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Subject

biostimulants effects on the germination of Cowpea (*Vigna Unguiculata* L.) under Salt Stress

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Dedication

To my parents for their endless love, encouragement and support

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To my promo " M2 vegetal production " for the best times that we spend together

to all my friends "Ahlam;khalida;rania;walaa;manal"

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Dedication

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AMRANE ABIR

I. I.ist of figures

II. List of tables

III. Acronyms and Abbreviations

%: percent

°C : degree Celsius

ABA: abscisic acid

ACC: 1-Aminocyclopropane-1-carboxylic acid

AS: acid salicylic

Ca+2: calcium ion

CDA: Canadian food inspection agency

Cl- :chlorure
Cm : centimetre

CO2 : carbon dioxide

CPs : copalyldiphosphate synthase

Cscp : cysteine protease

D : difference

DDL: data definition language

Dns : dinitrosalicylic acid

EBIC: European biostimulant industry consortium

FAO : food and agriculture organisation .

Faostat : food and agriculture organisation corporate statical database

G/L :gram in litre

g: gram

GA: gibberellic acid

GA-20-ox: gibberellic acid 20 oxidase

GA-2-ox : gibberellic acid 2 oxidase

GA-30-ox : : gibberellic acid 30 oxidase

H2CO3 : carbonic acid

H_2O_2: hydrogen peroxide

i.e :That is or id est

ITGMI: technical institute of market gardenningand industriel

JA : jasmonic acid

K+ :Potassium ion

KO: kaurene oxidase

KOA: kaurenoic corrosive oxidase

KS : kaurene synthase

M:millimolar

Meq : milliequivalent

min : minute

ml ; millilitre

Mm:millimetre**m**

N: normality

NA+2 : sodium ion

Nacl : sodium chloride

nm: nanometre

OMWW: Olive mill wastewater

PEG: polyethylene glycol

ph: potential of hydrogen

ROS : Reactif oxygen species

Sig : significance level

SPSS: statistical package for the social sciences

VPE: vacualor processing enzyme

Λ:Wavelength

Abstract

Salinity is one of the main factors limiting agricultural yields in arid and semi-arid ecosystems. Agriculture has recently seen some development in the use of biostimulants to stimulate plant physiology under biotic and abiotic stress. Our study aims to investigate the effect of using the aqueous leaf extract of *Pistacia lentiscus* L. and the centrifuged Olive mill wastewater on enhancing cowpea *Vigna unguiculata* germination under 150 and 250meq/l of NaCl. The results showed that salinity considerably reduces the final rate of grain germinationas well as the earliness of germination. Therefore, the aqueous leaf extract of Pistacia lentiscus L. and the centrifuged Olive mill wastewater seem to improve germination rates. The centrifuged olive mill wastewater appears to be more advantageous than the aqueous leaf extract of *Pistacia lentiscus* L. in limiting the effect of salt stress, since under the 250meq/l concentration. Moreover, the results showed that the activity of amylases extracted from germinating seeds is not influenced by different saline treatments. It can be inferred that salinity affects amylase functioning, not its synthesis.
Keywords: *vigna unguiculata*, germination, salt stress, biostimulant, aqueous extract of *pistacia lentiscus* L.leaves , centrifuged olive mill wastewater.

Résumé

La salinité est l'un des principaux facteurs limitant du rendement agricole dans les écosystèmes arides et semi arides. Récemment l'agriculture a connu un certain développement à travers de l'utilisation des biostimulants qui permettent de stimuler la physiologie de la plante soumise au stress biotique et abiotique. Notre étude vise à évaluer l'effet de l'utilisation de l'extrait aqueux des feuilles de *Pistacia lentiscus* L. et les margines centrifugées sur l'amélioration de la germination du niébé sous 150 et 250meq/l de NaCl. Les résultats montrent que la salinité affecte considérablement le taux final de la germination ainsi que la précocité de la germination. Cependant l'utilisation de l'extrait aqueux des feuilles de *Pistacia lentiscus* L. et les margines centrifugées semblent améliorer le taux de germination des graines. Ainsi que les margines centrifugées apparaissent plus avantageuses dans l'atténuement du stress salin. En outre les résultats indiquent que l'activité amylasique n'est pas influencée par la salinité ce qui peut expliquer par le fait que la salinité a affecter le fonctionnement des amylases.

Mots-clés : *vigna unguiculata*, stress salin, biostimulant, extrait aqueux de pistachier, margines centrifugées.

الملخص

تعد الملوحة أحد العوامل الرئيسية التي تحد من المحصول الزراعي في النظم البيئية القاحلة وشبه القاحلة. شهدت الزراعة مؤخرًا بعض التطور في استخدام المحفزات الحيوية لتحفيز فيزيولوجية النبات تحت الإجهاد الحيوي واللاحيوي . يهدف بحثنا إلى دراسة تأثير استخدام مستخلص الأوراق المائية لشجرة الضرو (*Pistacia lentiscus* L.) والمياه المستخلصة من انتاج زيت الزيتون على تعزيز نبات اللوبياء *Vigna unguiculata* تحت تراكيز 150 و 250 مكيف/لتر من NaCl . أظهرت النتائج أن الملوحة تقلل بشكل كبير من معدلات الإنبات النهائية للحبوب وكذلك سرعة الإنبات. وبالتالي، يبدو أن المحفزين مستخلص الأوراق المائية لشجرة الضرو والمياه المستخلصة من مصانع انتاج زيت الزيتون يحسنان معدلات الإنبات. يبدو أن المحفز الاخير أكثر ميزة من المستخلص المائي لاوراق الضرو في تقليل تأثير إجهاد الملح، وذلك تحت تركيز 250 مكيف/لتر. علاوة على ذلك، أظهرت النتائج أن نشاط الاميلاز المستخلصة من البذور المنبتة لا يتأثر بتراكيز الملح المختلفة. يمكن استنتاج أن الملوحة تؤثر في وظيفة الأميلاز ، وليس في تركيبها.
الكلمات الرئيسية: اللوبياء vigna unguiculata ، إجهاد الملح، محفز حيوي، مستخلص مائي من أوراق شجرة الضرو، المياه المستخلصة من مصانع انتاج زيت الزيتون .

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Introduction

The main factors that limit crop yields and the dispersion of wild plant species are abiotic stresses, which include cold, warm, drought, and salinity, as well as biotic stresses like bacteria, viruses, fungi,and parasites. Among the abiotic stresses, salt stress has been increasing due to global warming (Shrivastava, 2015), poor irrigation water management (Evelin *et al.*, 2019), and excessive chemical fertilization (FAO, 2021). The most common salt found in soil is NaCl (Evelin *et al*., 2019), which negatively impacts plant growth and development through water stress, cytotoxicity caused by excessive uptake of ions like Sodium (Na+) and chloride (Cl-), and nutritional imbalance(Isayenkov *et al*., 2019).

Legumes are the edible seeds found inside pod plants, and come in various shapes and colors such as lentils (green, red, or brown), peas (whole, split, or chickpeas), soybeans, and dried beans (red, white, black, etc.) and cowpea (Polak*et al*., 2015).

Cowpeas (*Vigna unguiculata* L. *Walp*), which belong to the legume family Fabaceae, have a multitude of functions for both humans and animals (OECD, 2016; Satishand Karunakara, 2017). They are particularly crucial for the livelihoods of the least developed countries. Cowpea seeds contain 53% carbohydrates, 24% crude protein, and 2% fat, making them an important source of human nutrition (FAO, 2012). Furthermore, cowpeas can also contribute to soil fertilization through symbiotic nitrogen fixation and serve as a high-quality animal feed due to the nutritional value of their leaves (Diouf, 2011).

Cowpeas are grown on approximately 14.5 million hectares worldwide each year (Bucar *et al*., 2016). In Algeria, cowpeas are cultivated in three main regions: the north (Cabilla, ElCala), the southwest, including the Saharan oases (El Golea, Adrar, and Bechar), and the southeast of the country (GianneTe, Taman Rasset) (Ghalmi, 2010; Gahlmi, 2011).

The germination stage is the initial phase that influences the subsequent growth stages of cowpea and other plant species. For cowpea, optimal conditions for germination include a temperature range of 25-30°C, moisture content of 12-15%, and a pH level of 6.0-7.0 (Singh *et al*., 2013). Hence, any constraint during the germination stage can have detrimental effects on the final grain production of crops (Sergio *et al*., 2016). For instance, salt stress can affect seed germination by exerting toxic effects on the developing seedlings through excessive uptake of sodium and chloride ions (Mbarki *et al*., 2020).

Using biostimulants appears to be crucial in mitigating the impact of salt stress on plants (Hamdani *et al*., 2020 ;Chouhim *et al*., 2022; Zuzunaga-Rosas *et al*., 2023,).Biostimulants are substances that promote plant growth and can include natural or synthetic soil conditioners, pesticides, or plant extracts. Some examples of biostimulants are seaweed extract, plant extracts like the Aqueous extract of *Pistacia lentiscus* L. leaves;

microorganisms and their extracts, amino acids, and hydrolyzed proteins (Benhamou and Rey, 2012). Additionally, phytohormones such as auxins, cytokinins(CTK), ethylene, gibberellins (GA), and salicylic acid (SA) have also been used as biostimulants to mitigate the impact of salt stress on plants (Tarakhovskaya *etal.*, 2007; Taiz *et al*., 2010; Huang *et al.*, 2015).Moreover, the treated olive mill wastewaters acted very favorably on the germination of the tomato seeds, irrespective of the dilution rate(Rais *et al.*,2017).

