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**USE OF SEAWEED AND PLANT  
EXTRACTS TO IMPROVE CALLUS  
INDUCTION IN TOMATO**

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## List of Abbreviations

BAP: 6-Benzylaminopurine

CL: Clove (*Syzygium aromaticum*)

CON: Control

DMSO: Dimethyl sulfoxides

DPPH: 1-diphenyl-1-2-picrylhydrazyl

IVC: In vitro culture

MPEs : Medicinal plant extracts

MS: Murashige and Skoog medium

NAA: Naphthalene acetic acid

PGRs: plant growth regulators

PCI: percentage of callus induction

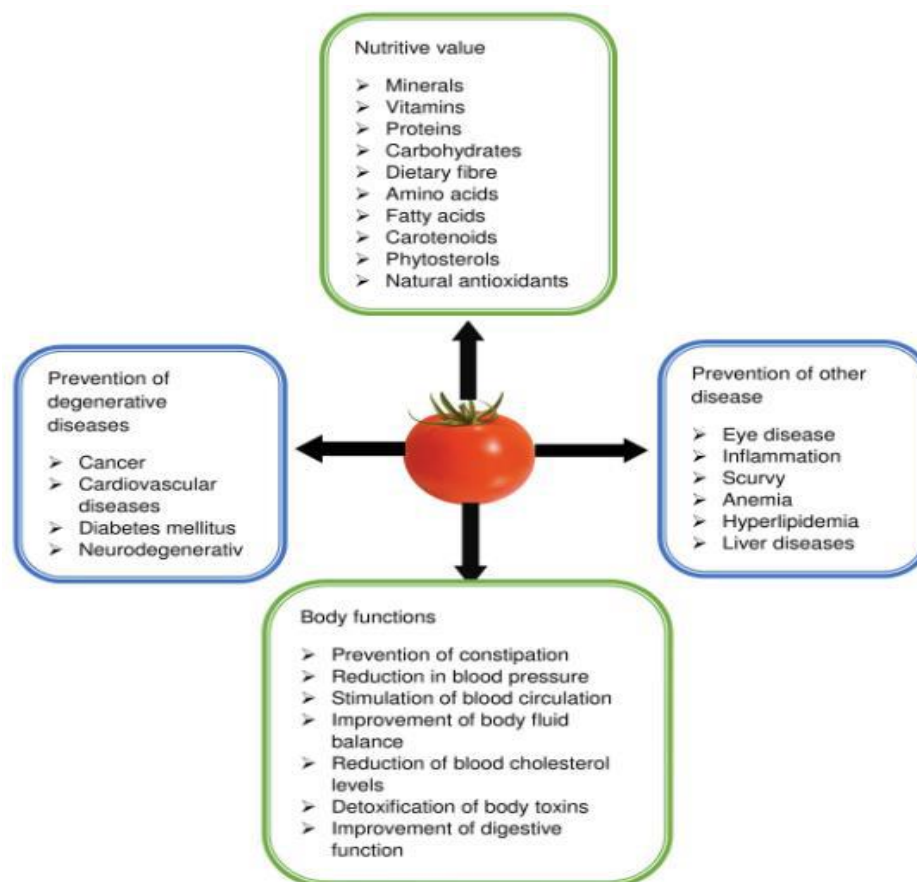
RM: *Rhamnus lycioides*

TF: Tafgha or *Rhaponticum acaule*

SWE: seaweed extract

2,4-D: 2,4-Dichlorophenoxyacetic acid

Tomato (*Solanum lycopersicum* L; *Solanaceae* family), is the most widely grown vegetable in the world. According to the latest **FAOSTAT** report (2022), world tomato production exceeded 189 million tons in 2022, with China, India, and Turkey being the largest producers. Tomato plays a vital role in keeping people safe and vigorous. Tomatoes are an important source of vitamin C, B, and an excellent source of  $\beta$ -carotene (**Raziuddin et al., 2004**).



