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FOREWORD

Environmental changes, particularly accelerating climate warming and the increasing frequency of extreme weather events, are putting immense pressure on terrestrial and marine ecosystems. Current climate perturbation, primarily driven by the constant intensification of human polluted activities, have far exceeded the thresholds of natural variability. Human-driven pressures are reshaping the natural world at alarming rates, and among them, climate change stands out as one of the most pervasive and far-reaching forces. These anthropogenic disturbances are profoundly transforming ecological processes, altering biogeographical patterns, and compromising the integrity, productivity, adaptive capacity of ecosystems on a planetary scale, and biodiversity. Climate-induced disruptions influence primary productivity, nutrient cycling, food-web dynamics, and the capacity of ecosystems to provide essential services such as clean air, water, and food

Rising global temperatures, ocean acidification, changing precipitation patterns, and increasingly frequent extreme weather events are transforming habitats and disturbing intricate biological interactions. These cascading effects accelerate species loss across terrestrial, freshwater, and marine systems, with consequences that reach far beyond environmental boundaries.

The International Union for Conservation of Nature (IUCN) estimates that 35% of birds, 52% of amphibians, and 71% of coral reefs are particularly vulnerable to climate change. The Intergovernmental Panel on Climate Change (IPCC), in its Fifth Assessment Report, confirms that human-induced climate disruption is intensifying the pressures already weighing on ecosystems and species—pushing our planet toward what may become the sixth mass extinction.

This proceedings, themed “Biodiversity and Climate Change,” brings together recent research, case studies, and innovative methodologies that explore the multifaceted impacts of a changing climate on ecosystems worldwide.



Table of Contents

Enhanced prediction of marine eutrophication: a case study in the lagoon of Bizerte.....	9
Hajer BENDAYA, Raida KTARI, Yosr GHOZZI, Rim KTARI & Béchir BEJAOUJ	
Diversity and spatial distribution of benthic macrofauna in the Kuriat Islands (central Mediterranean).....	13
Nawfel Mosbahi & Malak Chaarana	
Polychaetes Diversity and Functional Trait Composition in Tunisian ports (central Mediterranean Sea)	18
Nourhene Rebai, Nawfel Mosbahi & Lassad Neifar	
Mapping stocks and population structure of the clam <i>Ruditapes decussatus</i> along the Zarzis coasts, Tunisia.....	22
Abdelkarim DERBALI & Aymen HADJ TAIEB	
Qualitative Approach to Assessing the Risk of Contamination of Bivalve <i>Ruditapes decussatus</i> by Toxic Microalgae along the Sfax Coast	26
Fatma Abdmouleh Keskes, Lamia Dammak Walha, Asma Hamza & Hedia Hili Attia	
Influence of priming treatments on germination, seedling growth and tolerance to salt stress of fenugreek (<i>Trigonella foenum-graecum</i> L.)	30
Hamida BARHOUMI, Sawsen HNICHIR, Hanen EL ABED, Mayssa BELHASSAN, Takwa MHIRI, Bassem KHEMAKHEM, Olfa ELLOUZE & Amine ELLEUCH	
A comparative study of essential oil composition and antioxidant properties of <i>Pelargonium graveolens</i> in coastal and agricultural settings.....	34
Imen LAHMAR, Nawfel MOSBAHI, Karima BELGHITH, Lyubov YOTOVA & Naceur EL AYEB	
Exploring the biochemical complexity of <i>Rosemary</i> hydrolat.....	38
Imen LAHMAR, Nawfel MOSBAHI & Lyubov YOTOVA	
Identification and agronomic application of a rhizobacteria from date palm and its effect on <i>Pisum sativum</i>. L under salt stress	42
Sawsen HNICHIR, Hamida BARHOUMI, Mayssa BELHASSAN, Olfa ELLOUZE, Amine ELLEUCH & Bassem KHMEKHEM	
Germination and seedling growth of fourteen durum wheat varieties in comparison with <i>Vivasio triticum</i> under different cultivation systems.....	46
Takwa MHIRI, Hanen EL ABED, Aroua AMMAR, Hamida BARHOUMI, Olfa ELLOUZE & Amine ELLEUCH	
Effect of seed priming on germination and seedling growth of <i>Lathyrus sativus</i> L. cultivars under salinity conditions	50
Hanen El Abed, Hamida Barhoumi, Sawsan Hnichir, Arwa Ammar, Bassem Khemakhem, Alma Balestrazzi, Olfa Ellouze & Amine Elleuch	



Enhanced prediction of marine eutrophication: a case study in the lagoon of Bizerte

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Abstract

Marine pollution has emerged as a pressing global issue, posing significant threats to human health and marine life. One of the most concerning consequences is eutrophication, with coastal areas being the most impacted due to anthropogenic activities. This research aims to use advanced machine learning models to predict Chlorophyll-a concentration, a crucial input for the TRIX index and an indicator of eutrophication itself. The study seeks to gain insights into ecosystem dynamics and contribute to more effective assessments of eutrophication levels in the Bizerte Lagoon, Tunisia, utilizing physical-chemical data that were collected during the year 2012. In this regard, two approaches were followed. In the first one, we employ regression models including RF, SVR, and XGBoost. In the second one, we take into consideration the time aspect of the data and apply time series models such as SARIMA and ARIMA. The results indicate that the SARIMA model yielded superior performance with an R2* of 0.63, an MSE of 1.09, and an MAE of 0.82 compared to regression models.

Keywords: Machine learning, Marine pollution, Eutrophication, Time series models, Chlorophyll-a, Trophic index (TRIX)

1. Introduction

Marine pollution has become a global concern that poses serious threats to the environment, marine life, and human health. Many factors contribute to the increase in marine pollution, including anthropogenic activities (e.g., industry, sewage discharge, aquaculture, agriculture), oil spills, increased nutrients, and plastic waste; these contaminants lead to severe changes in the physical, chemical, and biological characteristics of marine environments [1].

In response to this, many efforts are being made to address this problem to reduce pollution levels in the Mediterranean Sea, such as from the United Nations Environmental Programme UNEP/MAP-Barcelona Convention Regional Plan on

Marine Litter Management in the Mediterranean.

Despite these efforts, pollution in semi-enclosed seas such as the Mediterranean Sea remains a significant challenge, due to the high population density that badly affects coastal water bodies' environmental status. Overall, human activities have led to an increase in pollution in the basin, resulting in a polluted environment where one of the significant threats is eutrophication [2]. Eutrophication is a term used to describe the negative consequences of human activities that introduce excess nutrients into aquatic environments leading to the loss of ecosystem integrity and biodiversity [3].

However, there is a need to provide an adequate assessment of the present state

of the marine environment to mitigate the impact of pollution. Traditionally, the assessment of aquatic ecosystem's pollution and eutrophication has relied on various indices and statistical methodologies, such as the Trophic Index (TRIX) [4], which is a widely used metric for assessing the trophic state of marine waters based on several key water quality parameters.

While the TRIX index is useful for assessing eutrophication in aquatic ecosystems, its application encounters limitations, such as managing large and complex datasets, addressing missing values, and adapting to the dynamic nature of marine environments. These issues make it difficult to obtain accurate, real-time insights using the TRIX index alone.

Consequently, there is a need for the integration of advanced models, such as ML approaches, which offer powerful tools for analyzing complex, large datasets and address the complexity and multifaceted nature of marine environments. In this regard, our research aims to develop an advanced ML-based tool, including regression models and time series models, to overcome the limitations of traditional methods and offer a reliable, real-time solution for monitoring marine water quality.

2. Materials and Methods

2.1. Study Area and Dataset

Environmental data were collected biweekly during 2012 in Bizerte Lagoon, Tunisia. A total of 92 samples were collected from a single sampling station located at the center of the lagoon. The dataset encompasses seven water quality parameters, including: temperature (T) and salinity (S), nitrite (NO₂), nitrate (NO₃), ammonium (NH₄), phosphate (PO₄), dissolved oxygen (DO), and chlorophyll-a (Chl-a).

2.2. Methodology

To achieve accurate predictions of chlorophyll-a (Chl-a) concentrations, we employed two complementary methodological approaches:

Regression-Based Prediction Models

This study builds on previous work with the Random Forest (RF) model [5], by integrating additional machine learning models to improve predictive accuracy. RF was selected for its robustness in handling nonlinear relationships, high-dimensional data, and its ability to reduce overfitting by averaging decision trees [6][7]. Support Vector Regression (SVR) was included for its strong generalization ability, particularly with small datasets, and its resistance to outliers [8]. Extreme Gradient Boosting (XGBoost) was chosen for its efficiency in managing large datasets and capturing intricate patterns through boosting [9].

Hyperparameter tuning was conducted to optimize each model's performance and enhance predictive accuracy.

Time-Series Forecasting Models

To incorporate the temporal dynamics of eutrophication, time-series forecasting models were applied. The Autoregressive Integrated Moving Average (ARIMA) model was used to predict Chl-a concentration trends and capture temporal dependencies (George Box.2013; Paul Newbold.1983). Additionally, Seasonal ARIMA (SARIMA), an extension of ARIMA, was utilized to account for seasonal variations in Chl-a levels [10]. Hyperparameter tuning was performed to determine the optimal autoregressive (p), differencing (d), and moving average (q) components for both models, ensuring accurate and reliable forecasting.

3. Results and Discussion

This section presents the predictive modeling outcomes and the resulting classification of water quality.

3.1. Model Results and Interpretation

Chlorophyll-a concentrations were predicted using both regression and time-series modeling approaches, based on seven water quality parameters. Among the regression models, the Random Forest algorithm yielded the best performance with an R^2 of 0.56, an MSE of 1.49, and an MAE of 0.96. In contrast, Support Vector Regression (SVR) and XGBoost models performed less effectively, with lower R^2 values of 0.17 and 0.31, respectively.

Notably, the time-series models demonstrated superior predictive power. The SARIMA model achieved the highest accuracy, with an R^2 of 0.63, an MSE of 1.09, and an MAE of 0.82, successfully capturing temporal dependencies and seasonal trends within the data.

3.2. TRIX Index Calculation

The predicted chlorophyll-a (Chl-a) values, obtained from the SARIMA model, were used to calculate the TRIX index, a key indicator of eutrophication. The results showed TRIX values ranging from 3.51 to 4.53, indicating a medium eutrophication level. While these values differ slightly from previously observed TRIX values (5.18 to 6.12), the predictive approach remains robust and provides valuable insights into water quality assessment. Despite the challenge of a small dataset (with only 92 observations), the results demonstrate the effectiveness of machine learning techniques in classifying water quality. However, further improvements, particularly using a larger dataset, could enhance the model's reliability and generalizability.

4. Discussion and Conclusion

This study expanded on previous work [5], by using the same dataset to predict chlorophyll-a (Chl-a) concentration in Bizerte Lagoon. While hyperparameter tuning improved the Random Forest (RF) model slightly ($R^2 = 0.56$), regression models like Support Vector Regression (SVR) and Extreme Gradient Boosting (XGBoost) struggled to account for temporal dynamics, reducing predictive accuracy. To address this, time-series models were employed, with SARIMA outperforming all regression models, demonstrating the importance of time-aware methods for Chl-a prediction. Using predicted Chl-a values, we calculated the TRIX index to assess the lagoon's trophic status and water quality effectively.

This research highlights the value of combining machine learning and time-series approaches for environmental data, noting the limitations of traditional regression methods in capturing temporal patterns. Despite promising results from a limited dataset (of only 92 observations), future work should focus on larger datasets and hybrid modeling techniques. This study contributes to marine ecology by offering a data-driven approach for eutrophication assessment, aiding in early detection and mitigation of aquatic ecosystem degradation.

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Diversity and spatial distribution of benthic macrofauna in the Kuriat Islands (central Mediterranean)

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Abstract

The Kuriat Islands, off the eastern coast of Tunisia in the central Mediterranean, are of international biodiversity interest. Their fragile ecosystems face biodiversity loss from multiple anthropogenic pressures. To assess benthic structure, functional diversity, and ecological quality, macrofauna were studied at 20 stations during two summer campaigns in 2023. A total of 4,289 individuals representing 76 taxa across six groups were recorded, dominated by mollusks (79%) and polychaetes. Multivariate analyses (Cluster, n-MDS, PCA) identified two benthic assemblages linked to habitat type and sediment characteristics. Biotic indices indicate an overall good to high Ecological Quality Status. This study suggests initiating a long-term monitoring program to improve our understanding of the temporal changes in this ecosystem and to recommend the necessary conservation measures in this area of high-value natural heritage.

Keywords: Kuriat islands, benthic communities, spatial distribution, environmental factors, ecological status.

1. Introduction

Coastal areas are generally highly productive and ecologically important [1] [2]. However, coastal ecosystems often form a mosaic of interlinked habitats that should not be studied in isolation. Each habitat has its own characteristics and hosts distinct benthic assemblages. Nevertheless, coastal habitats are threatened by anthropogenic stressors, including coastal development and habitat degradation [3] [4].

In the central Mediterranean Sea, Kuriat Islands (Gulf of Hammamet, Tunisia) represent a site of both international and national interest due to their marine and ornithological diversity. This archipelago represents the most important nesting zone of the sea turtle *Caretta caretta* in southern Mediterranean [5].

Likewise, the marine ecosystems of the Kuriat Islands are subject to the loss and degradation of biodiversity caused by many

natural and anthropogenic pressures such as overfishing, tourism activities, pollution, climate change, and the proliferation of the invasive species [6] [7].

A first ecological survey of benthic communities was conducted in summer 2023 to assess ecosystem structure, functioning, and health. Benthic organisms, living at the sediment-water interface, are sensitive indicators of environmental changes [8]. Their sedentary nature prevents escape from stress, and their long lifespans record both short- and long-term disturbances, making them suitable for low-frequency ecological monitoring [8] [9] [10]. Thus, the present report is the first study of macrozoobenthic communities in the Kuriat Islands, Monastir Bay. It aims to describe benthic structural diversity using taxonomic and ecological approaches and to assess the ecological status of the ecosystem, identifying key environmental factors shaping community distribution.