Our study aims to investigate the effects of using the *aqueous* leaf *extract* of *Pistacia lentiscus* L .leaves and the centrifuged Olive mill wastewater known as biostimulants, on enhancing cowpea germination under salt stress conditions.

Bibliographic synthesis

Cowpea
Vinga unguiculata L

1. Description of cowpea

Vigna unguiculata is a tropical herbaceous annual with very variable plant structure with definite or indeterminate growth. It can grow up to 60 cm tall (Borget, 1989). The taproot is generally well developed, exceeding 30 cm at the beginning of flowering (Chaux and Foury, 1994)

1.1.Leaves: The leaves are alternate, stalked and trilobed.

1.2.Flowers: the flowers are hermaphrodites of the classic legume, large (2 to 3 cm long), and vary in color from white to purple (Reamarkers, 2001).

1.3.Fruits: These are elongated cylindrical pods, more or less compressed, with sharp ends (Bourget, 1989).

1.4.seeds: their shape is generally elliptical or rounded. They're 5 to 6 mm wide. Each seed has a small, elliptical hilum surmounted by the micropyle, the color of the spot girding this hilum is a varietal characteristic, the germination capacity of the seeds lasts from 3 to 5 times (Santens, 1985).

1.5.Roots: The taproot is generally well developed, The roots bear nodes which contain nitrogen- fixing bacteria, nitrogen obsession is considered satisfactory (Mulongoy, 1985)

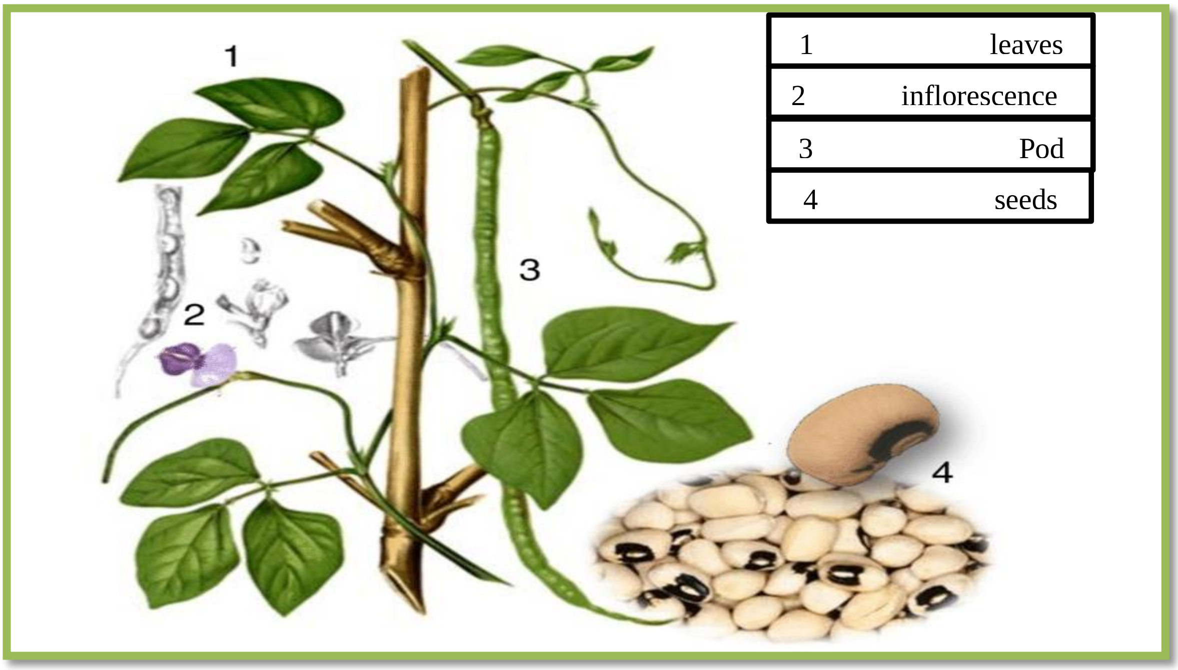

Figure 1: morphology of *vigna unguiculata*
(http://fr.wikipedia.org/wiki/Vigna_unguiculata_sesquipedalis 2012)

2. Classification of cowpea

Vigna unguiculata is a self-bred, diploid annual herbaceous plant with a chromosome number of 2n=2 x 11=22 (Maréchal *et al.,* 1978), classified as follows

Reign: plant
Phylum: Phanerogams (spermaphytes)
Class: Dicotyledons
Order: Leguminosae or Fabales **Family**: Leguminosae or fabaceae**Type** : *Vigna*
Species: *Vigna unguiculata subsp. Unguiculata (L) Walp*

3. Requirements of cowpea

Require temperatures between 25 and 35 degrees Celsius and yearly rainfall between 750 and 1100 millimeters, plains vegetation is ideal for the growth of the cowpea (Drahansky *et al* ., 2016).

4. Benefits of cowpea

Cowpea known for its

4.1.Economic Benefits

As an alternative, the cowpea is an essential pulse crop with significant nutritional and nutraceutical benefits for global food security and population health (Hall A., 2012). It is mostly planted for grain and leaves in less developed areas, with sporadic green pod plantings (GERRANO *et al*., 2017).

4.2.Soil Improvement

The cowpea plant greatly helps to the long-term viability of agricultural systems and the development of soil fertility in marginal lands by providing ground cover, fixing nitrogen up to 80%, controlling weeds, and reducing the need for and expense of nitrogen fertilizer (Asiwe *et al* .,2009;Beshir *et al* .,2019;kyei-boahen *et al* .,2017). Because it offers residual nitrogen obtained through the decomposition of its leaves litter, roots, and nodes, it is a crucial companion crop for cereal-pulse farming (okereke *et al* .,2006;FAOSTAT2013).

4.3.Nutritional value

The high nutritional importance of whole cowpea seed compared to the others vegetables, it is related to its richness in protein (50 -60%) and low fatty acid rate (1%) (Joes *et al*., 2014; Kirse *et al.,* 2015). Indeed, its protein rate presents 2 to 4 times compared to cereals and crops. It contains moderate amounts of fiber, phytochemicals, minerals and vitamins (trehan *et al*., 2015;Liyanage*et al*., 2014). Although whole grain cowpea is low in methionine and cysteine compared to animal source proteins, it is high in amino acids compared to cereals (Goncalves *et al*., 2016). It is crucial for the human diet as a source of macro- and micronutrients (bialostosky *et al*., 2002)

5. Production of cowpea *Vigna unguiculata*

5.1.production of cowpea *Vigna unguiculata* in the world

It is estimated that 14.5 million hectares of cowpea are grown each year worldwide, with a total annual production of 6.2 milliontons. Over the past three decades, world cowpea production has grown by an average of 5% per year (Bucar *et al.*, 2016).

5.2.production of cowpea *Vigna unguiculata* in AFRICA

Over 80% of Africa's production is produced in West Africa, which also accounts for over 84% of the continent's production areas and 83.4% of the world's cowpea output. According to (FAOSTAT ,2016), cowpea cultivation in Africa was estimated at 12.3 million hectares in 2014, with most production taking place in West Africa at 10.6 million hectares, especially in Nigeria, Niger, Burkina Faso, Mali and Senegal. Although Ethiopia is a center of diversity for Vigna (Beshir *et al*., cited 2019)

5.3.Production of cowpea in Algeria

In Algeria, a Saharan species possibly imported from northeaster Africa is currently considered the putative domestication area for the *Vigna unguiculata* cultivar. The original genetic population was preserved due to the extreme isolation of the oasis (Echikh, 2000) However; *Vigna* germ plasms have traditionally been preserved in different ecological conditions where they have accumulated very high genetic diversity. They grow in three mainregions: the north (Cabilla, El Cala), the south-west, the Saharan oases (El Golea, Adrar and Bechar) and the south-east of the country (Gianne Te, Taman Rasset). They are divided into three groups representing the flora *Unguiculata*, Bifora and Melanophtalmus (Gahlmi, 2011; Ghalmi 2010).

Known in Algeria as tedelegh or loubia, they are used in human nutrition as gradients, soups or teas, eaten as dried seeds or green pods, leaves and stems are often used for animal feed (Ghalmi, 2010**)**

6. Germination physiology

During germination, the seed rehydrates and oxidizes its reserves with oxygen for the necessary emergence. The permeability of the outer skin and its contact with soil particles determine the uptake and penetration of oxygen. Digest any type of retention (MICHEL, 1997) germination factor According to **(**Cüneyt Uçarlı,2020), is a complex multi-stage developmental process regulated by internal and external factors.