**Figure 1. Summary of health benefits of tomato (after Ali et al., 2020)**

Conventional propagation methods are very slow and propagation by tissue culture represents an important potential to effectively propagate it. The plant tissue culture techniques are an appropriate tool for improving many crops and vegetables (**Kader et al., 2015**) and also it is considered as an important tool for modern plant improvement programs to develop suitable cultivars in minimum time (**Tagi et al., 2002**).

Callus formation was the effective technique of tissue culture by which embryogenesis, organogenesis and cell suspension are formed when callus is transferred to suitable media (**Cardoza, 2016**). Callus is vacuolated cells contain regions of unorganized small meristematic cells cluster, that are induced by plant growth regulators (PGRs) from a cut region of the explants (**Efferth, 2018**).

Plant callus extracts are potential to be developed as ingredient in many nutraceutical and pharmaceutical products (**Babich et al., 2020; Kumari & Ramakrishna, 2024; Minutolo et al., 2020**). For example, tomato callus is supposed to contain protein-derivatives and or other components such as secondary metabolites that play a role in skin regeneration. Therefore, the production of calli is important to be studied for callus sustainable supply (**Setiaji et al., 2020; Nurchayati et al., 2023**).

Callus formation and tomato organogenesis rely on endogenous and exogenous growth regulators (**Osman et al., 2010**). Therefore, the effect of different PGRs has been extensively investigated on callus induction, shoot regeneration and hairy root formation in tomato (**Abd El-Hameid 2021; Shah et al., 2015; Sohail et al., 2015; Yaroshko et al., 2023**). According to **Coenen & Lomax (1997)**, callus induction requires cytokinins and auxins which are essential for cell growth by promoting cell division and elongation. Cytokinins in combination with auxins or alone in media have enhanced callus induction for many tomato cultivars (**Hanur & Krishnareddy, 2016**).

Phytohormones are the costly chemicals in plant tissue culture. According to **Jalal et al. (2015)**, plant extracts can be used as an effective alternative to phytohormones in tissue culture. Plant extracts has the promising potential for the induction of callus and direct organogenesis, thus could be used as an alternative to phytohormones such as 2, 4 D, BAP or kinetin (**Abed et al., 2006; Sohrabi et al., 2025; Tahiri et al., 2023**)

In recent years, the use of algae and plant extracts as *biostimulants* has become an increasingly important and fundamental approach (**De Saeger et al., 2020; Jalal et al., 2015; Sohrabi et al., 2025**). By using these natural substances, we can avoid harmful chemicals and promote healthier and more sustainable agricultural practices. Therefore, the expansion of these natural approaches is a positive development that can benefit both farmers and consumers alike.

The wide range of plant species in Algeria presents an attractive opportunity to further explore natural compounds that stimulate plant growth. For instance, several previous studies have evidenced the potential use of seaweed and plant extracts to increase the callus induction and plant regeneration of many crops (**Jalal *et al.*, 2015; Sohrabi *et al.*, 2025; Tahiri *et al.*, 2023**).

Therefore, the main objective of the present work is to investigate the impact of seaweed and 3 medicinal plant extracts on in vitro tomato seed germination and callus induction.

### 1. Medicinal plant extracts (MPEs) preparation

In this study, in addition to ‘*dalgin*’ (seaweed extract) (see **Attia *et al.*, 2023**) and *Syzygium aromaticum* cloves (**Yadav *et al.*, 2024**) purchased from the market, two local medicinal plants, namely: *Rhaponticum acaule* L (DC) (roots) and *Rhamnus lycioides* subsp. *oleoides* (leaves) were used. These plants are widely distributed across the Eastern North of Algeria, especially in the BLEZMA National Park ([https://en.wikipedia.org/wiki/Belezma\\_National\\_Park](https://en.wikipedia.org/wiki/Belezma_National_Park)) from where they were collected. The plant’s samples were crushed after drying at room temperature. For extraction, approximately 5 g of each plant powder (<0.2mm) were soaked in 50 ml of methanol (80%) for 24 h with constant stirring at room temperature. The mixture was then sieved over the Whatman No:1 filter paper to remove debris. The filtrate was evaporated to dryness using a rotary evaporator (40°C) and the residue was restored in 5 mL of DMSO. This filtrate represented the 100% crude extract. The 50 and 100 ppm concentrations of each extract were subsequently prepared by adding distilled water. Before use, the extracts were strained through a fine mesh cloth and sterilized using a membrane filter (0.45µm).