2. Material and methods

2.1 Study area

The Kuriat Islands are situated in the centre of Tunisia (Figure 1) (35° 48' 05" N, 11° 02' 05" E) and lie 18 km from the coast of Monastir. They consist of two small islands: the Small Kuriat (Kuria Sgira) which is ca. 0.7 km² and the Great Kuriat (Kuria Kbira) which is ca. 2.7 km² in area. Small Kuriat has a total of 1500 m of sandy beach situated in the north-eastern and eastern parts of the island whereas the rest of the coastline is rocky or marshy. Almost one-third of the Great Kuriat shoreline is rocky and large deposits of seagrass, *Posidonia oceanica*, and detritus further restrict the accessible nesting sites particularly on the south and the south-western beaches. The principal nesting beach lies on the western and southern coast and is almost 3000 m in length

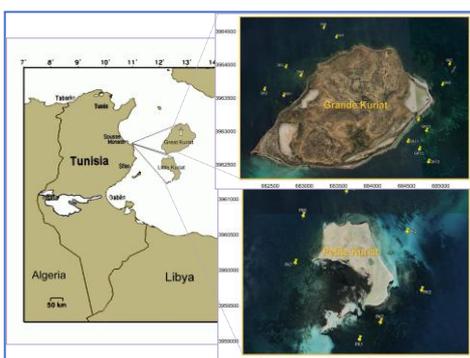


Figure 1: Map of the study area indicating the studied sites in Kuriat Islands.

2.2 Sampling design and sample treatments

Sampling was conducted from June to July 2023 at 20 stations (Fig. 1) by scuba diving using a metallic corer (0.0225 m²). At each station, five replicates were taken: four for biological analyses (0.09 m²) and one for sediment analyses (sediment type, organic matter, and chemical contamination). Biological samples were sieved (0.5 mm), preserved in 5% formaldehyde, stained with Rose Bengal, sorted, identified to the lowest taxonomic level, and counted. Physicochemical parameters were measured during sampling. The top 3 cm

sediment was analyzed for median grain size and organic matter (LOI at 450 °C for 4 h).

2.3 Data Analysis

Benthic community structure was assessed using diversity indices (S, H', J) and biotic indices (AMBI, BENTIX, BO2A) with PRIMER®-v6. Spatial patterns were analysed through cluster analysis, n-MDS, and PCA to link macrofauna distribution with environmental parameters.

3. Results

3.1 Benthic diversity

The taxonomic identification of collected invertebrates yielded 4,289 individuals and 76 macrobenthic taxa from six zoological taxa, unevenly distributed among stations, with molluscs dominating (79%). The most dominant species are *Tricolia speciosa*, *Smaragdia viridis*, and *Calliostoma zizyphinum*. Other groups, including annelids, crustaceans, and echinoderms, show lower diversity. Mollusc diversity is strongly linked to habitat type and *Posidonia oceanica* beds.

Species richness analysis in the Kuriat Islands shows significant spatial variation (ANOVA; $F = 24.1$; $p < 0.01$). In Great Kuriat, station GK8 recorded the highest richness with 27 species, dominated by *Tricolia speciosa* and *smaragdia viridis*. In Small Kuriat, station PK2 showed the highest value with 30 species, mainly represented by herbivorous mollusks such as *Tricolia speciosa*, *Calliostoma zizyphinum*, and *Gibbula ardens*.

Equally, benthic macrofauna abundance varies significantly between stations (ANOVA; $F = 114.2$; $p < 0.05$). Station GK1 shows a high abundance (494 ind/m²), mainly composed of mollusks, dominated by the bivalves *Loripes orbiculatus* (190 ind/m²) and *Truncatella subcylindrica* (115 ind/m²).

Shannon index (H') variation across the 20 surveyed stations around the Kuriat Islands revealed that the lowest values were recorded at station GK7 (1.636 bits.ind⁻¹), while the highest value was observed at station GK4 (2.532 bits.ind⁻¹).

Pielou evenness index showed significant variation (ANOVA; $F = 8.21$; $p < 0.05$). The lowest values were recorded at stations GK1 (0.59) and PK2 (0.54), whereas the highest values were observed at stations PK5 (0.89) and GK11 (0.98). The H' and J' indices are influenced by sample size and habitat type. Their values are generally low in transitional waters, such as lagoons, islands or estuaries, even when they are not disturbed.

3.2. Benthic assemblage's distribution

The study of the spatial distribution of benthic communities of the Kuriat Islands revealed two distinct benthic assemblages (Figure 2), each with a unique faunal composition. The first assemblage includes stations characterized by sandy bottoms. This group is dominated by the following mollusc species: *Tricolia speciosa*, *Gibbula ardens*, *Cerastoderma glaucum*, *Calliostoma zizyphinum*, *Loripes orbiculatus*, *Mesalia mesal*, and *Turritella communis*. The second assemblage consists of stations with rocky bottoms, where the organic matter content is relatively low. This group primarily hosts mollusc species such as *Tritia varicosa*, *Tricolia speciosa*, *Gibbula ardens*, *Cerastoderma glaucum*, and *Calliostoma zizyphinum*. The distribution of benthic assemblages seems to be entirely governed by the physico-chemical and edaphic characteristics of the environment.

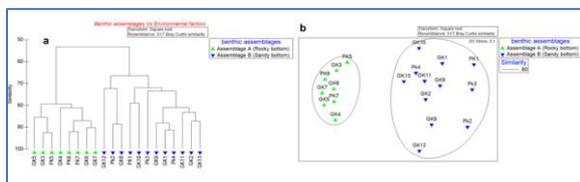


Figure 2: Cluster (a) and MDS (b) of macrobenthic communities at Kuriat Islands

3.3. Ecological Status of the Kuriat Islands

During this work, three biotic indices AMBI, M-AMBI, and the BENTIX index based on the pollution sensitivity of benthic macrofauna species, were used. These indices classify the sampled stations around the Kuriat Islands as being in a good ecological status.

4. Discussion

This study provides the first assessment of benthic communities and the ecological status of the Kuriat Islands. Seventy-six invertebrate species from six groups were recorded, with molluscs most abundant, followed by annelids (11%) and crustaceans (5%). While the faunal composition resembles other Mediterranean islands, biodiversity is lower than in the Kneiss (106 sp) and Kerkennah (183 sp) Islands, but higher than in Djerba (13 species) and Boughrara Lagoon (24 species). Differences in biodiversity likely result from variations in sampling effort, methodology, timing, and environmental or anthropogenic conditions. The spatial distribution of benthic communities at the Kuriat Islands revealed two main assemblages based on sediment type: one in sandy areas dominated by species like *Tricolia speciosa* and *Gibbula ardens*, and another in rocky areas with low organic matter, also dominated by similar mollusc species. The distribution of these assemblages is influenced by factors such as sediment characteristics, organic matter content, and other environmental conditions (Figure 3). Numerous ecological studies have shown that sediment characteristics, including sediment texture [8], organic matter content, substrate type [11] [12], water physico-chemical factors (e.g., salinity, dissolved oxygen, and pH), hydrodynamic factors, and food availability [13] are the main environmental factors controlling the distribution and spatial patterns of benthic communities in marine ecosystems. The ecological status of the

Kuriat Islands was assessed through the application of three biotic indices based on the pollution sensitivity of benthic macrofauna species. These indices classified the sampled marine ecosystem of the Kuriat Islands as being in good ecological status. The AMBI and BENTIX indices were used together because the effectiveness of the former can be reduced when the number of taxa and/or individuals in a station is low, whereas the latter is independent of substrate type and sample size [8].

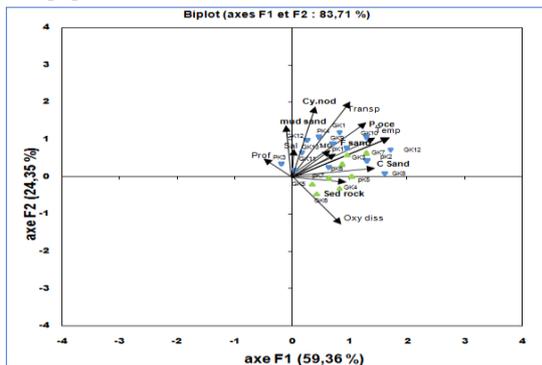


Figure 3: (PCA) (axis 1 and 2) of biological environmental variables at the 20 stations.

5. Conclusion

In conclusion, the Kuriat Islands' marine ecosystem is in good ecological status. The study highlights the importance of sediment type and environmental conditions in shaping benthic community distribution. It emphasizes the need for ongoing monitoring to better understand biodiversity dynamics and the impact of environmental changes. For long-term sustainability, it is crucial to implement conservation measures and collaborate with local stakeholders to protect the ecosystem from future pressures and ensure its resilience.

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Polychaetes Diversity and Functional Trait Composition in Tunisian ports (central Mediterranean Sea)

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ABSTRACT

Ports are among the most disturbed coastal ecosystems due to intensive anthropogenic pressures. The sediments in these areas are moderately polluted with organic matter, microplastics and heavy metals. Therefore, the value of the softbottom polychaete community as a bioindicator for these unique conditions was analysed. The aim of this study is to examine the spatial distribution, abundance, and assemblage structure of polychaete communities in Tunisian ports. This study was conducted in 12 different ports (marina, fishing and industrial) during 2019 and 2021. A total of 10,026 individuals and 112 species of polychaetes belonging to 34 families were collected. Among them, 22 exotic polychaetes taxa were recorded for the first time in Tunisian waters. Nereididae, Glyceridae, Eunicidae, Maldanidae, Terebellidae and Serpulidae are the dominant families (in number of species and abundance). The industrial harbours yield higher richness and abundance of non-indigenous species than the fishing harbours and marinas. Analysis of the trophic structure revealed that the majority of stations are strongly dominated by deposit feeders (51.2%), followed by carnivores (34.8%) and suspension feeders (14%). Two polychaetes assemblages were identified by the multivariate analyses (Cluster and n-MDS analysis), distinguished according to their environmental features (sediment type, organic matter, heavy metal pollution). The present study provides a data baseline for the implementation of environmental policies and management plans in the future.

Keywords : Ports, Polychaetes assemblage, sediment contamination, distribution.

1. INTRODUCTION

Ports face a wide range of environmental challenges, including the discharge of sewage waste and ballast water, the release of petroleum and its derivatives, and the use of antifouling paints. These impacts are often intensified by dredging activities, which can affect both the dredged areas and the disposal sites [1]. Harbours are particularly vulnerable due to their limited water exchange with the open sea, making them hotspots for the accumulation of various pollutants, particularly heavy metals, organic matter, and microplastics, which tend to accumulate in port sediments [2]. Among the benthic organisms

inhabiting these soft-bottom sediments, polychaetes are particularly prominent and are considered key representatives of these communities. Due to their sensitivity to environmental changes and their close association with sediment conditions, polychaetes are frequently employed as bioindicators to assess the ecological status of marine environments [3]. In this context, the present study aims to describe the diversity and structure of polychaetes communities and (2) to assess their ecological quality status (EQs) linked to different levels of anthropogenic stress and biological invasions, so as to determine the relationship between these polluted zones and the establishment of non-indigenous species.

2. MATERIAL AND METHODS

Sampling was conducted across 12 different Tunisian ports, representing marina, fishing, and industrial activities (Fig. 1A, B). The fieldwork took place over two years, from 2019 to 2021. At each port, three stations were selected to cover the entire harbour basin. Sampling was carried out using a motorized boat to access all stations. At each station, eight replicate samples were collected using a Van Veen grab with a sampling area of 0.04 m². of these, four replicates were used for biological analysis, and four additional replicates were taken for sediment analysis.

Polychaetes samples were sieved on a 1 mm mesh and the retained material was preserved in 70% ethanol. The exact location and depth of each station were recorded using a portable GPS (WGS84) and a depth sounder. In the laboratory, the benthic macrofauna was carefully sorted, identified where possible to the species level under a stereomicroscope, and counted. Taxonomic nomenclature was updated according to the World Register of Marine Species (WoRMS, last accessed in May 2024).



Figure 1: Location of Sampling ports along the Tunisian coasts A: Gulf of Gabes, B: Gulf of Hammamet.

3. RESULTS

3.1. Main faunal characteristics

A total of 112 polychaete species were recorded, comprising 10,026 individuals and representing 34 families. Among these, the most dominant families in terms of both species richness and abundance were

Nereididae, Maldanidae, Eunicidae, and Capitellidae (Fig. 2). In total, 22 non-indigenous polychaete species were identified, highlighting the high diversity and ecological significance of this group within the studied ports.

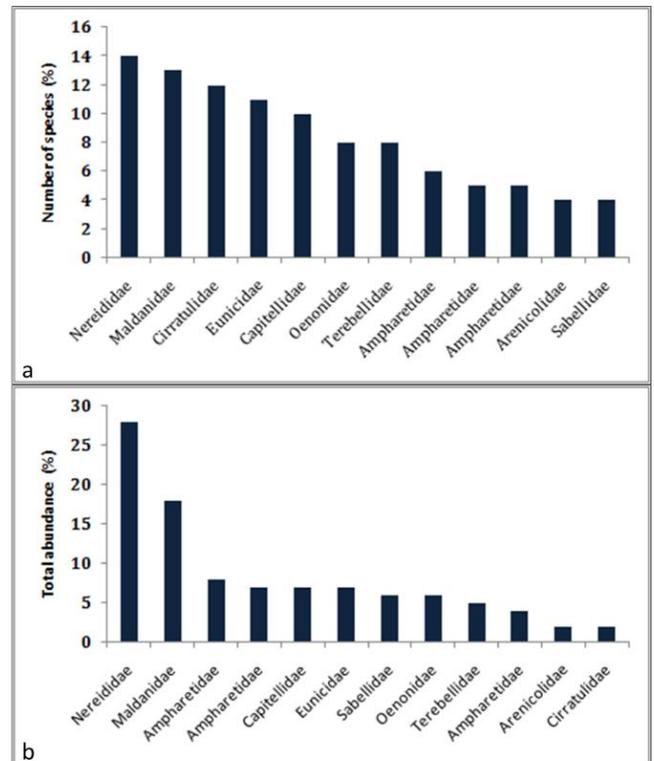


Figure 2: Dominance of polychaetes families in Tunisia ports (a: species number; b: abundance).

3.2. Spatial patterns of the Polychaete fauna in the Tunisian ports

Analysis of the trophic structure across all stations revealed a clear dominance of deposit feeders, comprising 51.2% of the polychaete community, followed by carnivores (34.8%) and suspension feeders (14%) (Fig. 3).