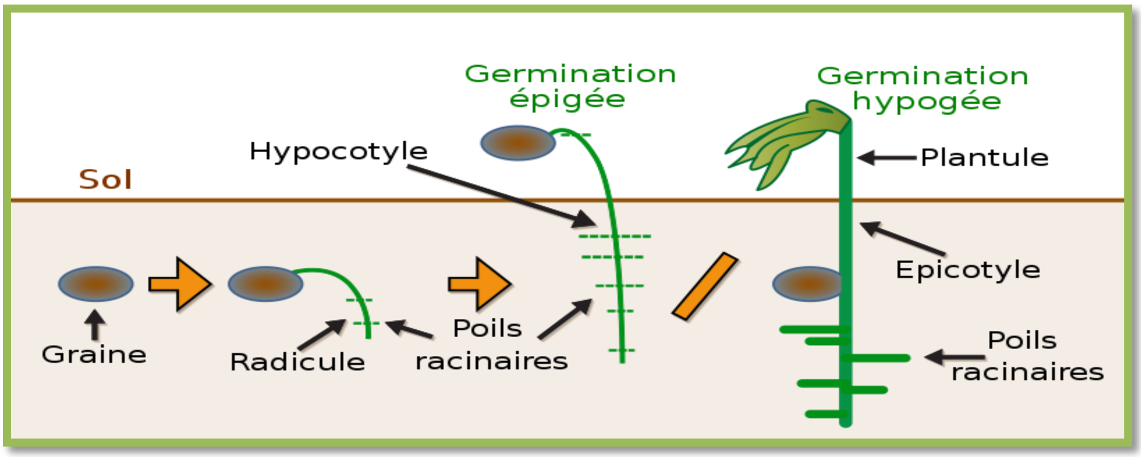

Figure 2 : (Germination-fr.svg 2019)

7. Germination stages

It consists of three consecutive main phases:

7.1.The first phase (admission stage)

Corresponds to high tissue hydration, accompanied by Breathing intensity (Heller *et al.*, 2004).it's about movement The direction in which water potential falls (Hopkins, 2003).

7.2.The second phase

It is characterized by high and consistent hydration and breathability. Water absorption is followed by a general activation of seed metabolism (Hopkins, 2003). At this stage, the seed can be important alter their viability (Heller *et al.*, 2004).

7.3.Growth period after germination

Characterized by rehydration and increased oxygen Rapid recovery of cell division and growth after consumption (Hopkins University, 2003).During this stage, tissue dehydration leads to seed death Germination is complete when the radicle emerges from the seed coat.

8. Germination of cowpea

Cowpea *(Vigna unguiculata)* is a legume crop that is widely cultivated for its edible seeds and leaves. The germination process of cowpea involves the emergence of a radicle andthe subsequent growth of the embryonic plant.(USDA;2021)The optimal conditions for cowpea germination include a temperature range of 25-30°C, a moisture content of 12-15%, and a pH of 6.0-7.0. (singh,D *et al* 2013)The seed coat of cowpea is relatively thick, and it may require scarification to enhance water absorption and allow for germination to occur.

This can be achieved through physical methods such as scratching the seed coat or through chemical treatments such as soaking the seeds in concentrated sulfuric acid or boiling water.(Ndakidemi, p.A ;2004)

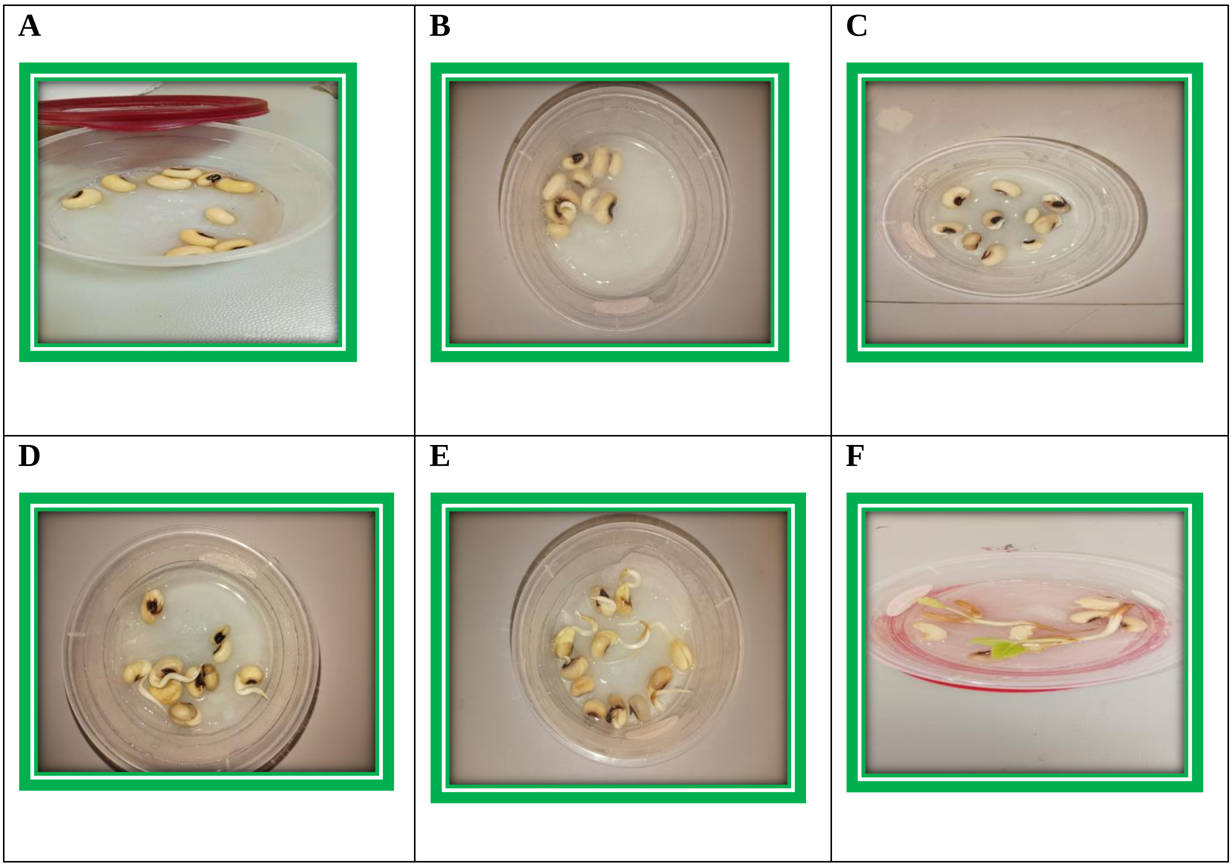

Figure 3: Germination Of Cowpea *Vigna unguiculata*(Amrane/Mis 2023)

Salt Stress

1. Definition of salt stress

Salinization plays an important role in soil degradation and threatens it a large fraction of the Earth's arable land (Souguir *et al*.,2013).This phenomenon corresponds to the excessive accumulation of highly soluble salts in the water soil surface, leading to a decrease in soil fertility (Souguir *et al*.,2013).

2. Causes of Salt stress

80% of the soil's salinity comes from natural sources, there are two types of salinity

2.1."Native" salinization

Due to salt formed during the weathering of rocks or external natural inputs, while 20% of salinized soils are of "anthropogenic" origin.

2.2."Secondary" salinization

Caused by human activities associated with agricultural practices, especially irrigation (Chamekh, 2010). Salinity results from alteration of saline source rocks by erosion factors, dissolution of chloride-rich runoff water Sedimentary rocks, sulfates and carbonates, leading to primary salinization, often caused by acid rain (H_2CO_3) and physical factors (Hammou, 2010;Bouchoukh, 2010).

According to (Noomene ,2011) states that secondary salinization is the result of human activities altering the hydrological balance between soil application of water (irrigation or rainwater) and plant water use (transpiration). According to the same author, the most common causes are:

- ✓ Land clearing and replacement of perennial vegetation with annuals,
- ✓ Use of saltwater irrigation,
- ✓ deficiency drainage and unbalanced irrigation systems

3. Physiological and biochemical response of plant to salt stress

3.1.Photosynthesis: Water uptake and transport

Under drought and salinity stress, plants experience reduced water uptake and transport, which can lead to water deficit and osmotic stress. Plants respond to these stresses

by reducing stomatal conductance, increasing root-to-shoot ratio, and producing osmoprotectants such as proline and sugars to maintain water balance (Chaves *et al*., 2003).salinity stress can also reduce photosynthesis by affecting various components of the photosynthetic machinery, including chlorophyll content, stomatal conductance, and CO2 fixation. Plants respond by reducing leaf area, altering the composition of pigments, and optimizing the photosynthetic process (Chaves *et a*l., 2003).

3.2.Antioxidant defense

salinity stress can lead to the accumulation of reactive oxygen species (ROS) in plant cells, which can damage proteins, lipids, and DNA. Plants respond by producing antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) to scavenge ROS and protect cellular components (Farooq *et al*., 2009).

3.3.Metabolite accumulation

Drought and salinity stress can lead to the accumulation of metabolites such as sugars, amino acids, and organic acids. These metabolites can act as osmoprotectants, regulate ion homeostasis, and provide energy for plant growth and metabolism (Zhu, 2016).