*Seaweed* (SW)



*Syzygium aromaticum* (Cloves, CL)



*Rhaponticum acaule*  
(Roots, TF=Tafgha)



*Rhamnus lycioides* subsp. *Oleoides*  
(Leaves, RM)

**Figure 2. Photos of the seaweed and the plant material used in the present study.**

## 2. Estimation of polyphenols

Total phenolics were assayed using the Folin-Ciocalteu reagent, following **Singleton et al. (1999)**. Different concentrations of gallic acid were used for the standard curve. The total phenols content was expressed as mg of gallic acid equivalents (GAE) per gram of dry weight through a calibration curve range of 50 to 400  $\mu\text{g}\cdot\text{ml}^{-1}$  using the equation  $A = 0.0178[\text{GAE}] + 0.0427$  ( $R^2 = 0.9904$ ).

## 3. Antioxidant activity (DPPH) assay

DPPH' assay was carried out according to (**Liu et al., 2004**) with some modification. The radical-scavenging capacity of the extracts was calculated with the following equation:

$$\text{Antioxidant activity (\%)} = (A_0 - A_s) \times 100 / A_0$$

Where  $A_0$  is the absorption of the blank (DPPH);  $A_s$  is the absorption of the extract sample.

## 4. Plant material

Tomato seeds (var. *super strain B*) were purchased from a local market.



**Figure 3. characteristics of tomato var. SUPER STRAIN “B”**

- \* Oval/square fruits hybrid, typical dual purpose.
- \* Mid-early maturity.
- \* Large fruited 120-140 g.
- \* Very productive strong plant with very good cover.
- \* Firm fruits, very good shelf life.
- \* Very fleshy and pulpy fruits.
- \* Uniform ripening.
- \* Super strain B, proven very good adaptability to the hot weather areas.

<https://unigenseedsitaly.com/en/seed/superstrain-b-f1/>

## **First experiment: Impact of SW and MP extracts on in vitro tomato seeds germination.**

- **Culture Conditions**

The basic culture medium used for germination was that of MS, the macro-elements of which were diluted by half (MS/2). After adding sucrose at 1% concentration, the pH of the culture media was adjusted to 5.8 before solidification with agar at 8 g·L<sup>-1</sup>. After being sterilized by autoclaving at 110°C for 20 minutes, the culture media corresponding to the treatments described above were distributed in culture plates (90 mm) which were filled up with 25 mL (or in test tubes filled with 10 mL).

- **Seed Disinfection and Germination test**

Tomato seeds (var. *super strain B*) were first soaked in tap water or one of the four plant extracts (50 or 100 ppm for each one) for 24 hours according to **Touati et al. (2015)**. After soaking duration surface sterilization of seeds was done by washing them with water containing a few drops of tween-20 for 3 minutes and then rinsing with tap water for 30 min before the disinfecting agent. This consists in immersion in ethanol at 70 ° for two seconds and then in a sodium hypochlorite solution (1 to 2%) for 15 to 20 minutes. The latter immersion is followed by several rinses with sterilized distilled water for 10 min each.

Each Petri dish was aseptically sown with 20 disinfected tomato seeds. The experiments were incubated at a temperature of 24 ±2°C in a culture chamber provided with neon incident light. The light intensity corresponded to a synthetically active radiation equivalent to 50 μE·s<sup>-1</sup>·m<sup>-2</sup>, with a 16 h light/8 h night photoperiod. Thus, germination treatments consisted of 01 tomato variety subjected to 9 different treatments (CON; SW0.1%; SW0.5%; TF50ppm; TF100ppm; RM50ppm; RM100ppm; CL50ppm; CL100ppm) with three replicates. The effect of SWE and MPE treatments was studied by calculating the final cumulative rate of germination (%) after 15 days of culture. The breakthrough of the radicle from the seed coats was used as the criterion for germination. Fifteen-day-old seedlings were used to measure the growth parameters (length and biomass).