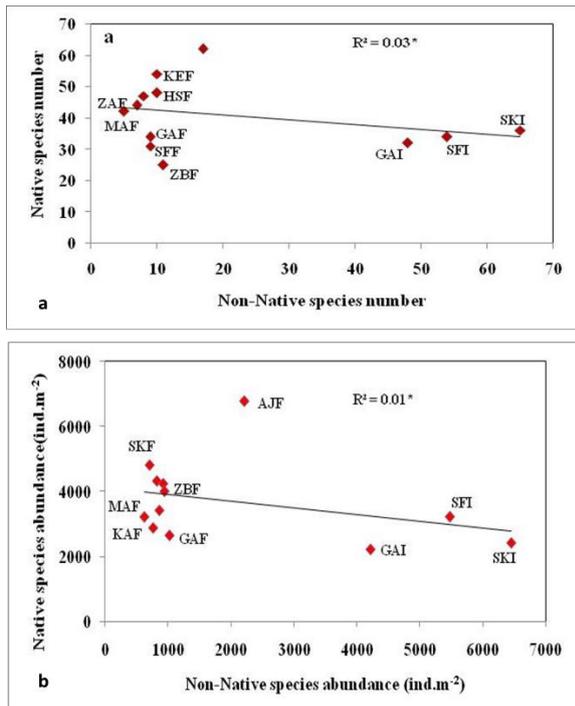


Figure 3: Scatter plots relationship between Native and Non-Native Species in the Tunisian ports.

3.3. Polychaete assemblages in the Tunisian ports

The dendrogram and MDS ordination (Fig. 4) revealed two distinct polychaete assemblages, differentiated based on environmental characteristics such as sediment type, organic matter content, and heavy metal pollution. The analysis also highlighted a clear separation between the two main groups, corresponding to commercial harbours and fishing harbours. Polychaete diversity was strongly influenced by port size and activity, indicating the significant role of anthropogenic pressure in shaping community structure.

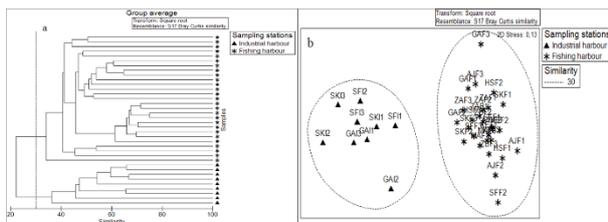


Figure 4: Dendrogram (a) and MDS ordination (b) of bray-Curtis similarities from abundance data.

4. DISCUSSION

Studying the impacts of anthropogenic disturbances and heavy metal pollution on harbour ecosystems is essential for developing effective environmental management strategies and protecting marine biodiversity [4]. Elevated concentrations of heavy metals in coastal environments can significantly affect the structure, diversity, and abundance of macrobenthic communities, ultimately disrupting the ecological functioning of harbour ecosystems [5]. This study provides an ecological risk assessment of heavy metal contamination and evaluates the environmental quality of central Tunisian harbours located in the central Mediterranean Sea. In terms of both species' richness and abundance, the commercial harbour was dominated by polychaetes. This predominance is consistent with observations in other harbour ecosystems, such as those in the Gulf of Gabes (Tunisia), various Algerian harbours, and Valdez Harbour in Alaska [6, 7]. Such dominance reflects the adaptability of polychaetes to disturbed and polluted environments, where they often thrive due to their ecological tolerance and diverse feeding strategies.

The ALEX (Alien Biotic Index) showed a significant positive correlation with several biological parameters (biological invasion) and environmental variables, including port surface area, temperature, salinity, organic matter content, mud percentage, and levels of chemical contaminants. Industrial harbours, in particular, emerged as hotspots for polychaete communities, especially for opportunistic species belonging to the Capitellidae and Nereididae families (Fig. 5). This highlights the strong influence of anthropogenic pressures and environmental degradation on the proliferation of tolerant and non-native taxa in these ecosystems. The effect of the frequency of marine traffic and environmental disturbances is highlighted by the high number of NIS recorded in the

industrial Tunisian ports. In general, elevated levels of metal contamination in ports ecosystems are closely linked to various anthropogenic activities. Over the past few decades, factors such as intense maritime traffic, cargo handling, ship maintenance, dredging operations, and the discharge of industrial and urban effluents have significantly contributed to the ecological deterioration of coastal marine environments.

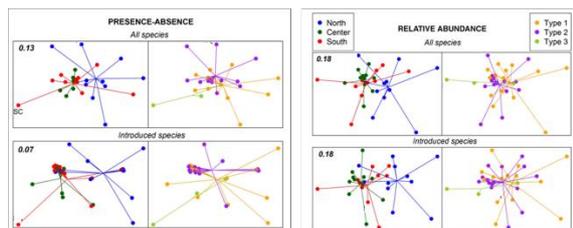


Figure 5: Non-MDS plots of the studied ports obtained from the relative abundance and the presence–absence data, for the whole dataset and for the introduced species.

5. CONCLUSION

This study highlights the significant variability in polychaete community structure across different port types along the Tunisian coasts. Sediments within commercial and fishing ports exhibited notably higher polychaete species richness and diversity compared to marinas, likely reflecting differences in environmental conditions and anthropogenic pressures. These findings underscore the ecological value of benthic communities particularly polychaetes as sensitive indicators of environmental quality in port ecosystems.

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Mapping stocks and population structure of the clam *Ruditapes decussatus* along the Zarzis coasts, Tunisia

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Abstract

The grooved carpet shell *Ruditapes decussatus* is one of the most prevalent bivalve species along the Tunisian coasts. Its exploitation status represents a major concern for the artisanal fishery in Tunisia. The status of this species remains poorly understood, and there is a lack of information regarding its abundance. This study aimed to investigate the stock assessment and population structure of *R. decussatus* in the coastal area of Zarzis (South of Tunisia). The results revealed that this bivalve had significant fluctuation in density depending on the location ranging from 0 to 10 inds. m⁻², while biomass values varied from 0 to 144 g. m⁻². The total stock amounted to 89.4 tons, with abundance levels exceeding 12.6 million individuals. The individuals varied in size from 4 to 56 mm. If managed properly, this clam is expected to meet the domestic market demand through artisanal fishery.

Keywords: Bivalve, *Ruditapes decussatus*, stock levels, distribution, size structure, South of Tunisia.

1. Introduction

The grooved carpet shell, *Ruditapes decussatus* (Linnaeus, 1758), is a commercially valuable bivalve mollusk that plays a significant economic role. It is exclusively harvested in the Gulf of Gabes. It can be found in sandy and muddy-sand sediments in bays, estuaries coastal lagoons and other sheltered environments.

The Tunisian authorities has sustained to maintain this sector by establishing a relatively comprehensive institutional and regulatory framework that complies with international standards, overseeing and regularly monitoring the harvest season, and launching several development projects.

Based on the available published literature, extensive works have focused on various aspects of the reproductive biology of *R. decussatus*, as well as ecotoxicological studies and pollution. [1,2,3]. Nevertheless, the available information on stock levels and distribution of *R. decussatus* is inadequate. What is known about this species is limited to the contributions focused on the coastal

areas of the Sfax and Gabes regions [4,5]. The literature review clearly indicates the lack of studies although the knowledge of stock assessment is considered essential for population dynamics, management and conservation of marine bivalves. The overall goal of the present study is to give new data about the status of *R. decussatus* along the Zarzis coasts depending upon the stock size, geographical distribution and population structure.

2. Materials and methods

The field sampling was conducted over a one-year period (2018) along the Zarzis coasts (Fig. 1). Transects were systematically conducted in the sampling area during low tide. Samples were collected every 50 meters along the transect lines, ranging from the highest to the lowest tide. Along transects, 4 to 10 replicates were collected in quadrats (0.25 m²) using a shovel. Large specimens were gathered by hand, while smaller ones were collected using a sieve with a 2 mm mesh size. In the laboratory, shell length (SL, mm, maximum

anteroposterior distance considered as reference length), shell height (SH, mm, maximum distance from hinge to ventral margin), shell width (SW, mm, maximum distance between the closed shell valves), were measured with a digital caliper to the nearest 0.01 mm. Additionally, the samples were weighed on a toploading digital balance (precision of 0.01 g) to determine the total fresh weight (TW).

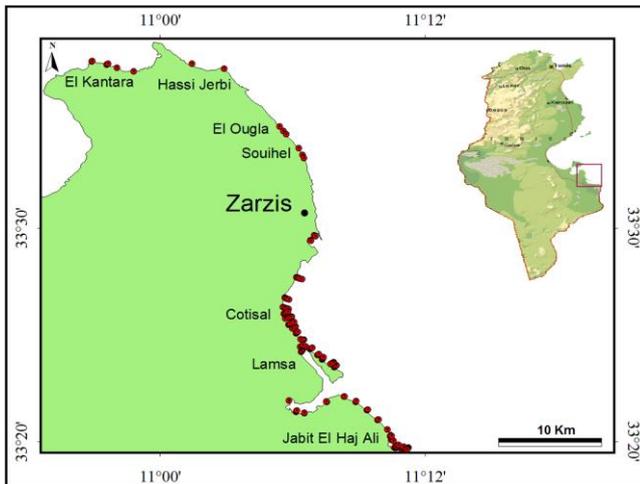


Figure 1: Geographic position of sampling transects in the littoral zone of Zarzis (South of Tunisia).

The obtained dataset were pooled to calculate mean density (inds. m^{-2}) and biomass (g. m^{-2}). Spatial distributions were mapped using ArcGIS 10.8, and stocks were assessed according to the following equation [6]: $B_i = N_i \times A_i/a_i \times 1/X_i$. Where

- B_i represents the total biomass of clams;
- N_i : mean abundance of all quadrats samples in each site;
- A_i : the whole study area;
- a_i : the quadrat swept area;
- X_i : the proportion retained.

For statistical analysis, data were tested for homogeneity of variance and normality using Leven's and Kolmogorov–Smirnov tests, respectively. The significance level used for the tests was $p < 0.05$.

3. Results and discussion

The clam *R. decussatus* was observed across the entire sampling area and in a variety of substrates. This includes muddy-sand substrate covered in some areas by the marine seagrass *Cymodocea nodosa* or *Zostera noltii* or by mixed vegetation consisting of these two seagrasses. In some localities, samples of *R. decussatus* were also found loose on the sandy bottom. Overall, 45 transects were conducted, ranging from the highest to the lowest tide, resulting in a total of 266 samples.

The total stock was estimated at 89.4 tons (total fresh weight), with an average biomass around 5.6 g/ m^2 and a total abundance reaching 12.6 million individuals. The population was limited to mean densities of 0.79 inds. m^{-2} . On the whole, there were significant variations in stock levels within the sampling area. The size frequency distribution was analyzed by combining data from the study area hosting the clam *R. decussatus*. Overall, there were significant fluctuations in the distribution of clams across the sampling areas, with a densities ranging from 0 to 10 inds. m^{-2} and biomasses from 0 to 144 g m^{-2} (Figs. 2-3). Densities of clams did not follow a normal distribution (Kolmogorov–Smirnov test, $p < 0.05$) and were not homogeneous (Leven's test, $p < 0.05$).

Sizes of specimens varied between 4 and 56 mm SL. The mean shell size (SL) was 26.45 mm and almost the entire population was assigned into size classes ranging from 19 to 45 mm, which represented 68% of all samples. Natural populations of *R. decussatus* exhibit significant spatial variability within and between sampling areas, as well as across different geographic regions characterized by diverse bottom characteristics, vegetation cover and physicochemical factors [4,5]. The major fundamental factors structuring bivalve populations include soft bottoms and climate change [7].

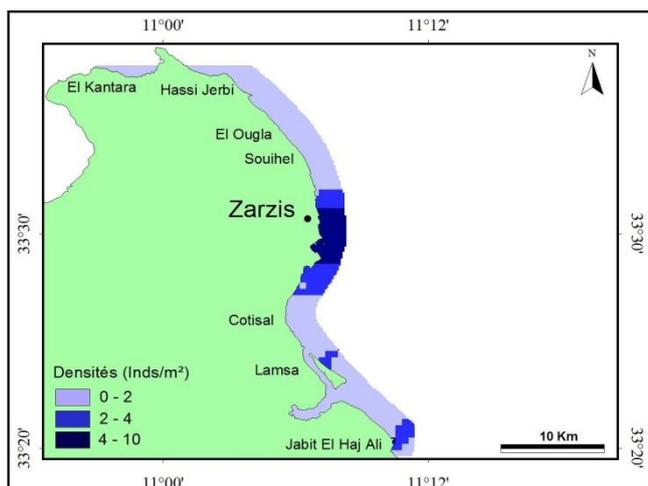


Figure 2: Spatial distribution of *Ruditapes decussatus* densities along the Zarzis coasts (South of Tunisia).

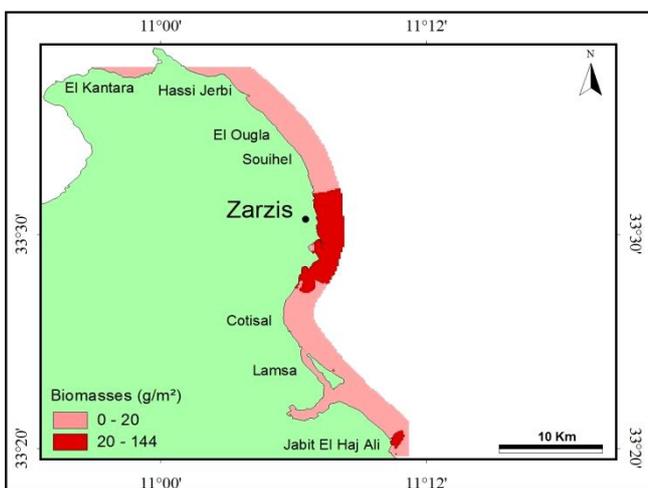


Figure 4: Spatial distribution of biomass of *Ruditapes decussatus* along the Zarzis coasts (South of Tunisia).

In the Gulf of Gabes, the natural populations of *R. decussatus* have been significantly overexploited for the past two decades. The fishing mortality (F) of *R. decussatus* ($F = 1.02 \text{ yr}^{-1}$) in the Gulf of Gabes was higher than that reported for the natural mortality ($M = 0.90 \text{ yr}^{-1}$) [8]. Moreover, the high exploitation rate ($E = 0.51$) indicates serious overexploitation of the stock. Indeed, previous studies suggested that harvesting activity of the clam *R. decussatus* at high tide creates strong disturbances and has a negative effect on the clam populations [9]. The depletion of stocks of *R. decussatus* can be due also to direct and indirect mortality

(e.g. destruction of tubes, exposure to predators, and loss of specimens from the unstable sediments via water currents) [10,11]. According to [12], the mortality rates in *R. decussatus*, especially in natural beds may results from a complex synergy of biotic and abiotic factors.

In conclusion, the present study provides an informative overview of the status of *R. decussatus* along the Zarzis coasts. Regarding the sustainable commercial exploitation of this resource, a guideline to prevent recruitment overfishing is imperative. Another guideline includes imposing closed seasons during the months of peak spawning activity. These guidelines can restore the stock to a sustainable status. Besides, the harvest effort should be under surveillance in order to determine adequate fishing technologies for a sustainable exploitation of clams. The adoption and implementation of rules to avoid grass beds and limit the size of clams or catch levels will be required to regulate the clam *R. decussatus* ranching and protect this exploitable resource. Further studies are required to identify the technological conditions necessary for setting up profitable exploitation and long term the possibility to farm this resource.