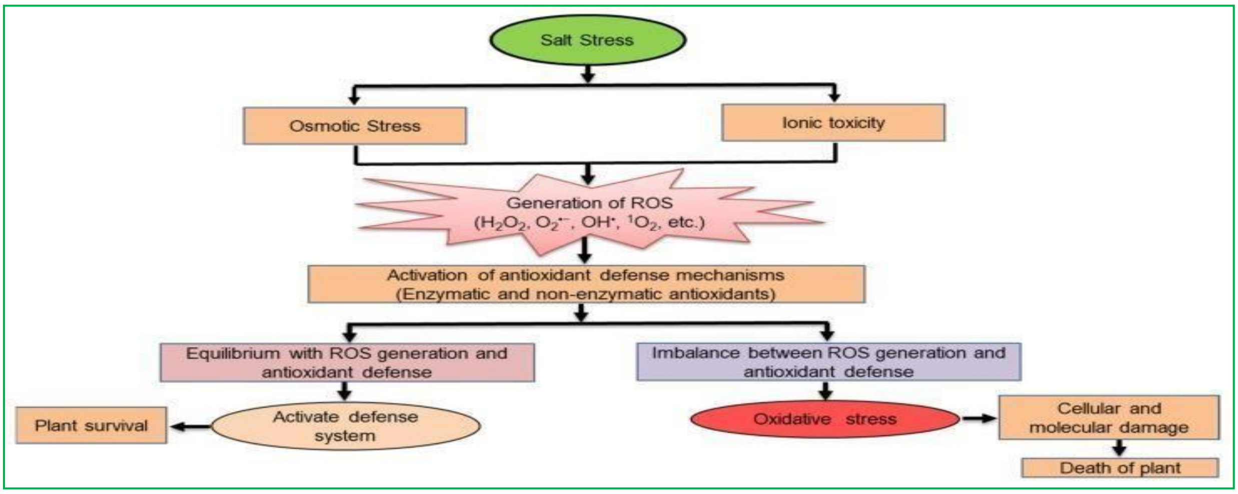

Figure 4: Oxidative stress and antioxidant defense under salinity.(Geilfus C.M.,2015)

4. Effects of salt stress on the germination

4.1.Delayed germination

High concentrations of salt can delay the germination of seeds by affecting water uptake and imbibition. Salt stress can cause osmotic stress and reduce the availability of water for germination, resulting in delayed germination (Bajji *et al*., 2002).

4.2.Reduced germination rate

Salt stress can also reduce the germination rate of seeds by affecting various physiological and biochemical processes. Salt stress can affect the integrity of cellular membranes, reduce enzymatic activity, and induce oxidative stress, all of which can lead to reduced germination rates (Gao *et al*., 2019).

4.3.Decreased seedling growth

Even if germination occurs, salt stress can significantly affect seedling growth. High salt concentrations can cause ion toxicity, osmotic stress, and nutrient imbalance, all of which can lead to reduced seedling growth and survival (Khan *et al.*, 2015).

4.4.Alteration of gene expression

Salt stress can also alter the expression of genes related to germination, including those involved in stress response, hormone signaling, and seed dormancy. Salt stress can induce the expression of stress-related genes and reduce the expression of genes involved in germination and growth (Niu *et al*., 2020).

5.Definition of hydrolase

Hydrolase could be a course of hydrolytic chemicals that are commonly utilized as biochemical catalysts which utilize water as a hydroxyl bunch giver amid the substrate breakdown. In basic words, a hydrolase is an chemical that catalyzes the hydrolysis of a chemical bond in biomolecules. This, in turn, partitions a expansive particle into two littler ones. Hydrolases are thus critical for the environment since they process huge particles into little parts for the blend of biopolymers as well as for the debasement of poisons. In organic chemistry, We mention examples of hydrolases such as : Esterases , Proteases , Glycosidases , lipases (Shukla,E.,2021)

6. Effects of hydrolases on salt stress

6.1.Improved salt tolerance

Several studies have shown that overexpression of certain hydrolases can improve salt tolerance in plants. For example, overexpression of the vacuolar processing enzyme (VPE) gene AtδVPEγ in Arabidopsis thaliana resulted in increased salt tolerance (Kariya *et al.*, 2009). Similarly, overexpression of the cysteine protease gene (CsCP) in hot pepper (Capsicum annuum L.) improved salt tolerance by regulating the expression of stress response genes (Kim *et al*., 2019).

6.2.Improved ion homeostasis

Hydrolytic enzymes may also play a role in regulating ion homeostasis under salt stress. For example, overe xpression of the vacuolar H+-pyrophosphatase (H+-PPase) gene AVP1 in Arabidopsis thaliana resulted in improved salt tolerance by maintaining ion homeostasis (Li *et* *al*., 2005). Similarly, over expression of the vacuolar proton pump pyrophosphatase (H+-PPase) gene from the halophyte Suaeda salsa (SsVP) in Arabidopsis thaliana improved salt tolerance by regulating Na+/K+ homeostasis (Wang *et al*. ., 2014).

6.3.Activation of antioxidant defenses

Salt stress can lead to oxidative stress in plants, leading to cell damage and cell death. Hydrolytic enzymes may play a role in activating antioxidant defense mechanisms to protect against oxidative damage. For example, over expression of the aspartic protease gene OsAP65 in rice improved salt tolerance by activating the antioxidant defense system (Kaur *et al*., 2017).

6.4.Modulation of hormonal signaling

Hydrolases can also modulate hormonal signaling pathways that are important for salt tolerance. For example, overexpression of the cysteine protease gene RD21A in Arabidopsis thaliana enhanced salt tolerance by modulating abscisic acid (ABA) signaling (Shindo *et al*.l., 2012).

7.Hydrolases function at the germination

A group of enzymes known as hydrolases is responsible for breaking down complicated compounds into their component parts by joining water molecules to the chemical bonds. Hydrolases are essential during seed germination because they help release

stored nutrients that the growing embryo needs for energy and building blocks.(Shindo *etal*.,2012)

8. Functions of amylases in germination

During seed germination, amylases play an important role as hydrolase in breaking down stored starch into glucose. Glucose is used as an energy source for the developing of plant embryo. When the seeds absorb water, amylase is activated, breaking down the starch granules and releasing glucose. This process is essential for seedling growth and survival. The role of amylases in seed germination has been extensively studied, and many researchers have reported the effects of amylase activity on germination. For example, a study by Li and colleagues (li *et al*; 2021) found that increasing amylase activity in rice seeds promoted germination and improved seedling vigor. Similarly, a study by Pagnussat and colleagues (pagnussat *et al*; 2019) showed that inhibition of amylase activity in Arabidopsis seeds delayed germination and decreased seedling growth.

Biostimulants

1. Definition of Biostimulants

The term biostimulant was coined in the early 1990s (yakhin*et al*., 2017)The term "biostimulant" is what gardening experts use to describe it Substances, not nutrients, that promote plant growth, soil conditioners or pesticides. These substance can be natural or synthetic Apply to seeds, plants or soil. (CDA, 2021) The EBIC concept of biostimulants includes products containing some of them Nutrients, unless the effect on plant growth is direct Fertilize. "Biostimulants have a different mechanism of action than fertilizers, Regardless of the presence or absence of nutrients in the product" (European Biostimulant Industry 2012b).

2. Effects of biostimulants on plants

- promote plant growth and development when used in small quantities
- Improved nutrition Nutrient uptake or reduction of nutrient losses to the environment, or both. or serve as earth Additives that improve the structure, function or performance of soil, thereby improving it Plant Response" (Biostimulant,2013)
- Stimulates seed germination and improves production quality Optimal conditions.
- Helps improve nutrient absorption. Certain Amino Acids in Biostimulants Can Do This Incorporates micronutrients, especially to help plants extract nutrients from high pH levels floor.
- Increased resilience to abiotic stresses such as climate change and minerals Lack of water, excessive salinity, drought or even excess water. These properties depend on the composition of the biostimulant (CDA, 2021).

3. Source and properties of biostimulants

There are different types of biostimulants.(Benhamou and Rey, 2012).

- Plant extracts
- Seaweed Extract
- microorganisms and their extracts, amino acids and hydrolyzed proteins,
- Humic substances or similar substances (humic acids, fulvic acids, lignosulfonates,etc.), non-nutrient minerals,
- Biomolecules (enzymes, vitamins, antioxidants, etc.)
- Phytohormones.

4.Plant extracts

Many bioactive components, such as different antioxidants, different minerals, and many osmoprotectants, are present in different plant extracts. These bioactive ingredients are Strengthen the plant defense system and effectively fight against multiple Stressors (Abd El-Mageed *et al*., 2017; Desoky *et al*., 2018a.,Rehman*et al*., 2018; Desoky *et al*., 2019)

To help stressed plants, extracts from the plant's different parts are used as bioactive stimulants.

The endogenous components of a plant's antioxidant defense system are inadequate to support healthy growth during the most stressful situations.

4.1.the aqueous extract of *Pistacia lentiscus* leaves L.

4.1.1.Geographical distribution of *Pistacia lentiscus* leaves L.