## Second experiment: Impact of SW and MP extracts on tomato callus induction.

- **Callus induction**

Hypocotyls or leaves (obtained from 21-day-old seedlings) of tomato were cut into 0.5 - 1 cm<sup>2</sup> sized explants, and cultured on the Murashige & Skoog (MS) medium (1962) supplemented with 30 g/L sucrose and 8 g/L agar (Sigma-Aldrich, USA).

Tomato explants were cultured on different culture media composed of MS medium without or with a combination of MPEs and PGRs (Table 01). The pH of the medium was maintained at 5.8 with 1.0 N NaOH or 1.0 N HCl before autoclaving at 121°C for 20 min. The cultures were incubated at 24 ±2°C temperature, under 16 h photoperiod and photosynthetic photon flux density (PPFD) of 50 μmol m<sup>-2</sup>×s<sup>-1</sup> provided by white fluorescent lights. For each treatment 4 explants per dish and 4 repetitions were investigated.

After 4 weeks of culture, the *callus induction frequency* and **weight** were recorded for each treatment combination. The percentage of *callus induction (PCI)* was calculated using the following formula:

$$\text{Callus induction (\%)} = (\text{number of explants induced callus} / \text{total number of explants cultured}) * 100.$$

**Table 1. Culture media composition for callus induction in tomato var. Super strain B**

Explant	Medium	PGRs and SWE and MPEs.
Hypocotyl	MH0	PGR- Free (control)
	MH1	H <sub>2</sub> O + NAA 0.2 ppm + BAP 2 ppm
	MH2	SW0.01% + NAA 0.2 ppm + BAP 2 ppm
	MH3	SA100ppm + NAA 0.2ppm + BAP 2 ppm
	MH4	RM100ppm + NAA 0.2 ppm + BAP 2 ppm
	MH5	TF100ppm + NAA 0.2 ppm + BAP 2 ppm
Leaf	ML0	PGR- Free (control)
	ML1	H <sub>2</sub> O + NAA 1 ppm + BAP 3 ppm
	ML2	SW0.01% + NAA 1 ppm + BAP 3 ppm
	ML3	SA100ppm + NAA 1 ppm + BAP 3 ppm
	ML4	RM100ppm + NAA 1 ppm + BAP 3 ppm
	ML5	TF100ppm + NAA 1 ppm + BAP 3 ppm

*PGRs: plant growth regulators; MPEs: medicinal plant extracts; SW: seaweed; SA: Syzygium aromaticum; RM: Rhamnus lycioides; TF: Rhaponticum acaule (Tafgha); MH: medium for hypocotyl explant; ML: medium for Leaf explant.*

***In vitro* seeds germination and seedling growth**

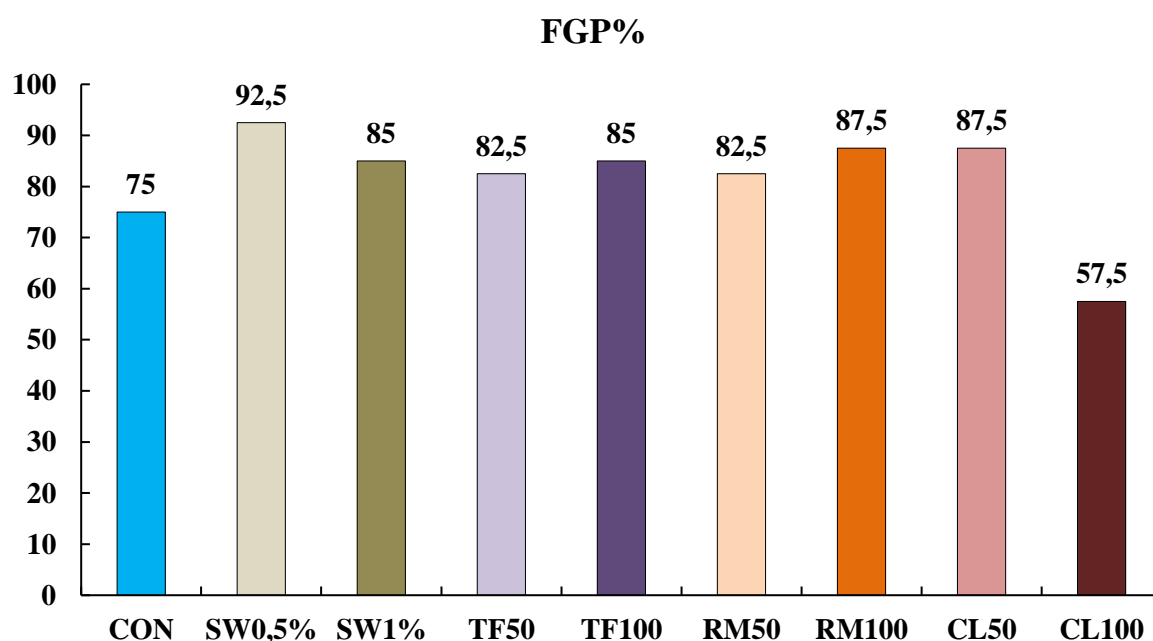
This experiment aimed to assess the *in vitro* germination and seedling growth of tomato (*S. lycopersicum* var. super strain B) following seed pretreatment with various plant extracts and a commercial seaweed extract (see Materials and Methods section).