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Qualitative Approach to Assessing the Risk of Contamination of Bivalve *Ruditapes decussatus* by Toxic Microalgae along the Sfax Coast

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Abstract

In the Gulf of Gabes the bivalve mollusk *Ruditapes decussatus* is subject to significant collection activities and holds considerable commercial value. As filter-feeding animals, these mollusks are exposed to harmful algal blooms (HABs), which pose a potential risk. The consequences of contamination of bivalve mollusks by HABs can affect both human and animal health and has implications for sustainable development. Therefore, assessing the risk associated with HABs exposure is crucial to safeguarding both public health and the economy. The framework was used to analyze a qualitative assessment of the risk aims the health risks associated with shellfish exploitation along the Tunisian coast, with the goal of developing early warning systems and minimizing the inherent risk of contamination. To assess the risk posed by toxic microalgae, we analyzed 10 years (2011-2021) of data from a toxic phytoplankton monitoring program conducted at four stations along the Sfax coast (S2, S4, S5, and S6). Over this period, *Karenia selliformis* and *Prorocentrum lima* were responsible for most alert situations. The risk assessment examined emission levels, exposition factors, and potential consequences by station and season. The results indicate that contamination risk for *Ruditapes decussatus* by *Prorocentrum lima* and *Karenia selliformis* ranged from very low (VL) to low (L).

Keywords: risk assessment; *Prorocentrum lima*, *Karenia selliformis*; Sfax Coast

1. Introduction

Since the 1990's, risk assessment (RA) has become a useful tool in animal health. RA is a crucial part of the risk analysis process, alongside hazard identification, risk management, and risk communication. It focuses on systematically collecting and evaluating data and evidence about a potential adverse event to provide a solid foundation for decision-making and implementing the most effective risk mitigation strategies [1]. Risk assessments can also serve as decision-support tools, helping decision-makers identify and implement the most effective control strategies and risk mitigation measures during an animal disease outbreak. The World Organization for Animal Health [2] RA framework is based on a model that

distinguishes between emission, exposition and consequence assessments. RA can be carried out using two general approaches, termed qualitative and quantitative. Qualitative RA uses non-numerical terms to communicate or describe levels of risk, such as high, medium, low, or negligible [3]. This paper presents the method used within the collegial scientific expertise of the French food safety agency (AFSSA, following and adapting the work of [4].

In Gabes gulf, the bivalve "*Ruditapes decussatus*" is the subject of significant harvesting activity and high commercial interest. Its fishing is conditioned by quality standards and by health status. However, the coast of Gabes gulf suffers from significant pollution, recurrent blooms of toxic microalgae and the transmission of

pathogens affecting the quality and quantity of exploited shellfish. Due to the potential extent of the damage generated by the various contaminants, an analysis of risk using an innovative approach is strongly recommended.

This approach, traditionally applied in terrestrial animal health, is used here for the first time in the marine environment and for shellfish. So, qualitative appreciation aims to analyze the health risk for the exploitation of shellfish resources on the Tunisian coasts in order to develop early warning systems and to minimize the inherent risk of contamination of shellfish. In order to understand the risk by toxic microalgae, we analyzed 10 years of data acquired a monitoring program of toxic phytoplankton.

2. Material and Methods

The database of the phytoplankton monitoring program in the Gulf of Gabes (2011–2021) was archived at 41.229.139.4:8081/INSTM_NEW/. We analyzed approximately 5,090 records. The study area focused on four stations located along the coast of Sfax (Fig. 1).

The Sfax coast is characterized by a semi-arid Mediterranean climate, with seasonal variations in water temperature, salinity, and wind patterns. This environment is subject to anthropogenic pressures, including industrial, urban, and agricultural pollution, resulting in elevated levels of contaminants in sediments [5].

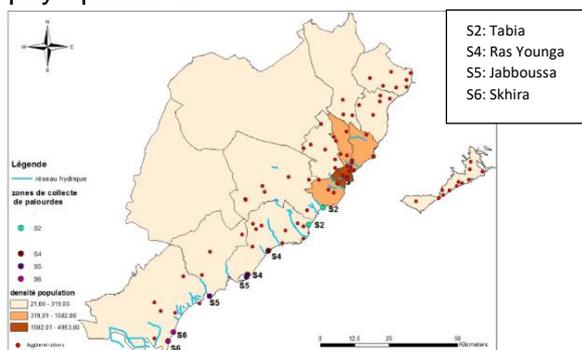


Figure 1. Position of clam production zones, location of major rivers, and population density in the Sfax region (CNVZ Opinion, Request No. 2013-SA-0001, enhanced)

Risk analysis was performed using a qualitative approach as described by [6]. (Fig.2)

The qualitative assessment of emission, exposition, and consequences was carried out using a grid based on the method described by the OIE (2007). However, the

assessment conducted in this study relied on the available databases (REPHY, REBIO...), data from DGSV and CRDA, a set of hypotheses constructed from national and international literature, expert opinions, and field observations



Figure 2. Risk Analysis

The method used to appreciate the risk, as proposed by the Animal World Health

Organization consisted of the following five steps (Fig.3).

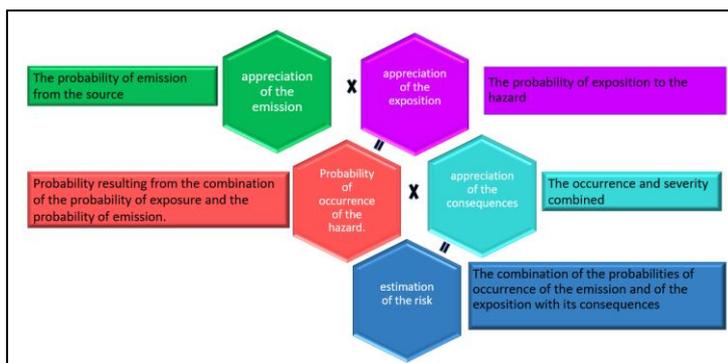


Figure 3. The steps of a risk appreciation, proposed by the Animal World Health Organization

3. Results and Discussion

The analysis of the REPHY database from 2011 to 2021 revealed that, across the four production areas, the highest percentages of alert situations were associated with two toxic phytoplankton species: *Prorocentrum lima* and *Karenia selliformis*. This finding supported the selection of these two species for this risk assessment study. It is well known that the growth, abundance, and distribution of phytoplankton are generally regulated by environmental factors [7]. These factors contributed to the risk dynamics associated with *Prorocentrum lima* and *Karenia selliformis*, encompassing

oceanographic, climatic, and biological factors, as illustrated in the conceptual graph (Fig.4).

Indeed, the factors were established based on national and international bibliographic references. Significant correlations between the species *Prorocentrum lima* and various physicochemical and meteorological parameters in the Sfax region, according to seasonal variations, have been established through the studies of [7] [8] and [9]. For *Karenia selliformis*, for example, the bibliographic references used for the emission factors were primarily the works of [5].

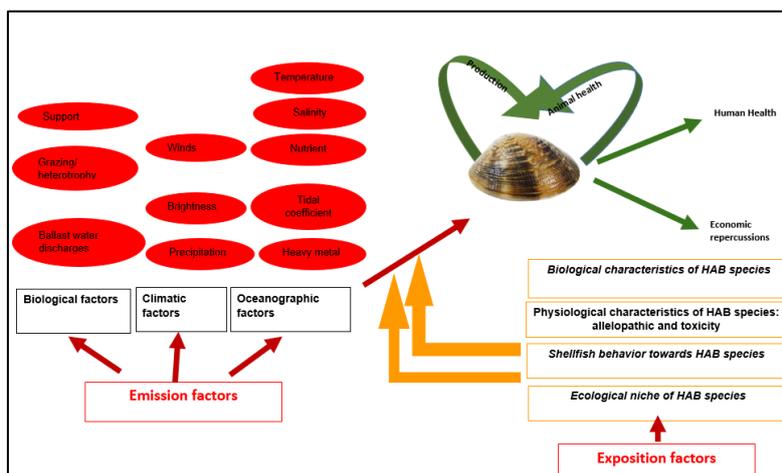


Figure 4. Conceptual graph of risk analysis by *Prorocentrum lima* and *Karenia selliformis*

The results of the assessment of emission factors by season and study station indicated that, for *Prorocentrum lima*, the emission risk level was high to very high at S2 and S6 in spring, at S4 in summer, and

at S5 in autumn. The emission risk of *Karenia selliformis* was high to very high at S2 and S6 in autumn. Additionally, the exposure risk level for *Prorocentrum lima* was high to very high at S5 in winter,

whereas for *Karenia selliformis*, the exposition risk was high at S6.

The appreciation of risk for humans and the clam (*R. decussatus*) by *Prorocentrum lima* ranged from very low (VL) to low (L) (table1), while for *Karenia selliformis*, the contamination risk ranged from very low (VL) to low (L) for the clam (*R. decussatus*) and from minimal (M) to almost negligible (AN) for humans (table 2).

Table 1. Risk assessment of *P. lima*

Outcome		Probability of occurrence															
		S2				S4				S5				S6			
		S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	E	A	W	Sp
Clam	VL	VL	VL	VL	VL	VL	VL	VL	L	L	VL	L	VL	VL	VL	VL	
Humans	VL	VL	VL	VL	VL	VL	VL	VL	L	L	VL	L	VL	VL	VL	VL	

Table 2. Risk assessment of *K. selliformis*

Outcome		Probability of occurrence															
		S2				S4				S5				S6			
		S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	E	A	W	Sp
Clam	VL	VL	VL	VL	VL	VL	VL	VL	L	L	VL	L	VL	VL	VL	VL	
Humans	AN	M	AN	M	AN	AN	AN	AN	AN	AN							

S: Summer; A: Autumn; W: Winter; Sp: Spring

The validation and final assessment of the results for these toxic dinoflagellate species, *Prorocentrum lima* and *Karenia selliformis*, which were the most abundant on the coasts of Sfax, proved to be a valuable asset for the shellfish farming sector. It contributed to the success of juvenile stocking at corresponding stations, stock regeneration, and the management of health risks.

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Influence of priming treatments on germination, seedling growth and tolerance to salt stress of fenugreek (*Trigonella foenum-graecum* L.)

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Abstract

Climate change is the main cause of abiotic stress, threatening crop yield, quality, and global food security. This study evaluated the effects of seed priming methods hydropriming, biopriming and hydro-biopriming on germination and growth of fenugreek (*Trigonella foenum-graecum* L.) under NaCl-induced salinity. Results showed that biopriming and hydro-biopriming significantly enhanced germination, growth, and salt tolerance, highlighting the potential of PGPR (Plant Growth Promoting Rhizobacteria) -based priming to improve crop performance under saline conditions.

Keywords: Priming; legume; *Trigonella foenum graecum* L.; Salt stress; Germination; Growth.

1. Introduction

Global climate change is a major cause of abiotic and biotic stresses that adversely affect agricultural productivity, often to an irreversible extent (Cao et al., 2023). Salinity stress disrupts biochemical, physiological, and metabolic processes, reducing plant performance and yield (Aydinoğlu et al., 2019). To enhance agricultural productivity and ensure food security, seed priming a pre-sowing treatment that partially hydrates seeds to activate pre-germinative metabolism without radicle emergence can be applied using various methods during this reversible germination phase (Tania et al., 2020). This study aims to evaluate the effects of hydropriming alone and combined with biopriming using *Bacillus subtilis* and a native strain (IG2) on fenugreek (*Trigonella foenum-graecum* L.) seeds under salt stress, as well as to compare the performance of the native strain with the reference *Bacillus subtilis* strain.

2. Materiel and Methods

2.1. Seed Material and Priming Treatments

This study evaluated the salt tolerance of the Tunisian fenugreek variety Rayhan using four seed priming treatments: untreated control (T1), hydropriming with water (T2), biopriming with (T3), and hydro-biopriming combining (T4). This study used *Bacillus subtilis* NCIB 3610 (ATCC 6051) as a PGPR and the native strain IG2, isolated from the date palm rhizosphere in Kébili (NCBI Accession: PRJNA1013824). Seeds were hydroprimed in water for 8 h or bioprimed in a bacterial suspension for 2 h in Petri dishes (Belhassan et al., 2024). In the field experiment, *Bacillus* spores were used for seed treatment.

2.2. Experimental Details

Three experiments were conducted. The first two, in Petri dishes and in the field, assessed four treatments with the reference strain *Bacillus subtilis*. The third, in Petri dishes under salt stress, compared the native strain IG2 with the reference strain. The field trial took place in Agareb on March 30, 2023,

where irrigation water salinity was about 6 g/L (≈ 100 mM).

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 20.0. ANOVA followed by Duncan's multiple range test ($p < 0.05$), with means denoted by different letters to indicate significant differences at the 5% level.

3. Results

In Petri experiment: Effect of seed priming in Root and Shoot Length of fenugreek

Growth responses varied with priming type and salt intensity, with significant reductions observed under NaCl stress.

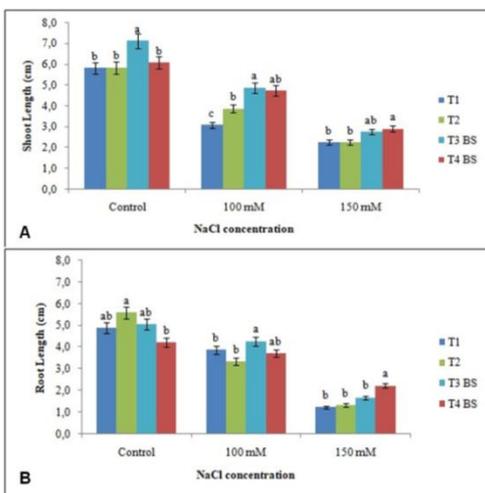


Figure 1: *In Vitro* Growth of Fenugreek Seedlings: Effect of seed priming with *Bacillus subtilis* on root (B) and shoot (A) length.

Priming treatments enhanced both root and shoot growth under salt stress, T3 enhanced root (4.3 cm vs. 3.9 cm) and shoot (4.9 cm vs. 3.1 cm) growth at 100 mM NaCl, while T4 showed the most pronounced improvement at 150 mM NaCl, increasing root (2.2 cm vs. 1 cm) and shoot (2.3 cm vs. 1.9 cm) lengths compared to the control.

In Field experiment: Effect of seed priming in Root and Shoot Length and seedling growth

The results clearly indicate that biopriming (T3) and hydro-biopriming (T4) improved seedling growth, with the longest roots observed in July. Shoot lengths increased from April to July across all treatments, with T3 and T4 showing the most significant growth.