Is widespread in dry areas of the Mediterranean region, including Asia, Europe, Africa, and the Canaries (Maameri - Habibatni, 2014) In the open, *Pistacia lentiscus L* grows in the garrigue and on any kind of soil in Algeria. It grows in semi-arid, subhumid environments, particularly in the Soummam basin with Aleppo pine, holm oak, and cork oak.(Bougherara and Merzougui,2014)

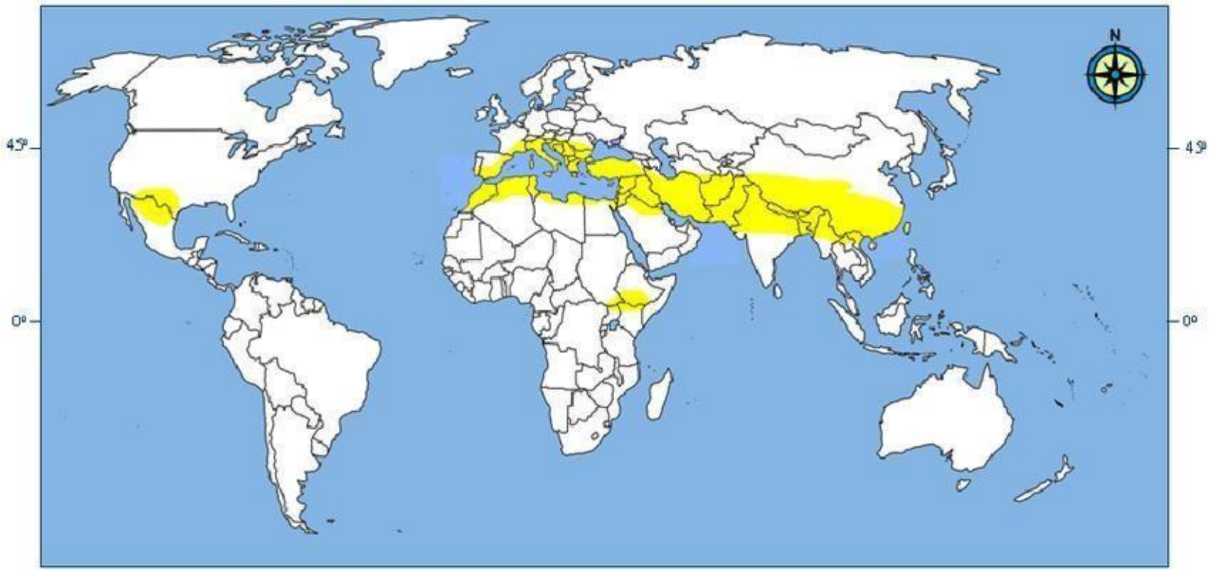

Figure 5: Range of the genus Pistacia lentiscus leaves L.(**https://www.researchgate.net 2009**)

4.1.2.The features of *Pistacia lentiscus* leaves L.

This tiny woody plant has parippinnate, evergreen leaves with 4 to 10 elliptical, leathery, and shiny leaflets. The stem is clearly winged (Hans, 2007).

Figure 6: Representation of mastic tree leaves(Chouhim)

Leaf extracts with high concentrations of total phenols, flavonoids, and tannins exhibit powerful antioxidant potential as well as significant H_2O_2 damage inhibition (Remila *et al.*, 2015)

4.1.3.The aqueous extract of *Pistacia lentiscus* leaves L.and its function as biostimulantat the germination

Pistachio extract has been studied for its potential use as a biostimulant to enhance plant growth and improve germination.

Several studies have investigated the effects of pistachio extract on plant germination. For example, one study found that treating tomato seeds with pistachio extract increased germination rate, seedling vigor, and root and shoot growth compared to untreated seeds (Shahabi *et al*., 2021). Another study reported similar results with pepper seeds, showing that pistachio extract increased germination percentage, seedling length, and fresh and dry weight compared to a control group (Mohammadi *et al.*, 2017).

In addition to enhancing germination, pistachio extract has also been shown to improve plant growth and yield. For instance, a study conducted on wheat plants found that foliar application of pistachio extract increased plant height, chlorophyll content, and grain yield compared to untreated plants (Mousavi *et al*., 2016).

The mechanism by which pistachio extract improves plant growth and germination is not fully understood, but it is thought to be related to its antioxidant and growth-promoting properties. Pistachio extract contains high levels of antioxidants, which can help protect plants from oxidative stress and promote healthy growth (Mousavi *et al.,* 2016).

4.2.Olive mill wastewater

Olive mill wastewater is a foul-smelling acidic dark liquid that is generated during the extraction of olive oil and has a significant negative impact on the environment (Dhaouadi and Marrot 2008)

4.2.1.Effects of the OMWW on the ecosystem

OMW is considered the most polluting effluent produced by the olive industries (Niaounakis and Halvadakis ,2006). It is characterized by high concentration of polyphenols which varies from 0.1 to 18 g/L (Davies *et al*. 2004., Hamdi 1993), acidic pH ranging between 3 and 6 (Azzam, Al-Gharabli and Al-Harahsheh, 2015), high concentrations of reduced sugars and high concentrations of organic matter represented by high values of COD that may exceed 220 g/L (Mantzavinos and Kalogerakis, 2005).

4.2.2.The physic-chemical properties of the OMWW

The amount and physico-chemical properties of the OMWW depend on the method used for the olive oil extraction. Today, centrifugal systems have replaced traditional pressure method. The three-phase centrifugal system generates a solid husk, oil, and OMWW while the two-phase centrifugal system releases a wet olive husk and oil. Relative to the two-phase centrifugation, the three-phase system utilizes 0.6–1.3 m3 of additional water during decantation that eventually increases the amount of OMWW (Gebreyohannes, Mazzei, and Giorno 2016).

4.2.3.The treatment of OMWW

In recent years, many management options have been proposed for the treatment of OMWW. Most of these methods aim to reduce the phytotoxicity of OMWW for reuse in agricultural applications, or alternatively to recover the biophenolic fraction due to its interesting pharmacological properties (Dhaouadiand Marrot 2008; El-abbassi, Khayet, and Hafidi 2011; Gebreyohannes *et al*. 2016; Servili *et al*. 2011). It is worth quoting that wastewater from olive oil mills can be interesting biological source of high added value compounds, such as hydroxytyrosol (3,4-di-hydroxyphenyl-ethanol) or other antioxidant phenolic compounds. In fact, the phenolic compounds are known as important natural antioxidants with nutritional and pharmaceutical properties (Tuck and Hayball ,2002).

Hydroxytyrosol and tyrosol are the main phenolic compounds in extra virgin olive oil.

Hydrophilic extracts of olive oil contain many phenolic compounds and phenyl-alcohols. Oleuropein, hydroxytyrosol and their derivatives are found in large quantities in olive leaves and olive fruits (Kashaninejad *et al.* 2020, 2021).

4.2.4.The effects of the centrifuged olive mill wastewater on the germination

According to (Gharibi *et al*., 2019) treating tomato seeds with the centrifuged olive mill wastewater hydrolat significantly increased seed germination percentage, seedling length, and seedling fresh and dry weight compared to untreated seeds. Another study reported that applying the centrifuged olive mill wastewater to wheat seeds enhanced seed germination, root and shoot length, and chlorophyll content(Salem *et al.*, 2018).

Experimentel part

Materiel and methods

II- Material and methods

1. Objective of this work

Our study aims to investigate the effects of using the aqueous extract of *Pistacia lentiscus* L. leaves and the centrifuged Olive mill wastewater known as biostimulants, on enhancing cowpea germination under salt stress conditions.

2. Plant material

The plant material includes seeds of the ecotype *Vigna unguiculata* native to Azazga, of white-beige colour with a hilum (attachment point to the pod) white surrounded in black, supplied by the technical institute of vegetable and industrial crops of Algiers (ITCMI).

Figure 7: seeds of the cowpea variety "*Vigna unguiculata*"(Mis/Amrane 2023)

3. Setting up of the experiment

The experiment was conducted in the laboratory of Science and Technology at TISSEMSILT university

The cowpea seeds were disinfected with 1% sodium hypochlorite by soaking them for 3 minutes, then rinsed with distilled water several times to remove traces of chlorine. The seeds were placed in sterile 9 cm diameter and 1 cm plastic packs lined with two layers of blotting paper at a rate of 10 seeds per plastic pack. Then, for the experimental device, the packs were divided into three treatments according to the salinity level, control (0 meq), 150meq and 250meq of NaCl; that is 3 repetitions for each treatment. In this block, the saline solutions constituting the different germination media are prepared from distilled water.

Two other similar devices were involved in the study, the first depending on the application of the aqueous extract of *pistacia lentiscus* L. leaves , and the second device concerns the centrifuged olive mill wastewater .

In each pack the seeds are soaked to 20 ml of the saline solution. During germination tests, the packs are kept at a temperature of 26°C.

Figure 8: Arrangement of cowpea (*Vigna unguiculata*) grains in plastic packs.(Amrane/Mis 2023)

Figure 9: Distribution of Vigna unguiculata seeds in the oven.(Mis/Amrane 2023)

3.1.Preparation of the aqueous extract of *Pistacia lentiscus L.*

Fresh and mature *Pistacia lentiscus* leaves L. have been collected in the vicinity of the town of Tiaret. After collection, 200g of leaves were washed and stored overnight at freezing temperatures, resulting in 125 ml of the aqueous extract of the *Pistacia lentiscus* leaves L. The extract was diluted 30 times with distilled water to prepare a 1:30 aqueous extract of *pistacia lentiscus* L.leaves solution(Latif, 2016).

Figure 10: preparation of the aqueous extract of the leaves of the pistachio*(Pistacia lentiscus* L.).(Amrane/Mis 2023)

Figure 11: filtration of aqueous extract of the leaves of pistachio *(Pistacia lentiscus L.).(*Mis/Amrane 2023*)*

Figure 12:storing the solutions in the refrigerator.(Amrane/Mis 2023)

3.2.Preparation of centrifuged olive mill wastewater

Olive mill wastewater (OMW) is obtained by olive oil extraction systems. It contains high concentrations of organic compounds mainly phenols which represent a source of pollution for the environment. Therefore, there is a need for their treatment or their valorization (Rais *et al.*,2017; Azzam *et al.*, 2020).The treatment was carried out by centrifugation at 8,000g for 10 minutes and the supernatant was recovered. This operation was repeated 4 times and the supernatant was used in in seed germination tests.