**▪ Seed Germination**

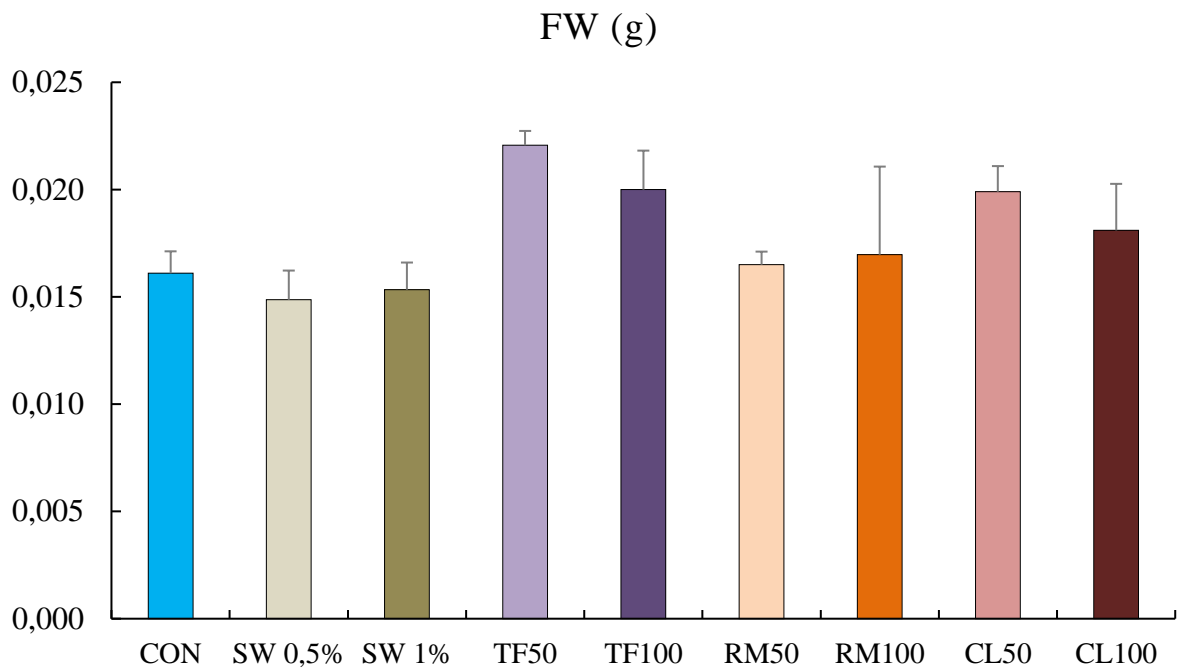
As shown in Figure 4, almost all seed pretreatments increased tomato seed germination compared to the control (seeds treated with distilled water).

The highest final percentage of germination (FGP) was recorded in response to seaweed extract pretreatment (SW0.5%), reaching 92.5%, followed by RM100 and LC50 extracts, both at 87.5%. Conversely, pretreatment with CL100 had a negative effect on germination, with a PGF of only 57.5%.