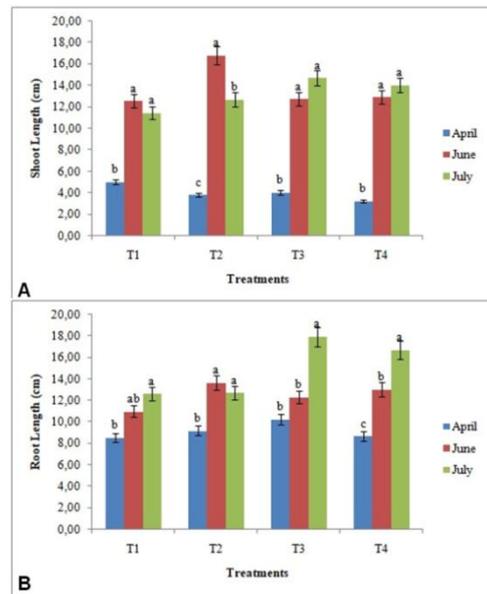


Figure 2: Effect of seed priming with *Bacillus subtilis* on root (B) and shoot (A) growth of fenugreek in field conditions.

Total Seed Yield and Harvest Mass

Total plant biomass and seed yield differed among treatments, with T3 and T4 significantly boosting both biomass (347 g and 398 g) and seed production (176 g and 226 g) compared to the control T1 (145 g biomass, 80 g seeds), while T2 showed slightly lower values. These results indicate that T3 and T4 significantly enhanced overall growth and reproductive output, with T4 being the most effective.

Table1: Total Biomass in Field Conditions.

Treatments	Total Seed Yield	Harvest Mass
T1	80± 1.32 ^c	145± 2.93 ^c
T2	63± 1.26 ^d	131± 2.31 ^d
T3	176± 2.18 ^b	347± 4.77 ^b
T4	226± 2.29 ^a	398± 4.36 ^a

In Petri experiment: Effect of IG 2 in the Root and Shoot and seedling growth compared to *Bacillus subtilis*

The analysis of the results showed that at 100 mM NaCl, T4IG2 showed the highest shoot (4.9 cm vs. 3.1 cm) and root (4.8 cm vs. 3.8 cm) lengths, whereas at 150 mM NaCl, T4 treatments with both strains provided moderate improvements compared to the control.

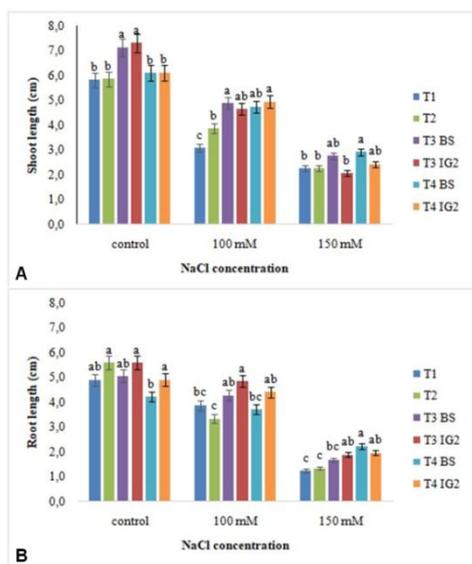


Figure 3: Effect of seed priming with *Bacillus subtilis* and IG 2 Strain on root (B) and shoot (A) growth of fenugreek in field conditions.

4. Discussion

High salinity markedly reduced seed germination and growth, as seen in fava beans (Biswas et al., 2023). This effect is attributed to elevated osmotic pressure, which restricts ion uptake and results in the accumulation of Na⁺ and Cl⁻ in the embryo, causing metabolic disruption and ultimately cell death (Hajlaoui et al., 2007). Our findings demonstrate that biopriming significantly enhances germination and growth, aligning with previous research that shows PGPR

inoculation improves shoot and root length, biomass, and salinity tolerance in *Lepidium sativum* (Mohamed et al., 2022). The inoculation effect on plant growth varied by bacterial strain. IG2 notably improved fenugreek germination and growth, showing effects similar to *Bacillus subtilis*, especially under salt stress. Our results on hydro-biopriming are consistent with previous studies demonstrating that this combined treatment improves germination and growth in chickpea (Sufyan et al., 2020).

5. Conclusion

Priming significantly improved the salinity tolerance of *Trigonella foenum-graecum* L. Among the treatments, biopriming and hydro-biopriming were the most effective, producing the highest root and shoot lengths. The IG2 strain promoted plant growth under saline conditions similarly to *Bacillus subtilis*, likely due to its PGPR activity as an indigenous rhizosphere bacterium.

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A comparative study of essential oil composition and antioxidant properties of *Pelargonium graveolens* in coastal and agricultural settings

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Abstract

Understanding how environmental conditions influence the chemical and biological properties of medicinal plants is essential for optimizing their valorization in pharmaceutical and food industries. *Pelargonium graveolens*, a species known for its therapeutic potential, was cultivated in two distinct regions of Chebba, Tunisia; an agricultural site and a coastal saline site having a high electrical conductivity (10.4 ms m^{-1}). This study aimed to evaluate the plant's morpho-physiological responses and essential oil composition under these contrasting conditions. GC-FID analysis revealed notable variations in the essential oil profiles: geranyl acetate, cis-ocimene, and trans-rose oxide predominated in the agricultural region, while geranyl acetate, trans-rose oxide, and cis-ocimene, were more abundant in the saline coastal region. Furthermore, salt-stressed plants exhibited enhanced antioxidant activity, demonstrated by reducing power assays. These findings suggest that salinity stress may boost antioxidant capacity, offering a promising approach for enhancing the functional value of *P. graveolens* in natural product applications.

Keywords: *Pelargonium graveolens*; salinity conditions; hydro-distillation; antioxidant activity

1. Introduction

Phytochemical research demonstrates the therapeutic relevance of plant metabolites, especially in medicinal plants rich in primary and secondary compounds [1]. *Pelargonium graveolens*, a medicinal herb with therapeutic properties, was cultivated in several countries like Tunisia, Egypt, and India, and was known for its antibacterial properties, which make it valuable in water treatment and lead-contaminated soil remediation [2]. Its essential oil, rich in terpenoids, is widely used in the flavor, perfume, and aromatherapy industries, exhibiting antibacterial, antifungal,

antioxidant, and anti-glucosidase properties. Additionally, it has health benefits, such as improving circulation, relieving anxiety, and addressing conditions like cancer and diabetes [3]. *Pelargonium graveolens* is also known for its tolerance to salinity stress, a major agricultural challenge, with strategies to mitigate osmotic and ionic stresses [4]. This study investigates the impact of environmental habitat and soil characteristics on *Pelargonium graveolens* in Tunisia, examining its morphological, physiological, and antioxidant properties.

2. Material and Methods

2.1. Plant material and soil sampling

The studied plant was cultivated in two distinct habitats within the Chebba region of Tunisia. The first cultivation site (35°14'N, 11°04'E) was situated in an agricultural zone, while the second site (35°14'N, 11°09'E) was a coastal area located 300 meters from the sea. Plant samples were collected during the peak flowering stage. Soil samples were taken from a depth of 20 cm. The pH value was measured. Soil salinity was evaluated using an electrical conductivity meter. Organic matter (OM) content was determined using the Walkley-Black method [5]. Indeed, essential oils were extracted using hydro-distillation in a Clevenger-type apparatus. Gas chromatography (GC) analysis was performed using a Hewlett-Packard 5890 system equipped with an HP-5MS capillary column and a flame ionization detector (FID).

2.2. Morpho-Physiological measurements

Plant height was recorded using a meter rod. The weights of leaves and stems were

determined using a physical balance. The relative water content (RWC) and electrolyte leakage were evaluated [6; 7].

2.3. Antioxidant activity

The antioxidant potential of *Pelargonium graveolens* essential oil was evaluated using reducing power assays, based on the reduction of Fe^{3+} to Fe^{2+} , monitored at 700 nm [8].

3. Results

The soil exhibited a slightly alkaline pH. Electrical conductivity (EC) measurements indicated the lowest salt content at site 1 (3.1 ms m^{-1}) and the highest at the coastal site (10.4 ms m^{-1}), making site 1 a control region. Organic matter was highest at site 1 (1.58%) compared to site 2 (0.61%). In addition, *Pelargonium graveolens* exhibited morphological variations based on habitat (Figure 1).

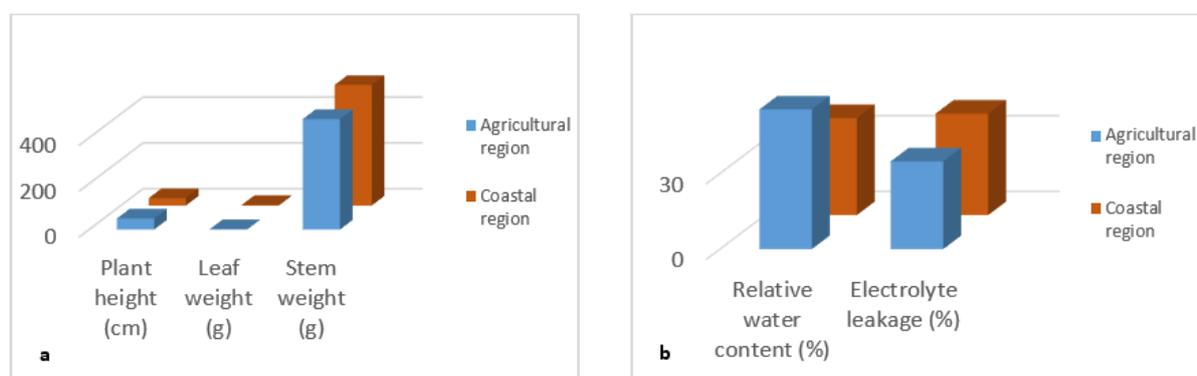


Figure 1. Morphological (a) and physiological (b) parameters of *Pelargonium graveolens* at different regions

Plants from site 1 showed greater growth, with no wilting observed at site 2. Site 1 plants had greater height (48.4 cm vs. 32.5 cm), while site 2 plants had higher leaf (2.3 g) and stem weights (526 g). Site 2 plants showed reduced water content (38.5%) and increased leakage (40.3%) (Figure 1).

GC-FID analysis revealed significant variations in oil composition between the two

regions. In the agricultural area, geranyl acetate, cis-ocimene, and trans-rose oxide were the dominant compounds, whereas in the coastal region, geranyl acetate, trans-rose oxide, cis-ocimene, and menthol were more prevalent. Percentages were presented in Figure 2.

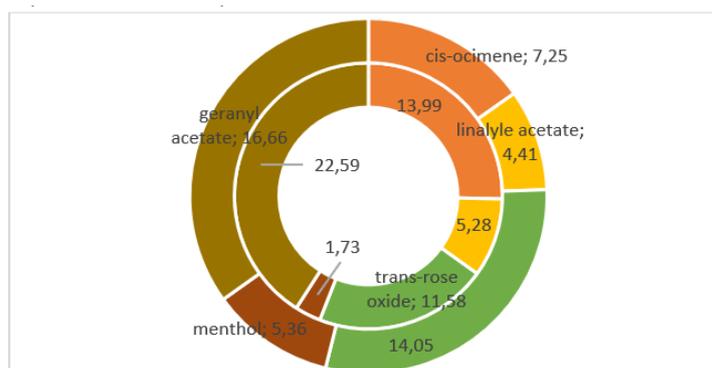


Figure 2. Graph of the distribution (%) of main components identified in the essential oil of *Pelargonium graveolens* at different regions. From inside to outside: agricultural, coastal region.

The geranium from the agricultural site exhibited the lowest antioxidant activity in reducing power activity ($IC_{50} = 106 \mu\text{g/mL}$) compared to site 2.

4. Discussion:

Alkalinity was mainly driven by the presence of bicarbonates and high limestone content. Bicarbonate accumulation raised pH levels, limiting nutrient availability for plants. The abundance of limestone further enhanced alkalinity by releasing alkaline compounds into the soil [9]. Electrical conductivity (EC) measured the salt content in soils and irrigation water. High EC levels indicated excessive salinity, which can hinder plant growth by limiting water availability. However, regular monitoring helped manage salinity and maintain soil health [10]. Organic matter enhanced soil fertility and CO_2 sequestration, allowing Tunisian geranium to grow in poor soils. Plants under salinity stress suffered from nutrient deficiency, membrane damage, and cell narrowing, leading to reduced growth due to osmotic stress and ion toxicity. Moreover, *Pelargonium* plant resistance to salinity in coastal regions may depend on genetic traits and environmental conditions. Genetic factors may influence salt tolerance, while soil composition, rainfall, and temperature can play a crucial role. Understanding these interactions helped in

selecting salt-tolerant species for cultivation [11]. Relative water content was inversely correlated with electrolyte leakage in stressed plants. Salinity delayed water retention, reducing relative water content and osmotic potential while increasing electrolyte leakage [12].

High salinity showed in site 2 may reduce the ability of phenolic compounds to neutralize reactive oxygen species (ROS), leading to oxidative stress. As ROS accumulate, they accelerate lipid peroxidation, causing membrane instability. This triggered enzymatic and non-enzymatic defense mechanisms to counteract oxidative stress. Antioxidant enzymes played key roles in detoxifying ROS by converting harmful oxygen radicals into less toxic molecules, reducing cellular damage [13]. The activation of antioxidants varied with salinity levels and plant species. Some plants rely more on enzymatic responses, while others depend on non-enzymatic mechanisms. Understanding these strategies is key to improving plant resilience in saline environments [14].

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Exploring the biochemical complexity of Rosemary hydrolat

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Abstract

Valorizing by-products of medicinal and aromatic plants offers a sustainable path for enhancing their economic and ecological impact. In this context, *Rosmarinus officinalis*, known for its adaptability and ecological relevance, was assessed for its extract potential in Tunisia. The study focused on hydrolat derived from plants cultivated in the Metouia oasis, an arid region marked by irregular and scarce rainfall. This research aimed to evaluate the hydrosol's phenolic content, antioxidant capacity, and enzyme activity to determine its potential applications. The hydrosol exhibited a notable total phenolic, flavonoids and tannins content, as well as a promising antioxidant activity. Additionally, this work reports for the first time the presence of lipoxygenase activity in plant hydrosol with an amount of 2.24 U/mg protein. These findings highlight the potential of *R. officinalis* hydrosol as a valuable natural resource for applications in healthcare, cosmetics, and other biotechnological sectors.

