions are created in the plant's environment, and a production increase is observed.

The study focused on planting and cultivating the native Mediterranean species *Lavandula angustifolia* and *Thymus vulgaris*. Both plants have been widely known throughout the centuries for their medicinal properties and flavor, as they are used as spices. *Lavandula angustifolia* features some drought tolerance toward water stress, while *Thymus vulgaris* is semi-sensitive to drought. Sensitivity to dry conditions was desirable as those plant species would work as indicators for the effectiveness of *AGLEV® SI 200* and *Micosat F-Olivo* in the dry conditions of Oreites, where *Thymus vulgaris* shrubs were planted, and the wetter conditions of Vavla, where *Lavandula angustifolia* shrubs were planted. Four different treatments were implemented in both experimental sites: the control (C), the attapulgite (A), the microbe (M), and the combination of attapulgite and microbes (AM). Measurements examining the growth and soil moisture of the plants were obtained to show the effect of *AGLEV® SI 200* and *Micosat F-Olivo*.

From comparing the shrub volume to Flower Beds in Vavla, it appeared that Flower Bed 1 had a significantly lower mean shrub volume than the other three Flower Beds (Figure 63). The only difference between Flower Beds was that 24 out of 28 plants were initially pruned in Flower Bed 1. It seems that pruning negatively affected the base shrub growth of *Lavandula angustifolia*. According to Crișan et al. 2023, plants extract nutrients from the soil to develop and grow. By pruning a plant, the nutrients drawn from the soil are removed, and the soil must replenish the lost nutrients, causing decreased rates of flowering and harvesting. This could potentially explain why the shrubs of Flower Bed 1 had significantly lower volume than other Flower Beds. Generally, pruning in combination with fertilization is beneficial, as it stimulates flowering and revitalizes *Lavandula angustifolia* plants (Crișan et al. 2023).

The combination of *AGLEV® SI 200* and *Micosat F-Olivo* significantly increased the base shrub volume of *Lavandula angustifolia* plants in Vavla (Figure 64, Figure 65). Additionally, the comparison between treatments and shrub biomass in Oreites showed that the AM treatment had the highest median compared to other treatments, although no statistical significance was observed (Figure 60, Figure 61). This increase may indicate that significant results could have been observed if the shrubs continued to survive and their biomass was calculated later. Watering during the dry season could have helped the shrubs survive and obtain these biomass measurements, thus prolonging the experiment. Zhao et al. 2018 found that rhizobacteria and attapulgite combination treatment showed optimal growth promotion on *Brassica napus*. Dai et al. 2019 found that attapulgite improved the microbial environment of the soil in corn roots. The potential of AM treatment must be further examined regarding its significance in future research.

Treatment M showed increased shrub volume with no significance towards the C treatment in Vavla (Figure64, Figure 065). In Figure 66, the M treatment showed an increase regarding the mean maximum height growth rate in Vavla, although there was no significance. These results indicate that the potential of the M treatment regarding plant growth should be further examined. Generally, all the microbial strains of Micosat F-Olivo have beneficial effects on plants. According to El-Komy et al. 2022, two native *Trichoderma* strains both separately and combined reduced the *Fusarium* root and stem rot in cucumber plants in Saudi Arabia, thus assisting their growth. These findings are promising for organic agriculture in semi-arid regions and show that native microbial strains are more effective because they are adapted to these conditions. Tarafdar, Rao 2001 used an AMF (Arbuscular Mycorrhizal Fungi) *Glomus* strain for cluster bean cultivation in arid soil. It was observed that AMF treatment alone or in combination with FYM (Farmyard Manure), R (Rhizobium), and both with FYM and R, showed a greater number of spores population per 100 grams of soil, with values of 263, 293, 271, 345, respectively. Those numbers were greater than the control (no treatment) with a value of 89 per 100 grams of soil or treatments that contained only nitrogen, phosphorus, FYM, R, and FYM with R, with values of 105, 138, 125, 96, and 105 spores per 100 grams of soil, respectively. The treatment implementation of AMF, FYM, and R combined resulted in higher cluster bean dry matter production and seed yield. *Pseudomonas* sp. and *Bacillus* sp. are plant growth-promoting bacteria that assist plant performance in salinity stress conditions. Furthermore, they both solubilize and offer zinc to plants when degraded, and they are proven to increase phosphorus efficiency in wheat by 18%–30% (Kumar et al. 2023). *Bacillus* sp. also has nitrogen fixation properties and can effectively restore cadmium and nickel-contaminated soils. *Streptomyces* sp. belongs to the *Actinomycetes* phylum and is reported to degrade polycyclic aromatic hydrocarbons, such as pyrene, phenanthrene, and naphthalene (Kumar et al. 2023).

Treatment A also showed increased shrub volume with no significance towards the C treatment in Vavla (Figure 64, Figure 65). In Oreites, the A treatment displayed the highest median soil moisture, but there was no significance regarding those results (Figure 62). Perhaps if the soil moisture measurements were obtained during the dry season, the results could have shown significant results. Yang et al. 2022b mention that attapulgite soil use in an optimal percentage of 3% increased the clay content of sandy soil by 53.7%, adding moisture to it. This suggests that attapulgite is beneficial for potential water use reduction and plant growth promotion in semiarid and arid regions.

The average soil moisture didn't show any significance between treatments in both sites (Figures 62, 68, and 69). This result was expected, as the surface soil moisture was measured and is only natural for the surface moisture to be homogenous throughout the experimental area. This measurement was conducted mainly to ensure that there is homogeneity in terms of surface soil moisture, so no biases would affect the experiment.

Table 12 shows the mean coverage of wild vegetation inside the experimental area in Vavla. Mainly species from Apiaceae, Fabaceae, and Poaceae family were observed. Future population count and analysis could show whether treatment affects the population of wild vegetation. According to the literature, the species found have promising soil and other properties. Horstmann et al. 2020 found that *Streptomyces* isolates from rhizospheres of *Fabaceae* plants positively affected the growth of soybean plants, revealing the potential of biofertilizer formulation. Thiviya et al. 2022 mention that the Apiaceae family plants are widely known for containing bioactive phytochemicals, and their extracts possess many properties, such as herbicidal, antibacterial, insecticidal, and antifungal, making them a great candidate for future applications in agriculture. Stapleton et al. 2010 found that members of the *Poaceae* family possessed soil disinfestation potential against soilborne pest organisms, reducing 1) the effect of tomato root galling by *M. incognita* in a percentage of 49-97%, and 2) the recovery of *S. rolfsii* and *P. ultimum* fungi in soil by 0–100%. Overall, the potential of wild vegetation is endless, and more research is needed to uncover more benefits and properties.

Conclusions

The AM treatment was effective compared to the C treatment for shrub growth in Vavla. In addition, it showed an increase in shrub biomass in Oreites compared to the rest treatments, but it wasn't significant. The A treatment showed an increased shrub volume in Vavla compared to the C treatment, but it wasn't significant. Furthermore, it displayed the highest median soil moisture in Oreites, although not significant. The M treatment showed increased mean maximum height growth rate and shrub volume in Vavla, compared to the C treatment, but those increases weren't significant. The potential use of *AGLEV® SI 200* and *Micosat F-Olivo* should be further examined for positive effects on dry soils. Their combination (AM treatment) must also be further examined, as it showed significant results in the present study. Lastly, the study of wild vegetation present in cultivation fields can lead to the potential uses of their bioactive compounds in agroecology as sustainable methods of agriculture.

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