Figure 13:centrifuged olive mill wastewater.(Mis/Amrane 2023)

		
centrifugation	**Olive mill wastewater**	**Stirrer**

Figure 14:preparation of centrifuged olive mill wastewater.(Amrane/Mis 2023)

Figure 15:centrifuged olive mill wastewater.(Mis/Amrane 2023)

4.Parameters measured

4.1.The final germination rate

This rate is obtained by the number of seeds germinated from the beginning to the endof germination.

4.2.Cowpea seed amylase activity

4.2.1. Imbibition test

The second part is based on the evaluation of the imbibition of seeds germinated in different germination medium, by weighing the seeds every day, until the appearance of the radicle (beginning of growth). This test is necessary to determine the appropriate time for the extraction and analysis of amylases.

Figure 16:Evaluation of the imbibition seeds of cowpea.(Amrane/Mis 2023)

4.2.2Assay of amylase activity of germinating seeds

During seed germination, amylase activity is essential for the remobilization of carbohydrate resources, stored in the form of starch.

The amylase activity is measured according to the method of (Bernfeld .,1955). The principle of this method is based on measuring the reducing power of maltose released during the enzymatic hydrolysis of starch. The color intensity is proportional to the amount of maltose released (Bernfeld, 1955). The enzymatic activity is expressed per µmole of maltose released per minute.

4.2.2.1.Extraction of the enzyme complex

The extraction of the enzymatic complex is produced in two stages of the seed germination process, 24 and 48 hours. The extraction substrate consists of 4 g of seeds from different germination media.

The whole is ground in 12 ml of acetate buffer solution at pH 4.8, and filtered. The filtrate is collected in a 1.5 ml eppendorf tube, and then centrifuged for 10 min at 8000 g. The supernatant is recovered (extracted A)

Figure 17:Extracted A (Mis/Amrane 2023)

4.2.2.2.Amylase activity assay

In the test tubes, add to 1ml of the extract (A), 0.5ml of 1% starch solution based on starch and acetate buffer solution pH 4.8; Vortex and incubate in a water bath at 25°C for 10 min. Add 0.5 ml of reagent (A) containing 28 ml NaOH (2N), 0.4 g dinitrosalicylic acid (DNS), 12g tartrate (K/Na), 40ml distilled water, allowing hydrolysis inhibition and simultaneous determination of the maltose formed. The whole is placed in a water bath at 100°C for 5 min, then cooled. The reading of the optical density was done with a spectrophotometer at the wavelength λ=530 nm.

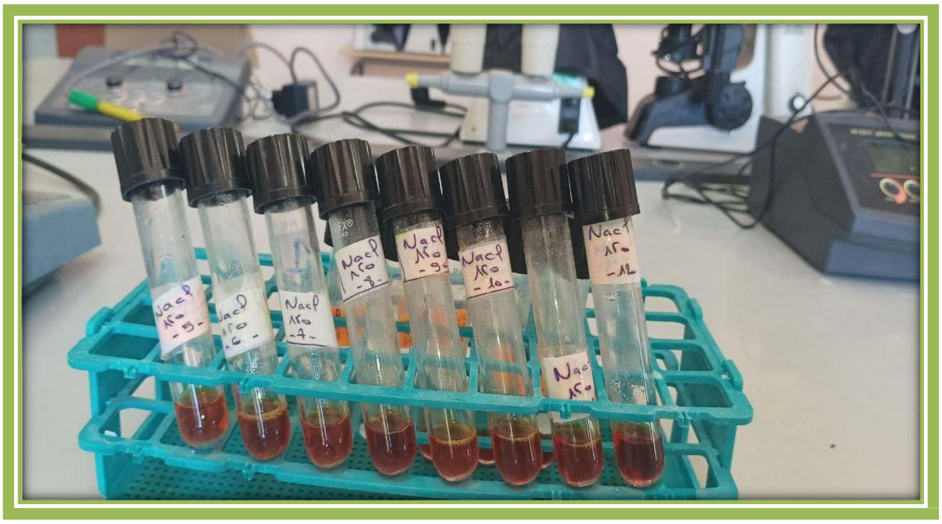

Figure 18: Tubes of amylase activity assay.(Amrane/Mis 2023)

5.Performing the calibration curve

The alkaline reduction of 3-5 dinitrosalicylic acid by maltose, causes an orange coloration whose intensity is proportional to the concentration incarbohydrate. A maltose stock solution (1g/l) is used in the test tubes standard range in acetate buffer (acetic acid and sodium acetate, pH 4.8) at dilutions of 1/10.1/5.1/3.1/2 to a final volume of 1ml. 0.5 ml of each dilution in test tubes, to which 0.5 ml of reagent (A) is added, the whole is mixed delicately. The tubes are placed in a boiling water bath for 5mn, then cooled by a jet of cold water and into which 5 ml of distilled water is added. The dosage is spectrophotometer at a wavelength of λ=530 nm.

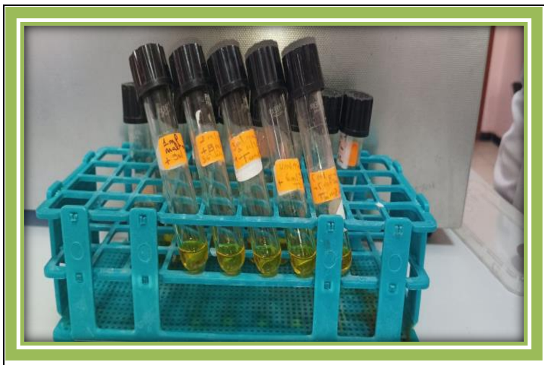	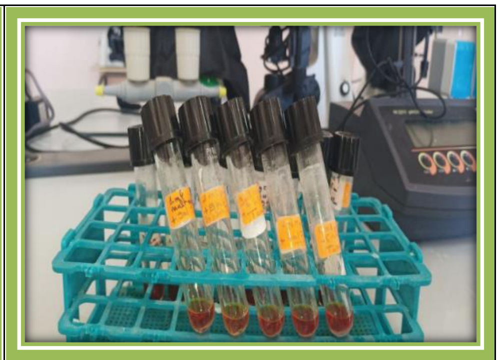
Before	After

Figure 19: Test tubes before and after putting them in the water bath (AMRANE/MIS 2023)

6.Statistical Analysis

The statistical analysis of the data was conducted using the 2 factors(ANOVA) at a 95% security threshold using SPSS version 20 software.

Interpretation of the results

Interpretation of the results

1. The physico-chemical parameters of germination

1.1Final germination rate

1.1.1. finale germination rate under the salinity in the presence of the aqueous extract of *Pistacia lentiscus L.leaves*

Table 1: Testing the effects of aqueous extract of pistacia lentiscus L.leaves on salt stress

Source	**DDL**	**D**	**Sig.**
NaCl	2	32,481	,000
Aqueous extract of pistacia lentiscusL.leaves	1	,148	,707
NaCl * aqueous extract of *pistacia lentiscus* L.leaves	2	,037	,964

The analysis of the obtained results (Tab.01), indicates that the salinity by the application of increasing concentrations of NaCl has a difference very highly Significant on the final rate of germination of *vigna unguiculata* ecotype kabyle seeds ($p<0.001$). As well as the contributionof the aqueous extract of the leaves of Pistachio mastic tree in the presence of NaCl in the media of germination has a significant effect ($p<0.05$).

The results in the figure n°20 indicate that the final seed germination rate decreases clearly with increasing NaCl concentrations. Under concentrations of 150 meq/l of NaCl, the final seed of germination decreases by 90% compared to the control (0 meq/l).

While the addition of the aqueous extract of pistachio mastic tree in the germination media of seeds stressed with 150meq/l of NaCl, caused a significant increase of 33.33% in the final rate of germination of seeds compared to those receiving the saline solution. That is an improvement rate of 23.33% recorded in the treatments of 150meq/l of NaCl compared to the control.

It is thus noted that under the concentration of 250meq/l of NaCl no seed germinated

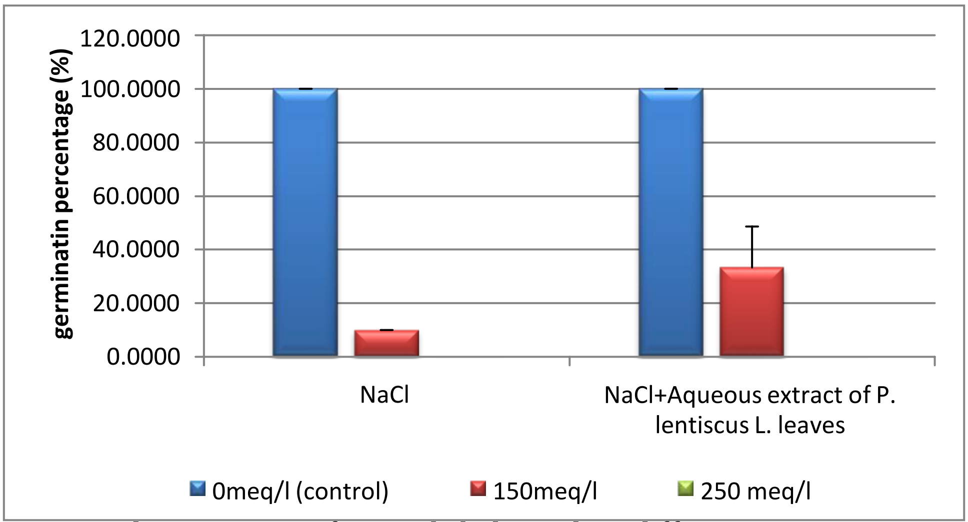

Figure 20: Total germination of Vigna kabyle seeds in different concentrations of NaCl combined with aqueous extract of *Pistacia lentiscus* L. leaves.