Keywords: *Rosmarinus officinalis*; hydrosol; secondary metabolites; antioxidant potential; lipoxygenase activity.

1. Introduction

Hydrolats, separated from essential oils after distillation, are known as hydrosols or aromatic waters. While essential oils were well-studied, research on hydrolats remains limited [1]. In Mediterranean countries, they were used in beverages, as flavoring agents, and as traditional remedies for ailments like chronic pain, depression, and dysmenorrhea [2]. They were effective as natural disinfectants in food preparation, effectively eliminating harmful microorganisms and ensuring hygiene. Their antiseptic and anti-inflammatory properties make them beneficial for skincare, helping to soothe irritation, and promote healing. Additionally, due to their antispasmodic effects, they were useful in aromatherapy and therapeutic applications [3].

Tunisian *Rosmarinus officinalis* L., mainly found in Seliana and Kef, covers approximately 39,000 hectares. It was known for its antimicrobial properties against bacteria, fungi, and viruses, aiding in infection prevention. Its antidiabetic properties helped regulate blood sugar and improved insulin sensitivity. Additionally, it inhibited tumor growth and supported cardiovascular health [4]. This study aims to analyze the phenolic composition, antioxidant potential, and enzymatic activities of rosemary hydrosol from Metouia oasis.

2. Material and Methods

2.1. Plant material and extraction process

Fresh Tunisian *Rosmarinus officinalis* was collected from the Metouia oasis. The aerial parts of flowering rosemary plants were

harvested and subjected to water-steam distillation using a semi-industrial Clevenger-type apparatus. pH was measured and the collected hydrosol was stored at 4°C for further analysis.

2.2. Secondary metabolites and antioxidant activity

The total phenolic content of hydrosols, total flavonoid and condensed tannins content were quantified [5; 6; 7]. Antioxidant activity was evaluated using the DPPH radical scavenging method [3].

2.3. Enzymes activities

The protein content of *Rosmarinus* hydrosol was quantified [8]. Lipoxygenase activity was assessed in borate buffer with linoleic acid as a substrate [9].

3. Results

The hydrosol exhibited a total phenolic content of 55.76 µg/mL, a total flavonoid content of 14.24 µg/mL and a total condensed tannin of 8.77 µg/mL (Figure 1). The antioxidant activity, assessed through the DPPH assay, showed a scavenging capacity of 36.08%.

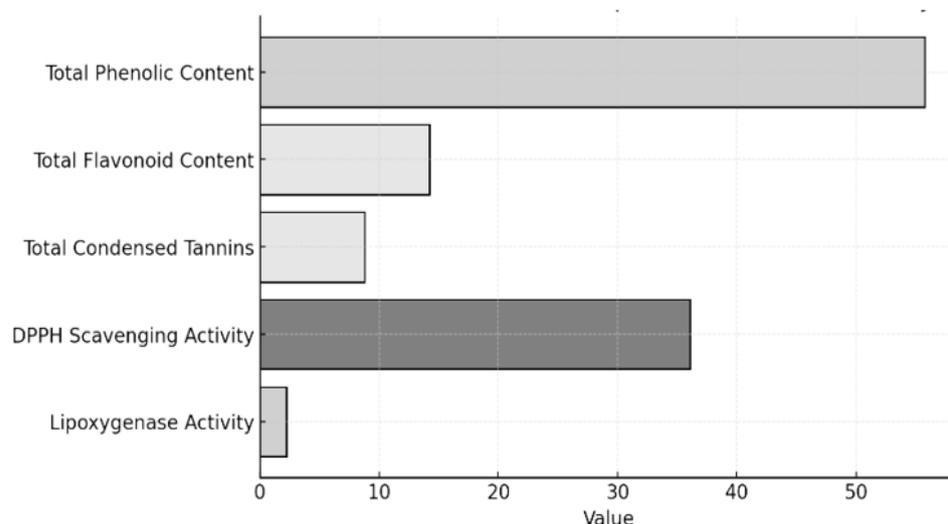


Figure 1: Biochemical composition and enzymatic activity of *Rosmarinus officinalis* hydrosol

Indeed, the findings highlighted the enzymatic profile of the hydrosol obtained through water-steam distillation. The lipoxygenase activity was in order of 2.24 U/mg protein.

4. Discussion

The examined *Rosmarinus officinalis* aromatic water had an acidic pH, influenced by the plant species and the chemical composition of its volatile fraction. Due to their acidic properties, aromatic waters are highly valued in natural skincare for their ability to restore the skin's acid mantle after

cleansing, helping to maintain pH balance, protect against harmful microorganisms, and enhance skin hydration and resilience [10].

The *Rosmarinus officinalis* hydrosol exhibited a total phenolic content higher than that of *Calendula arvensis* hydrosols, highlighting its potential as a rich source of bioactive compounds with antioxidant properties [11]. The total flavonoid and tannins content hydrosol follows a pattern similar to that observed in other medicinal and aromatic plants. Flowers generally contained the highest flavonoid

concentrations due to their role in plant defense mechanisms and pollinator attraction [12]. Environmental stressors such as drought, temperature fluctuations, and soil composition could affect the biosynthesis and accumulation of polyphenols, underscoring the role of ecological factors in determining the chemical composition and therapeutic potential of *R. officinalis* hydrosol.

Phenolic compounds exhibited strong antioxidant activity due to their hydroxyl groups, which help neutralize free radicals. The antioxidant properties of plant-derived hydrosols vary based on factors such as extraction methods, plant origin, and storage conditions [13]. The studied hydrosol demonstrated a promising DPPH radical scavenging activity, indicating its potential as a natural antioxidant. The correlation between total phenolic content and antioxidant capacity suggested the involvement of additional bioactive compounds or mechanisms [14]. These properties make phenolic-rich hydrosols valuable for applications in food preservation, pharmaceuticals, and cosmetics.

Results showed a good activity of Rosmary in lipoxygenase, which responsible for oxygenating fatty acids and playing a critical role in producing secondary metabolites and has various industrial applications. Lipoxygenase has been used in food technology for strengthening and bleaching agent and in cancer prevention [15]. Various factors influenced enzyme activities, such as extraction conditions, plant organ, geographical habitat, and collection period. The enzymes discussed have diverse biotechnological, industrial, and therapeutic applications making Tunisian *Rosmarinus officinalis* as a valuable natural source of bioactive molecules.

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Identification and agronomic application of a rhizobacteria from date palm and its effect on *Pisum sativum*. L under salt stress

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Abstract: Seed germination is a critical process in the life cycle of plants, vulnerable to various biotics (pathogens, insects, etc.) and abiotic stresses (drought, salinity, heavy metals, etc.). The objective of this study was to isolate a novel extremotolerant bacterium (IG2), then characterize and evaluate its ability to improve *Pisum sativum* seedlings growth under salts stress compared with different priming techniques. Results indicated that IG2 strain showed high tolerance to heat stress (37°C-45°C), pH (6-10), salt stress (0.2-1M) and heavy metals. Additionally, IG2 exhibited various hydrolytic enzyme activities, including amylolytic and proteolytic activities. The inoculation of *Pisum* seeds with IG2 strain combined with hydropriming considerably has improved the growth parameters (root and shoot length) compared to *Bacillus subtilis*. These results confirm that IG2 strain held promise for improving seedling growth and resilience to abiotic stresses.

Keywords: Plant Growth-Promoting Rhizobacteria, salt, IG2, *Pisum sativum*, *Bacillus subtilis*

1. Introduction

The sustainability of Mediterranean ecosystems and agroecosystems is increasingly threatened by accelerating climate change which led to a rise in abiotic stresses such as drought and salinity that negatively affect plant growth and development (Chandran et al., 2021). Seed priming has emerged as one of the promising approaches to address these challenges and enhance crop productivity. As one of the most widely used pre-sowing techniques, priming modulates the metabolic activities involved in germination before the radicle emergence during the reversible phase of germination, when the seed can return to its initial state without damage (Boucelha et al., 2015). Among the various priming techniques, the use of plant growth-promoting rhizobacteria (PGPR) is among the most effective (Paravar et al., 2023). PGPR are typically defined as microorganisms that colonize the

rhizosphere or the interior of plant tissues, enhancing plant growth through a range of mechanisms (Chandran et al., 2021). The use of PGPR has become a promising alternative for mitigating the adverse effects of salinity-induced plant stress (Hamoun et al., 2016). In this context, the first part of this study focused on the characterization of a bacterial strain isolated from the rhizosphere of date palm, including an analysis of its enzymatic activities and its tolerance to abiotic stress. The second part investigated the influence of different seed priming techniques including hydropriming, bio-priming with a characterized PGPR bacterium, *Bacillus subtilis* NCIB 3610 (also known as ATCC 6051) and the characterized bacteria, and a combined hydro-bio priming on the growth of *Pisum sativum* L. seeds under salt stress.

2. Material and Methods

Bacterial Strains

Two bacterial strains were employed in this study. The first was *Bacillus subtilis* NCIB 3610, a well-characterized PGPR, provided by the Italian team of the PRIMA Benefit-Med project in a spore's formulation. The spores were rehydrated in sterile distilled water containing 10% saccharose prior to use. The second strain, designated IG2, is an indigenous isolate obtained from the rhizosphere of *Phoenix dactylifera* L. (date palm) trees in the Kébili region of Tunisia (Belhassan et al., 2024).

Isolation and Characterization of the IG2 Strain

Rhizospheric soil was collected for bacterial isolation using a serial dilution plating technique. Following incubation, morphologically distinct colonies were selected, purified, and preserved in glycerol stocks. The IG2 strain was deposited in the microbial culture collection of the Faculty of Sciences of Sfax. Morphological characterization included Gram staining and screening for hydrolytic enzyme activities (amylase and protease). For enzymatic assays, IG2 was spot inoculated on LB agar plates containing 1% starch or 4% milk protein and incubated at 40 °C for 48 h. Enzyme activity was confirmed by the appearance of clear halos around colonies. Physiological characterization included assessments of salt tolerance, pH range, and temperature sensitivity. IG2 was cultured on LB agar supplemented with varying concentrations of NaCl (0.2–2.0 M), and at pH values ranging from 5 to 12. Thermal tolerance was evaluated at temperatures ranging from 37 °C to 55 °C (optimal pH 8), with growth recorded after 24–72 hours of incubation (Belhassan et al., 2024).

Plant Material and Seed Priming Treatments

Seeds of *Pisum sativum* L. (Forrimax accession) were supplied by the BENEFIT-

Med project partners. Seeds were surface sterilized using sodium hypochlorite solutions at 50%, 20%, and 10% concentrations for 3, 10, and 30 minutes, respectively, followed by three rinses in sterile distilled water. Bacterial cultures were grown in LB broth at 40 °C for 48 h, centrifuged, and resuspended in sterile water to a final optical density of OD₅₉₅ = 0.3 (≈10⁸ CFU/mL), measured using a UV–Vis spectrophotometer. Seeds were divided into four treatment groups: **T1** (Control): Untreated seeds. **T2** (Hydropriming): Seeds were soaked in distilled water for 8 hours at room temperature in darkness, then dried at 25 °C for 24 hours. **T3** (Biopriming): Seeds were soaked for 2 hours in bacterial suspensions of either *B. subtilis* or IG2 (containing 10% saccharose for *B. subtilis*), then dried under the same conditions. **T4** (Hydro-biopriming): Seeds were subjected first to hydropriming, followed by biopriming as described above (Belhassan et al., 2024; Boucelha & Djebbar, 2015).

In Vitro Germination and Salt Stress Assay

Ten seeds from each treatment group were placed on filter paper in sterile Petri dishes. For salt stress conditions, 10 mL of 100 mM NaCl solution was added to each dish, while distilled water was applied to the control plates. The experiment was replicated three times. Morphological parameters were recorded after 10 days, including root and shoot length (cm) and germination rate (%), calculated using the following formulas:

Germination percentage = (Number of germinated seeds / Total seeds) × 100

Statistical Analysis

Data were statistically analyzed using IBM SPSS Statistics (version 20.0). Duncan's multiple range test was employed to assess

significant differences in seedling root and shoot lengths across treatments. Statistical significance was determined at $p < 0.05$.

3. Results and Discussion

Characterization of the Indigenous Bacterial Strain IG2

The indigenous bacterial strain IG2 was isolated from the rhizosphere of date palm trees in the Kebili region of Tunisia, a hotspot of plant-associated microbial diversity. To characterize this strain, a series of physiological, biochemical, and environmental tolerance assays were conducted. Growth tests (Fig 3) revealed that IG2 grow within a temperature range of 37°C to 45°C, with optimal proliferation at 37°C, classifying it as thermotolerant. pH tolerance assessments on Luria–Bertani (LB) medium demonstrated growth from pH 6 to 12, with maximal activity at pH 8, indicating its adaptation to alkaline environments. Additionally, IG2 demonstrated high halotolerance, growing at NaCl concentrations up to 1 M (Fig 2). This characteristic is critical for application in saline soils and is likely driven by the synthesis of Osmo-protectants and exopolysaccharides that mitigate osmotic stress (Ali et al., 2022; Chandran et al., 2021). The IG2 strain exhibited significant amyolytic activity, confirmed by clear halo formation on starch-enriched agar following iodine vapor staining. Proteolytic activity was also confirmed on skim milk agar, evidenced by casein hydrolysis zones, highlighting IG2’s capacity to contribute to nitrogen cycling and plant development. Furthermore, IG2’s resistance to abiotic stressors was investigated, focusing on heavy metal toxicity (Fig 3). While cadmium ($CdCl_2$) proved highly inhibitory, IG2 tolerated zinc ($ZnSO_4$) up to slightly above 2 mM and copper ($CuSO_4$) up to 2 mM, with morphological changes suggesting stress-induced polysaccharide production. Nickel ($NiCl_2$) resistance was notably high, with growth sustained up to 10 mM, indicating

the strain’s robustness in contaminated environments. Such resistance is comparable to well-known heavy-metal-tolerant strains like *Cupriavidus metallidurans* CH34 (Belhassan et al., 2024).

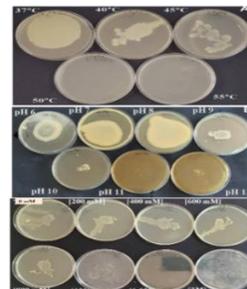


Fig. 2: Effect of temperature (A), pH (B) and salt stress (C) on bacterial growth.

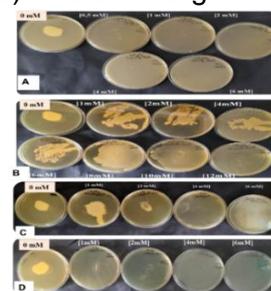


Fig.3: Growth of IG2 strain under different heavy metals concentrations. A: Cadmium; B: Nickel; C: Zinc and D: Copper.

In Vitro Evaluation of IG2 Strain Inoculation on *Pisum sativum* seeds Germination Under Salt Stress

Germination rates (Fig 4) exhibited significant variation, with T3BS achieving 100% germination under non-stress conditions, whereas T2 resulted in the lowest rate (83%). Under salt stress, T4IG2 markedly improved germination (97%) relative to the untreated control (73%).

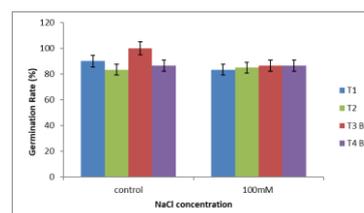


Fig.4: Germination Rate of *Pisum sativum* seeds under salt stress.

Assessment of early seedling development (Fig 5) revealed that T4IG2 consistently promoted the greatest root elongation, with mean root lengths of 7.6 cm under control conditions and 5.6 cm under salinity, both exceeding respective controls. Furthermore, IG2 inoculation enhanced root hair formation and primary root extension, indicative of improved adaptive capacity. These findings are supported by Gliz and Mahloul (2022), who reported that PGPR isolated from the rhizosphere of *Vicia faba* improved root development in *Linum usitatissimum* through enhanced branching and elongation. Shoot growth was also improved by IG2 treatments, particularly under salt stress, with enhanced leaf elongation and biomass accumulation. These effects may stem from IG2-induced changes in gene expression related to growth and metabolism (Chandran et al., 2021).

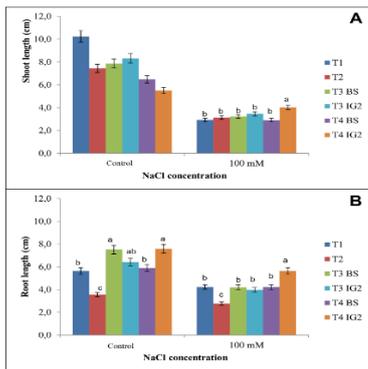


Fig.5: Effect of IG2 strain on shoot (A) and root (B) length on Pisum seedlings under salt stress (100 mM). Different letters indicate significant differences among the treatments at P<0.05.

Conclusion

IG2 exhibited key traits of an effective PGPR: abiotic stress resistance, enzymatic activity, and a strong ability to enhance seedling establishment and growth across multiple plant species. Its performance under saline conditions highlighted its promise for agricultural applications in marginal soils. Further greenhouse and

field trials are recommended to validate its efficacy in diverse agro-ecological settings.

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Germination and seedling growth of fourteen durum wheat varieties in comparison with *Vivasio triticum* under different cultivation systems

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Abstract

Cultivation systems could strongly affect crop production. This study was conducted to evaluate the effects of different cultivation methods on germination parameters and seedling physiological characteristics across fourteen durum wheat (*Triticum durum* Desf.) varieties and one triticale variety (*Vivasio triticum*). The experiment compared three distinct treatments: a conventional system irrigated with tap water, a conventional system irrigated with a nutrient solution, and a soilless hydroponic system utilizing a perlite substrate.

The obtained results showed that the addition of the nutrient solution improved the germination rate and the plant growth parameters of different cultivars, especially Monastir, Razzek, Dhahbi, Khiar and *Vivasio triticum*, while the hydroponic system using perlite substrate showed the best results with Salim and *Vivasio triticum*.

In conclusion, the comparison of the fifteen cultivars showed that Karim was the most promising wheat variety compared to the others. The use of Naturamin®NPK and perlite substrate improved plant growth and productivity. This enhancement depends on the varietal diversity of wheat, highlighting the importance of genetic diversity in cultivation strategies.

Keywords: Wheat, Perlite, Hydroponic system, Nutrient solution, Germination, Plant growth, Spikes production.

1. Introduction

Cereals play a crucial role in the global economy. Wheat is the second-largest cereal, accounting for 33% of total cereal production (USDA, 2019). It serves as the primary food source for over 36% of the world's population (Hasanuzzaman et al., 2017).

Intensive selection and cultivation of selected phenotypes have transformed populations of these wild progenitor species into domesticated varieties of the cultivated species we know today.

As Tunisia boasts great genetic richness in durum wheat, analyzing this diversity would significantly contribute to preserving the multiplicity of the national heritage and avoiding fluctuations in cereal production due to the unsuitability of some introduced varieties under Tunisian climatic conditions. Therefore, this study aimed to evaluate the effects of perlite substrate in hydroponic

system and nutrient solution in conventional system on the germination and seedling growth of wheat varieties.

2. Material and Methods

2.1. Seed source

Fourteen wheat genotypes with distinct genetic and adaptive backgrounds were selected. These included six Tunisian improved varieties (INRAT100, Razzek, Dhahbi, Maali, Salim, and Azmour), one Tunisian local variety (Msakni), five French improved varieties (Portodur, Sculptur, Aventadur, Carioca, and Monastir), and two Mexico-Tunisian improved varieties (Karim and Khiar). Their performance was compared to *Vivasio triticum*, a hybrid of wheat and rye.

Seeds were kindly provided by the National Institute of Cereal Crops (INGS) in Bousalem. They were collected in 2023 and stored at 4 C until utilization.

2.2. Experimental setup and recording of data

The experiment was laid out in a Randomized Complete Block Design (RCBD), having 3 treatments: (control group (T1), hydroponic group (T2), and nutrient solution test group (T3) where we used the Naturamin@NPK additive. Treatments were replicated ten times with twenty seeds per treatment for each cultivar, at a rate of 2 seeds per cavity in a germination plate. The plates contained either peat (T1 and T3) or perlite (T2).

Germination percentage (GP) was recorded every 5 days, and the experiment ended after three successively stable GPs that were retained as the germination capacity (GC). Mean daily germination (MDG %) is calculated as the ratio of the germination percentage to the total number of germination days at the end of the experiment.

Seedling growth was evaluated after three weeks and again after six and eight weeks of treatment for the surviving groups. Plantlets' heights, shoot length, root length, and number of leaves was quantified in the first plant growth stages, then number of spikes and thallus, and total biomass measurement were added in the advanced plant growth stages.

The data obtained for all treatments of the fifteen cultivars were analyzed using the one-way ANOVA test, followed by Tukey's multiple comparisons test to determine specific group differences. Statistical analyses were performed using GraphPad Prism 8."

3. Results

3.1. Effects of nutrient solution and perlite substrate on seed germination parameters

Table 1 presents the effects of nutrient solution additive and hydroponic cultivation using a perlite substrate on seedling germination metrics of fifteen different cultivars.

The nutrient solution and perlite substrate

improved the germination rate and capacity in many cultivars but caused a delay in germination speed and the mean daily germination ratio. Under T1, the Tunisian variety *Razzek*, the French variety *Aventadur*, and the Mexico-Tunisian variety *Karim* showed the highest germination performance compared to the other cultivars. *Vivasio triticum* exhibited the best germination under T2, while *Razzak* and *Dhahbi* varieties showed the best results under T3.

3.2. Effects of nutrient solution and perlite substrate on seedling growth biometrics

Figure 1 illustrates the effects of nutrient solution (T3) and perlite substrate (T2) on seedling growth parameters after 3 weeks. The nutrient solution significantly enhancing stem length, root length, and seedling height in most varieties, particularly Monastir, *Razzek*, *Dhahbi*, *Khlar*, and *Vivasio triticum*, compared to T1 and T2. Perlite substrate significantly improved the growth in *Portodur*, *Dhahbi*, *Monastir*, and *Vivasio triticum* while reducing water requirements. Statistical analysis revealed significant increases in leaf count for *Portodur*, *Dhahbi*, *Khlar*, and *Vivasio triticum* under T2 and T3 compared to the control ($p < 0.05$, Tukey's test). These findings highlight the potential of optimized growth media to enhance early wheat development.

Figure 2 demonstrates the effects of nutrient solution and perlite substrate on seedling growth metrics after six weeks. Significant improvement in growth parameters in T3 group compared to T2 group. The nutrient solution promoted superior growth and improved plant viability. T1 and T2 plants showed increased fragility during the observation period. Only T2 and T3 survived after six weeks, and only T3 plants reached the two-month sampling period. This underscores the nutritional efficacy of the supplemented solution for durum wheat cultivation.

Figure 3 showed the effects of nutrient solution on growth parameters among the fifteen cultivars after 8 weeks of sowing.

Statistical analysis revealed significant varietal differences ($p < 0.05$) in all measured parameters compared to earlier growth stages. Portodur demonstrated superior growth metrics among French varieties, while Salim outperformed other Tunisian cultivars. The Mexico-Tunisian variety Karim exhibited the highest overall performance, and fresh and dry biomass accumulation compared to all other cultivars. In contrast, *Vivasio triticum* showed the lowest seedling growth and spike production but produced the highest number of leaves and thallus. The Tunisian varieties, Msakni, Salim, and Azmour displayed exceptional spike production, suggesting their potential for grain yield optimization under nutrient-enhanced cultivation systems.

4. Discussion

The present study demonstrated that growth media composition and nutrient supplementation significantly influence germination, early seedling development, and long-term growth performance in durum wheat. The response to different cultivation systems was found to be cultivar-dependent.

Agricultural perlite is characterized by its high-water absorption capacity, long term fertilizer retention, as well as its high cation exchange capacity and neutral pH, which allows for better irrigation control, and helps preserve the nutrients, including potassium in a available form over extended periods without fixing which can explain the observed improvement in germination rate and plant growth under T2 (Sina et al., 2022).

Naturamin®NPK is a nutrient solution that contains free amino acids and is enriched with phosphorus and potassium, which is recommended for crop activity stimulation in periods of increased energy demand, helping the crop to overcome stress (Yang, 2021; Yuan, 2017). The observed improvement in germination and growth metrics under T3 aligns with previous reports on enhanced plant growth in nutrient rich environments (Fathidarehnejeh et al., 2023). The long-term (6-8 week)

observations highlight the sustainability benefits of nutrient solution, with maintained plant viability and continued growth performance. The cultivar-specific responses observed, including Karim's exceptional biomass production and the spike productivity of Msakni, Salim, and Azmour, emphasize the genetic basis of nutrient utilization efficiency (Sina et al., 2022).

5. Conclusion

The Mexico-Tunisian variety Karim exhibited the most promising growth and productivity metrics among all tested genotypes. Naturamin®NPK and perlite substrate significantly enhanced germination, and plant growth parameters across multiple cultivars, suggesting particular promise for controlled-environment agriculture applications. This improvement varied depending on genetic background, highlighting the critical role of varietal diversity in wheat's response to optimized growth conditions.

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nutrient and water use efficiency in hydroponic systems.

Tables

Table1: Effects of nutrient solution and perlite substrate on seed germination parameters (T1: Control group; T2: Hydroponic group; T3: Nutrient solution test group; GP%: Germination Percentage; CC%: Germination capacity; N.G.D: Number of germination days; MDG%: Mean daily germination)

Species	Portulaca	Spinach	Tomato	Razze	Dha	Sculp	Carrot	Kari	Maal	Khlar	Cario	Men	Sali	Msa	Azm	Tritic
GP	100	70	100	80	80	100	100	80	60	80	90	90	90	90	60	90
T2 Day	40	60	45	50	60	75	60	55	40	60	55	65	60	40	60	40
T3 Day	40	60	90	90	70	60	60	60	40	70	90	80	50	50	40	40
T1 GP	100	70	100	80	80	100	100	80	60	80	90	90	90	90	60	100
T2 Day	75	90	80	65	85	75	85	80	60	60	70	85	80	70	95	95
T3 Day	50	60	100	100	70	60	70	60	50	70	90	80	60	70	70	70
T1 GP	100	70	100	80	80	100	100	80	60	80	90	90	90	90	60	100
T2 Day	75	90	85	70	85	80	90	80	60	65	90	90	80	70	100	100
T3 Day	70	70	100	100	70	70	60	50	70	90	80	60	70	70	70	70
T1 GC	100	70	100	80	80	100	100	80	60	80	90	90	90	90	60	100
T2 GC	75	90	85	70	85	80	90	80	60	65	90	90	80	70	100	100
T3 GC	70	70	100	100	70	70	60	50	70	90	80	60	70	70	70	70
T1 N.G.D	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
T2 N.G.D	10	10	12	11	10	11	11	10	10	12	12	11	10	10	10	14
T3 N.G.D	13	12	10	10	5	11	10	5	10	5	5	5	10	10	10	10
T1 MDG	20	14	20	16	16	20	20	16	12	16	18	18	18	18	12	10
T2 MDG	7,5	9	7,08	6,3	8,5	7,27	8,18	8	6	5,41	7,5	8,18	8	7	7,14	7,14
T3 MDG	5,38	5,83	10	10	14	6,36	7	12	5	14	18	16	6	7	7	7

Figures

Figure1: The effects of the nutrient solution and perlite substrate on seedling growth metrics after 3 weeks. (a. Plumle length; b Radicle length; c. Seedling length; d. Number of leaves; T1: Control; T2: Hydroponic group; T3: Nutrient solution group) (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001)

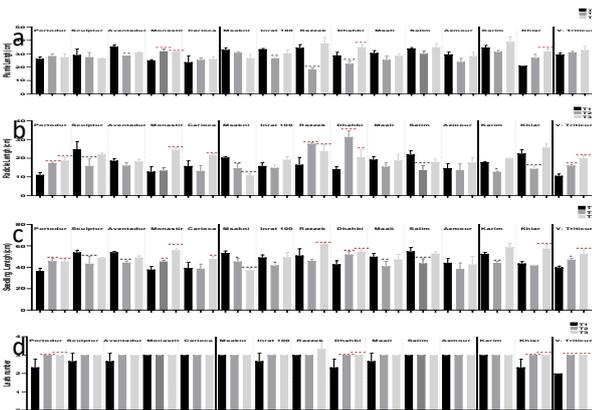
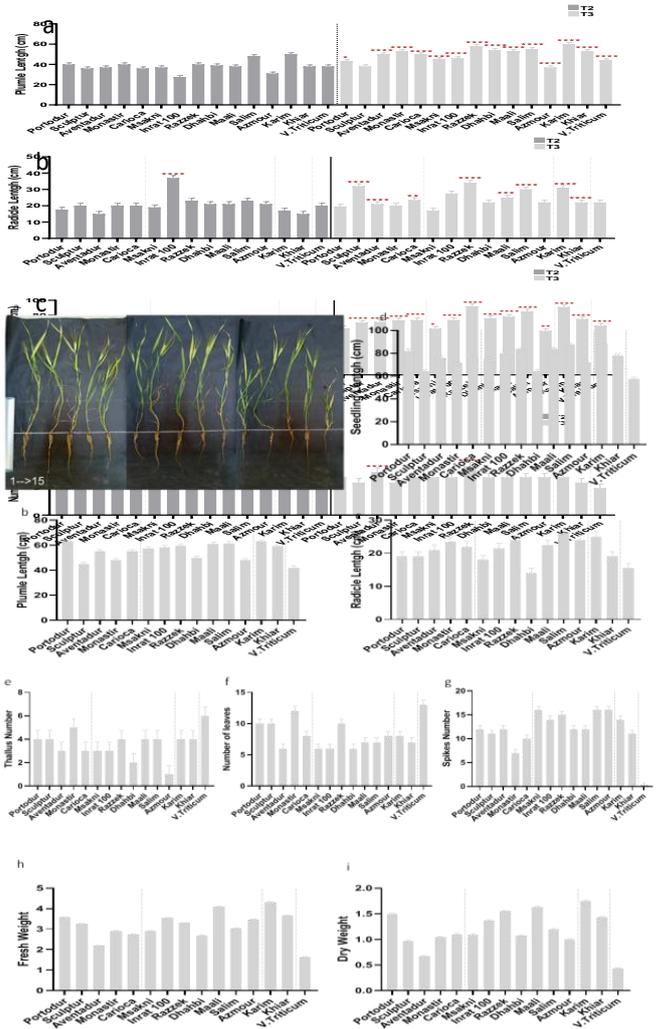