1.1.2.final germination rate under salinity in the presence of centrifuged olive wastewater

Table 2: Testing the effects of the centrifuged olive mill wastewater on salt stress

Source	ddl	D	Sig.
NaCl	2	32,808	,000
Centrifuged olive mill wastewater	1	,346	,567
NaCl * centrifuged olive mill wastewater	2	,115	,892

Statistical analysis of the results (tab n°2) indicate that salinity has a very highly significant effect on the final rate of germination ($p<0.001$). While the application of centrifuged olive wastewater mill in the presence of NaCl in seed germination media has a very highly significant effect by reducing effect of salt stress ($p<0.001$).

The results of the figure n°21 show that the final rate of germination of *Vigna unguiculata* seeds decreases very significantly as a function of the increase in NaCl concentrations. Under concentrations of 150 meq/l of NaCl, the level decreases by 90% compared to the control (0 meq/l).

However under the salt stress of the level of 150meq/l of NaCl, the final rate of germination increases by 100% following the exogenous contribution of the centrifuged olive mill wastewater That is an improvement rate of 10% recorded in the treatments of 150meq/l of NaCl compared to the control. Therefore no seed germinated under the concentration of 250meq/l of NaCl. While in the presence of centrifuged olive *vigna unguiculata* seeds recorded a germination rate of 26.67%.

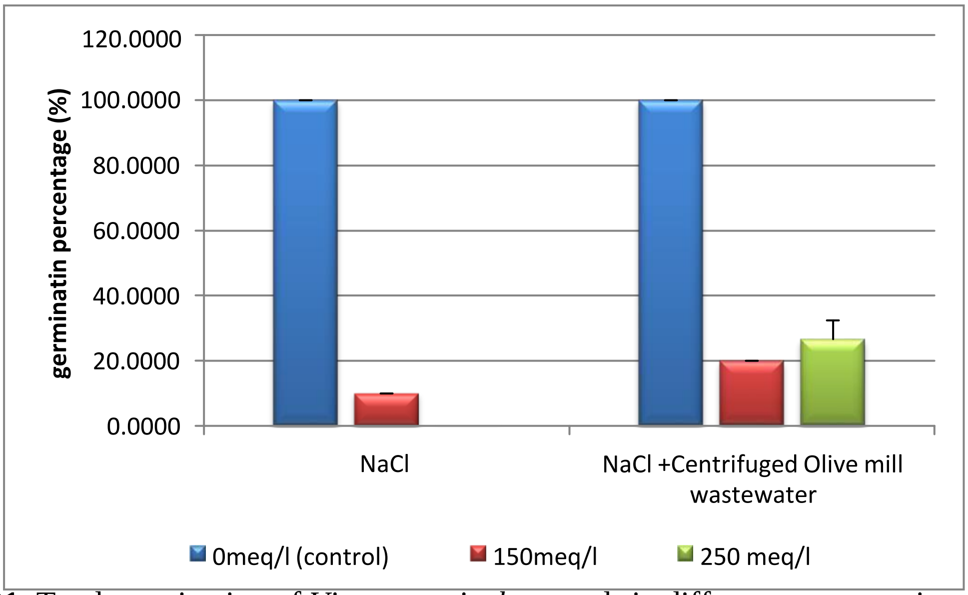

Figure 21: Total germination of *Vigna unguiculata* seeds in different concentrations of NaCl combined with centrifuged olive mill wastewater.

2. precocity of germination

2.1.final germination rate under salinity in the presence of the aqueous extract of the *pistacia lentiscus* L.leaves

Table 3:testing the effects of aqueous extract of pistachio on salt stress

Source	ddl	D	Sig.
NaCl	2	32,481	,000
Aqueous extract of pistacia lentiscusL.leaves	1	,148	,707
NaCl * aqueous extract of pistacia lentiscus L. leaves	2	,037	,964

The statistical analysis of the results (tab n°3) shows that the precocity of the germination of *vigna unguiculata* seeds is considerably very high significantly affected by salinity (p<0.001). As well as the contribution of the aqueous extract of the leaves of the pistachio mastic tree in the presence of NaCl in the germination media caused a significant effect (p<0.05).

The results of the figure n° 22 indicate that the precocity of germination of the seeds of *vigna* unguiculata decreases very much according to the increase in the concentrations of NaCl. Under concentrations of 150 meq/l of NaCl, the rate decreases by 93.34% compared to the control (0 meq/l of NaCl).

While under the concentration of 150meq/l of NaCl, the final rate of germination increases by 100.3% following the addition of the aqueous extract of the leaves of the pistachio mastic tree. That is an improvement rate of 13.64% recorded in the treatments of 150meq/l of NaCl compared to the control. On the other hand, the germination of the seeds subjected to the concentration 250meq/l of NaCl is nil.

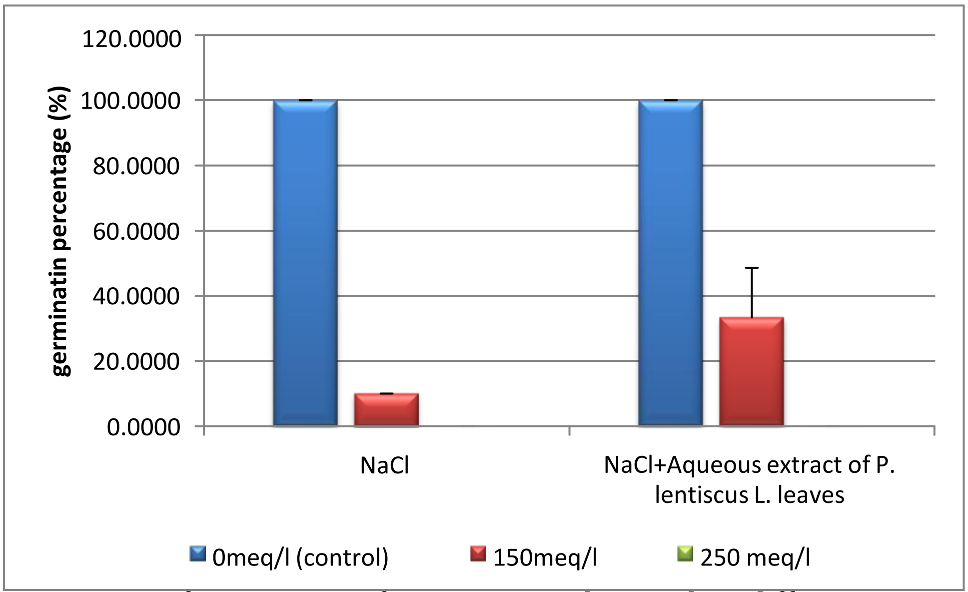

Figure 22: Precocity of germination of *Vigna unguiculata* seeds in different concentrationsof NaCl combined with aqueous extract of *Pistacia lentiscus* L. leaves.

2.2. final germination rate under salinity in the presence of centrifuged olive wastewater mill

Table 4: Testing the effects of the centrifuged olive wastewater on salt stress

Source	Ddl	D	Sig
NaCl	**2**	**,2653**	**,000**
Centrifuged	**1**	**,121**	**,000**
olive NaCl * centrifuged Olive	**2**	**,49**	**,000**

The statistical analysis of the results (tab n°4) indicate that the precocity of the germination of the seeds of *vigna* kabyle effects very highly significant on the salinity ($p<0.001$). While the application of centrifuged olive wastewater mill in the presence of NaCl in the seed germination media slightly minimized the reducing effect of salt stress the centrifuged has a very high significant effect ($p<0.001$).

The results of the figure n°23.show that the precocity of germination of *vigna* Kabyle seeds decreases very significantly as a function of the increase in NaCl concentrations. Under concentrations of 150 meq/l of NaCl, the rate decreases by 93.34% compared to the control (0 meq/l).

Under the saline stress of the level of 150meq/l of NaCl, the precocity of germination increases by 200.3% following the exogenous contribution of the centrifuged olives mill wastewater . That is an improvement rate of 13.34% recorded in the treatments of 150meq/l of NaCl compared to the control. Therefore, the germination of the seeds subjected to the concentration 250meq/l of NaCl is nil.

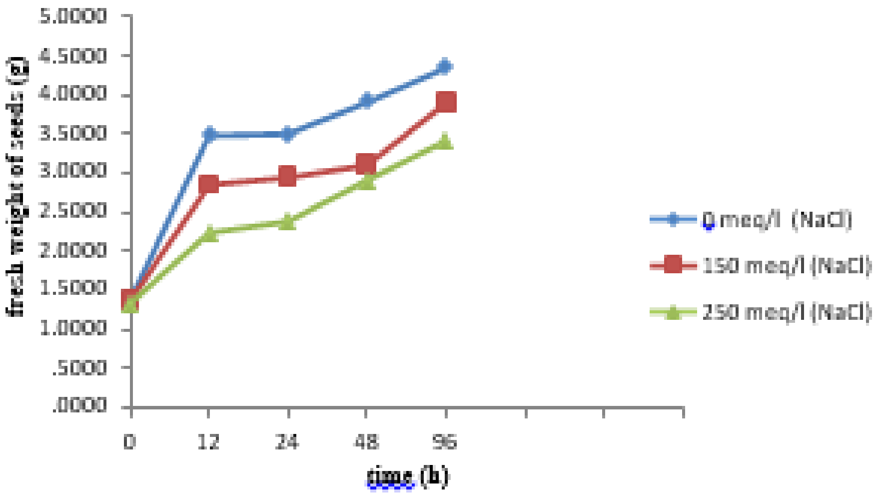

Figure 23: Fresh weight (g) of vigna kabyle seeds as a function of soaking time under different saline concentrations.