Figure 2: The effects of the nutrient solution and perlite substrate on seedling

growth metrics after six weeks (a. Plumle length; b Radicle length; c. Seedling length; d. Number of leaves) (T2:



Hydroponic group; T3: Nutrient solution test group) (*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001)

Figure 3: The effects of the nutrient solution on seedling growth metrics and spikes production after eight weeks (a. Plant growth; b. Plumle length; c. Radicle length; d. Seedling length; e. Thallus number; f. Number of leaves; g. Spikes number; h. Fresh weight; i. Dry weight)

Effect of seed priming on germination and seedling growth of *Lathyrus sativus* L. cultivars under salinity conditions

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Abstract

Salinity severely limits global crop productivity by negatively affecting seed germination and seedling growth. Orphan legumes like *Lathyrus sativus* L. (grass pea) are valued for their climate resilience but have underdeveloped seed systems. Seed priming is an effective method to enhance seed quality and early seedling vigor. This study evaluated the effects of hydropriming, biopriming and hydro-biopriming on germination and seedling growth of two grass pea cultivars Maleme-107 (LatM) and Sofades (LatS) under salinity (50, 100, 150 mM NaCl) in vitro and field conditions. Priming treatments improved germination rate, root and shoot length, and seedling biomass. Hydropriming enhanced LatM germination under all conditions, while hydro-biopriming significantly increased germination and root length in LatS under salt stress. Field trials confirmed priming increased emergence, plant height, root length, and dry weight compared to controls, with LatS showing higher emergence than LatM. Overall, seed priming is a cost-effective and scalable approach to improve orphan legume performance in saline environments.

Keywords

Grass pea; Seeds, Hydropriming; Biopriming; Hydro-priming; Salinity; Growth.

1. Introduction

Salinization is becoming the most critical environmental threat to the Mediterranean basin, intensified by climate change. By 2050, the region is expected to face a 4–30% reduction in rainfall and a temperature rise of 2 to 4 °C, alongside a sea-level increase of about 35 cm by 2100 (Hayder et al., 2024), which will exacerbate salinization of coastal and inland water resources. These changes will expand marginal lands with low agricultural productivity, threatening food security and ecosystem health (Hayder et al., 2024). Tunisia exemplifies the regional salinity challenges, with two-thirds of its territory

classified as semi-arid and the remainder as arid. To compensate for scarce rainfall, irrigation is widely practiced; however, about 52% of groundwater sources contain total dissolved salts ranging from 1.5 to 3 g/L, and two-thirds of deep aquifers exhibit even higher salinity levels (Farzaman et al., 2023). Such elevated salinity levels create unfavorable hydrological conditions that inhibit normal plant growth by reducing seed germination rates and prolonging germination time (Daghari et al., 2022). Innovative agronomic strategies are needed to sustain crop production under saline stress, with seed priming techniques including hydropriming, biopriming, and hydro-biopriming-emerging as effective

ways to enhance germination, seedling vigor, and stress tolerance (Jeppesen et al., 2020).

In Tunisia, the cultivation of orphan legumes such as *Lathyrus sativus* L., commonly known as grass pea, offers a promising avenue for sustainable agriculture in saline-prone areas. Grass pea is an annual legume valued for its resilience under adverse climates and its nutritional benefits (Banerjee et al., 2020). It is widely cultivated in arid and semi-arid conditions, making it a suitable candidate for saline environments. However, its seed systems remain underdeveloped, limiting its widespread adoption (Banerjee et al., 2020).

The objective of this study is to evaluate the effects of different seed priming techniques on the emergence and seedling growth of two *Lathyrus sativus* L. cultivars Maleme-107 (LatM) and Sofades (LatS) under salinity stress *in vitro* and field conditions.

1. Material and Methods

After sterilization, seeds were primed according to the protocol described by the University of Pavia, Italy (Paparella et al., 2015).

Seed germination was evaluated *in vitro* under salinity stress conditions using NaCl concentrations of 50, 100, and 150 mM. The NaCl solutions were prepared in pure distilled water. Pure distilled water was used as control for the study in triplicate. Seeds were placed on filter paper in each square Petri dish, and 10 mL solution was used as prepared above. After 10 days, germination rate, along with shoot and root lengths, was recorded to assess the effects of priming on salinity tolerance.

Both primed and non-primed seeds were sown in the field at the Agareb region (34.748117, 10.543664; Tunisia) on March 30, 2023. Surface irrigation technique was occasionally employed as needed. The

water used for irrigation had a salinity level of approximately 6 g/L. Field emergence, growth, and development under natural conditions were monitored to evaluate the practical benefits of seed priming on crop performance.

2. Results

1. *In vitro* performance of seed priming

1.1. Effects of seed priming on germination rates of Lat M and Lat S cultivars under varying salinity stress levels

The results showed that seed priming improved germination rates of both Lat M and Lat S cultivars across all tested salinity levels (0, 50, 100, and 150 mM NaCl) (Figure 1). While germination rates declined with increasing salinity, primed seeds maintained higher germination percentages than non-primed seeds, especially at the highest salinity (150 mM NaCl).

1.2. Effects of seed priming on seedling growth (root and shoot length) of Lat M and Lat S cultivars under salinity stress

The results showed that seedling growth, measured by root and shoot length, was adversely affected by salinity in both cultivars; however, priming treatments significantly enhanced seedling vigor under saline conditions (Figure 2). LatS accession showed a stronger positive response to priming, maintaining longer roots and shoots compared to LatM, highlighting varietal differences in stress tolerance and priming efficacy.

2. *In vivo* performance of seed priming

2.1. Effect of priming treatments on germination rates of Lat M and Lat S seeds

The first parameter analyzed was the percentage of plant emergence from soil. As shown in Figure 3, the grass pea accessions Lat M and Lat S did not show an enhancement in the percentage of soil emergence in response to all the seed priming treatments, compared to control (T1).

2.2. Effect of seed priming on plant growth in grass pea accessions Lat M and Lat S

Biometric analyses were conducted on samples collected at various time points throughout the trial (Figure 4). The results revealed that the vegetative growth of LatM was significantly enhanced by the treatments compared to LatS (Figure 4). Specifically, for LatM accession, the biopriming treatment (T3) significantly increased root and shoot length relative to both the control group and other treatments. In contrast, for the LatS accession, the hybrid priming treatment (T4) was identified as the most effective protocol for promoting seed germination and overall seedling growth in this variety.

2.3. Effect of seed priming on seedling biomass total fruit weight of *Lathyrus sativus* L. accessions

Seed priming treatments have a significant positive effect on seedling biomass parameters, specifically dry weight and fresh weight, across all grass pea cultivars (Table 1). Based on the results collected at the Tunisian pilot site at the harvest stage, the most consistent and beneficial effect was observed with the biopriming treatment (T3). This treatment notably enhanced total fruit weight in both grass pea accessions Lat S and Lat M, with 26.3-fold and 9.7-fold increases, respectively (Table 1).

3. Discussion

The results demonstrated that seed priming significantly enhanced germination rates and seedling growth of *Lathyrus sativus* cultivars Lat M and Lat S under salinity stress, both *in vitro* and *in vivo*. Primed seeds of both cultivars exhibited higher germination percentages than non-primed seeds across all NaCl concentrations, indicating that priming effectively mitigates the inhibitory effects of salt stress on germination. Lat M showed a stronger positive response to priming,

maintaining longer root and shoot lengths than Lat S, suggesting varietal differences in priming efficacy and salinity tolerance (Goufa et al., 2025).

Contrary to *in vitro* results, seed priming did not significantly improve soil emergence rates for either cultivar in the field trial, which may reflect more complex environmental interactions affecting emergence beyond seed vigor alone. However, priming enhanced vegetative growth parameters. This suggests that while priming may not always increase the emergence percentage under field conditions, it can improve subsequent seedling growth (Goufa et al., 2025).

Seed priming treatments positively influenced seedling biomass and total fruit weight, with biopriming producing the most pronounced yield that increases-26.3-fold in Lat S and 9.7-fold in Lat M. These improvements reflect priming's role in enhancing physiological and biochemical mechanisms such as antioxidant enzyme activities, ion homeostasis, and reserve mobilization, which collectively improve plant growth and productivity under salinity stress (Sghayar et al., 2023).

Seed priming is known to initiate pre-germinative metabolic processes, improve water imbibition, activate antioxidant defenses, and reduce ionic toxicity by modulating Na⁺, K⁺, and Ca²⁺ uptake, thereby enhancing seedling establishment and stress tolerance (Goufa et al., 2025). The varietal differences observed in this study are consistent with reports that priming effects can vary depending on genetic background and priming agent used (Sghayar et al., 2023).

4. Conclusion

Seed priming significantly enhanced germination and seedling growth of LatM and LatS cultivars under salinity stress *in vitro*. While it did not improve soil

emergence in the field, priming boosted vegetative growth and biomass, especially with biopriming and hybrid treatments. Importantly, biopriming greatly increased total fruit weight in both cultivars. Overall, seed priming is a promising strategy to improve salinity tolerance and yield in grass pea.

Acknowledgments

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Tables

Table 1: Effect of different priming treatments on seedling biomass the fruit weight of *Lathyrus sativus* L. accessions LatM and LatS. T1: Unprimed; T2: Hydropriming; T3: Biopriming and T4: Hybrid priming.

Accession	Treatments	April		June		July	
		Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Total seedling dry weight of product (g)	Total fruits weight (g)
Lat M	T1	1.03	0.23	2.71	1.40	367	4
	T2	1.28	0.22	2.90	0.60	430	24
	T3	1.45	0.29	2.96	0.64	370	39
	T4	2.07	0.37	2.85	0.71	348	13
Lat S	T1	0.61	0.16	0.88	0.35	160	6
	T2	0.50	0.12	1.99	1.07	413	3
	T3	1.02	0.29	1.37	0.57	946	158
	T4	0.98	0.22	1.66	0.58	399	3

Figures

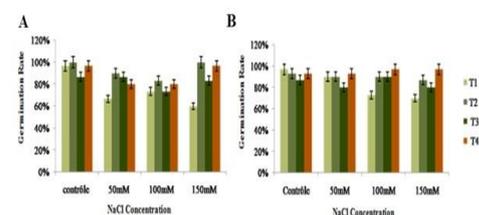
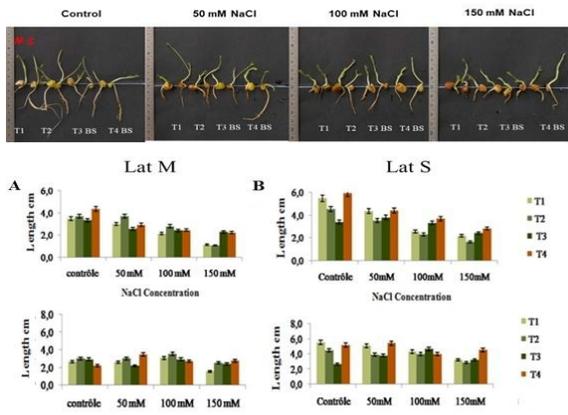


Figure 1: Effect of priming treatments on germination rates of Lat M (A) and Lat S (B) cultivars under different salinity levels *in vitro*. T1: Unprimed; T2: Hydropriming; T3: Biopriming and T4: Hybrid priming.



T2: Hydropriming; T3: Biopriming and T4: Hybrid priming.

Figure 2: Effect of seed priming on seedling root (A) and shoot (B) length in Lat M and Lat S cultivars under salinity stress *in vitro*. T1: Unprimed; T2: Hydropriming; T3: Biopriming and T4: Hybrid priming.

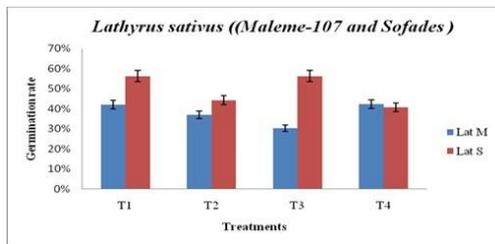


Figure 3: Percentage of plant emergence recorded in the field trial at the Tunisian pilot site. T1: Unprimed; T2: Hydropriming; T3: Biopriming and T4: Hybrid priming.

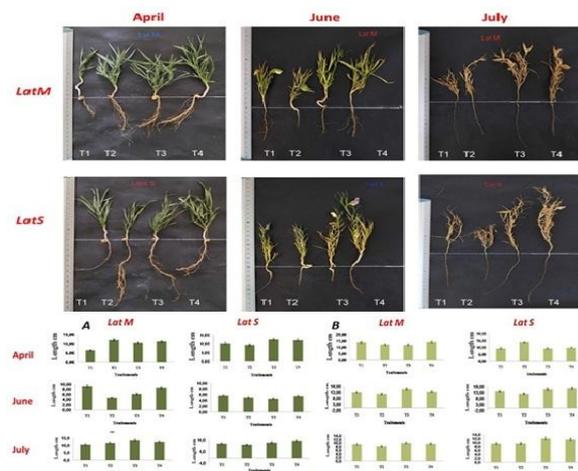


Figure 4: Growth response of grass pea accessions LatM (A) and LatS (B) to different seed priming treatments measured by root and shoot length at 1-, 3-, and 4-months post-sowing. T1: Unprimed;