The figure 21 indicates that the first phase lasts 12 hours during which the fresh weight of Kabyle vigna seeds subjected to salinity increases rapidly. The maximum seed weight is recorded in the control (3.48 g), it decreases with increasing NaCl concentrations. Under the concentration 150 and 250meq/l, the weights of the seeds register respectively reduction rates of 16.67% and 35.92% compared to the control. The second phase is characterized by seed weight stability between 12h and 24h. Beyond that, the fresh weight of the seeds increases differently. Indeed, after 96 hours of germination, the weight of control seeds recorded a maximum value of 4.34g. Under the effect of concentrations of 150 and 250meq/l of NaCl, the weight decreases respectively by 10.14% and 21.43% compared to the control (0meq/l of NaCl). According to the imbibition curves (fig 21), we choose 24 hours of germination of the Kabyle vigna seeds as being the optimal duration at which we proceed to the extraction of the amylases.

3. The activity of amylases extracted from vigna kabyle seeds subjected to salinity in the presence of centrifuged olive mill wastewater

The results indicates that the variations in the activity of the amylases extracted from the germinating seeds are not influenced by the different saline treatments ($p > 0.05$). As well as the exogenous contribution of the centrifuged olives mill wastewater does not show any effect on the variations of the amylase activity ($p > 0.05$).

The figure n°24 shows that the evolution of amylase activity is not uniform, it increases non-significantly according to the increase in NaCl concentrations. This activity registers a value of 1310.341 µmol/min in the control seeds (0 meq of NaCl). The activity increases by 1.35% compared to the control (0 meq of NaCl) under the concentration of 250meq/l of NaCl.Under the concentration of 150 and 250meq/l of NaCl in the presence of centrifuged olive wastwater, the activity of amylases increases in a non-significant way respectively by 4.51% and 3.84% compared to the control (0meq/l of NaCl).

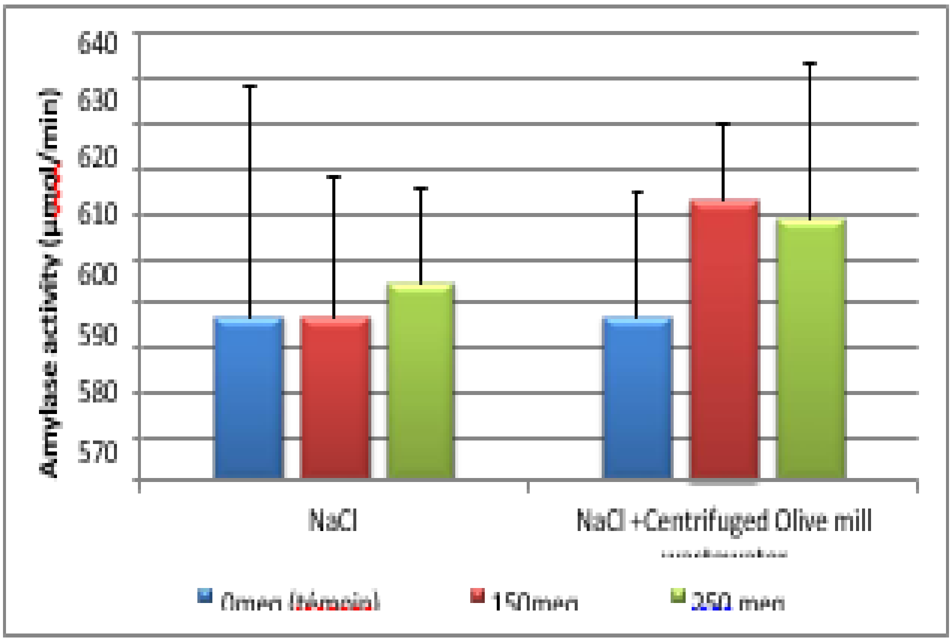

Figure 24:Amylase activity after 24 hours of germination of *vigna unguiculata* seeds in different concentrations of NaCl combined with centrifuged olive mill wastewater.

The amylase activity of seeds subjected to NaCl in the presence of centrifuged olive water increases non-significantly compared to that of seeds receiving concentrations of 150 and 250meq/l of NaCl, recording respectively rates of increase of 4.51% and 2.45 %.

Discussion

Discussion of the results:

Seed germination is a crucial phase in the life cycle of plants as it marks the transition from seed dormancy to seedling establishment (Baskin *et al.*, 2020) Likewise, germination rate is an important parameter to measure seed strength and models have been developed to calculate it (Jardim *et al*., 2021; Chao *et al.*, 2021).However, germination can be hindered by various abiotic stresses, such as salt stress, which negatively impacts the germination rate of seeds by affecting cellular membrane integrity, enzymatic activity, and inducing oxidative stress (Gao *et al*., 2019). Our study specifically focused on cowpea (*Vigna unguiculata* L.) and demonstrated that salinity reduced the final germination rate in concentrations of both 150 and 250 meq/l. According to(Zörb et *al*., 2019) and (Shahet*et al.*,*2020*), salinity is a major stress factor responsible for inhibiting germination percentage and delaying crop germination time.

Salt stress affects the water status of plants (Kamran et *al*., 2020 ;Sofy *et al*., 2020).Nevertheless, the application of biostimulants, including centrifuged olive mill wastewater and aqueous extract of *Pistacia lentiscus ,/* leaves, has shown promising results in improving the germination rate of *Vigna unguiculata* seeds. These aqueous plant extracts mitigate the negative effects of salt stress on growth, as supported by several studies. For example, a pot experiment conducted by (Latif *et al.*,2016) investigated the effect of foliar spray with *Moringa oleifera* leaf extracts on common bean plants (*Phaseolus vulgaris* L.) grown under salt stress. The results suggested that Moringa leaf extract, rich in amino acids, ascorbate, zeatin, minerals, and other growth-promoting compounds, activated antioxidants in plants, alleviating oxidative damage and improving physiological and molecular attributes under adverse environmental conditions.

A related study by (MultuDurak *et al.*,2021) demonstrated that treatment of maize seeds with weeping willow leaves and bark as biostimulant agents enhanced seedling growth and establishment under both control and stress conditions.

Furthermore, the addition of centrifuged olive mill wastewater to *Vigna unguiculata* seeds exhibited positive results. Under a concentration of 150 meq of NaCl, the final germination rate increased by 100%. A study conducted by (Rais *etal.*,2017) showed that treated olive mill wastewater significantly improved germination in tomato seeds, regardless of the dilution rate. This improvement can be attributed to the high concentrations of organic and inorganic compounds, such as polyphenols and salts, which may have phytotoxic effects on germinating seeds. These compounds can inhibit germination, affect root development, or induce oxidative stress in seedlings.

Aqueous extracts, including the aqueous extract of pistachio lentisque leaves and centrifuged olive mill wastewater, contain various compounds such as phytohormones, antioxidants, phenolic compounds, and enzymes. These compounds have specific physiological effects on germination by regulating different aspects, including embryo growth, dormancy release, and seedling establishment (Loffler *et al.*, 2019).

During seed germination, amylase activity plays a crucial role in mobilizing carbohydrate resources stored as starch. Analysis of the results indicates that the activity of amylases extracted from germinating seeds is not influenced by different saline treatments. Additionally, the exogenous contribution of centrifuged olive mill wastewater does not affect amylase activity. It can be inferred that salinity can affects amylase functioning, not its synthesis, as demonstrated in a study by (Kaplan *et al.*,2004). Maltose, which accumulates due to b-amylase induction, has been shown to protect proteins, membranes, and the photosynthetic electron transport chain under acute temperature stress.

Conclusion

Salinization has occurred in many of Algeria's arid regions. It leads to severe soil degradation and considerably limits agricultural production, particularly of glygophytes. These include cowpea, which it is particularly crucial for the livelihoods of the least developed countries. Our study aims to investigate the effects of using the aqueous leaf extract of *Pistacia lentiscus* L. and the centrifuged Olive mill wastewater known as biostimulants, on enhancing cowpea germination under 150 and 250meq/l of NaCl.

The results showed that salinity considerably reduces the final rate of grain germination as well as the earliness of germination .Nevertheless, the application of biostimulants, such as centrifuged olive mill wastewater and the aqueous extract of *Pistacia lentiscus* L. leaves, demonstrated positive effects on the germination of *Vigna unguiculata* seeds. While the aqueous leaf extract of *Pistacia lentiscus* L. and the centrifuged Olive mill wastewater have a significant effect on the germination because it contain various compounds, including antioxidants and phenolic compounds, soluble sugars, which contribute to the improved germination process and the other development stages . The centrifuged olive mill wastewater appears to be more advantageous than the aqueous leaf extract of *Pistacia lentiscus* L. in limiting the effect of salt stress, since under the 250meq/l concentration.

In the second part, the results showed that the activity of amylases extracted from germinating seeds is not influenced by different saline treatments. Additionally, the exogenous contribution of centrifuged olive mill wastewater does not affect amylase activity.

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