Overall, the most effective treatments were SW0.5% (92.5%), RM100 ppm and LC50 ppm (87.5% each). The optimal percentage of germination with TF extract was recorded at the concentration of 100 ppm. Statistical analyses demonstrated a significant effect ( $P < 0.05$ ) on tomato seed germination.



**Figure 04. Germination percentage (%) of tomato seeds (var. super strain B) treated with different plant extracts. Each value is the average of four repetition.**



**Figure 5. Biomass or fresh weight of tomato seedlings (var. super strain B) treated with different plant extracts. Each value is the average of three repetition  $\pm$  SD.**

- ***Seedling growth***

Seed pretreatments appears to affect the seedling growth of tomatoes to varying degrees. For instance, pretreatments with CL50, CL100, TF50, and TF100 significantly increased the fresh weight of tomato seedlings (Figure 5).

The most notable effects were observed with TF50 (22 mg), CL50 (20 mg), and CL100 (18 mg).

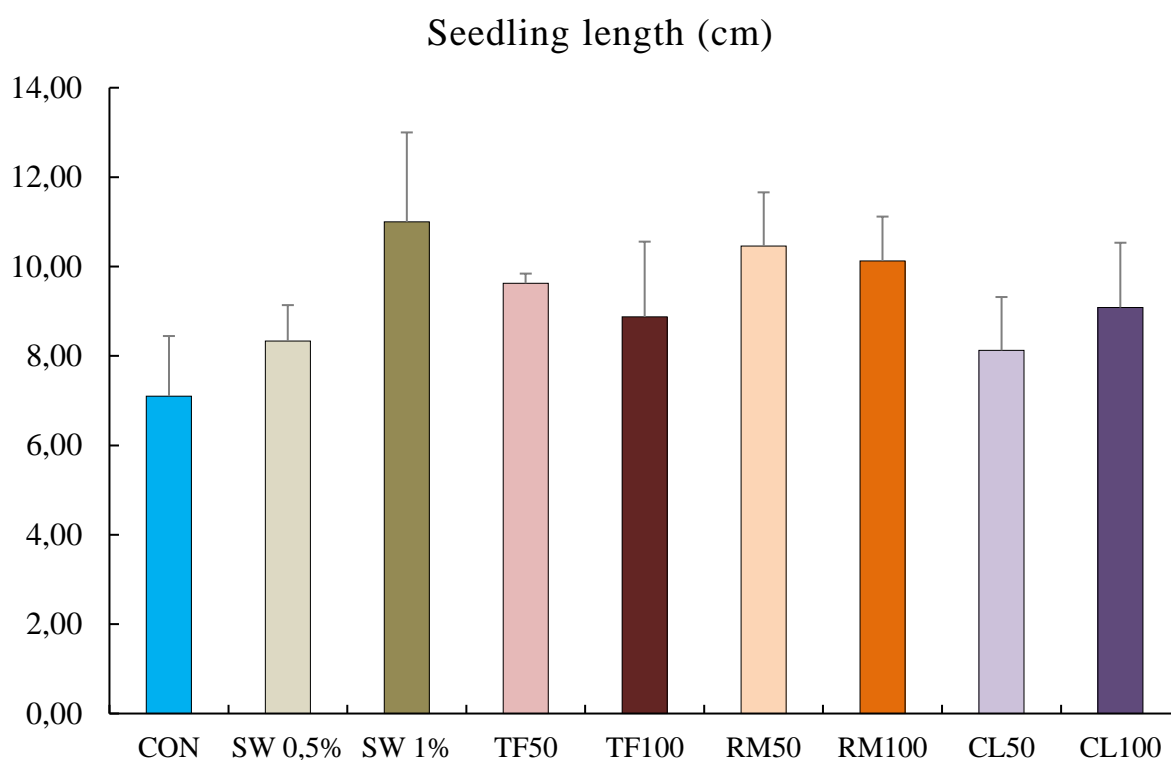
Conversely, pretreatment with the algae extract at both concentrations resulted in a slight decrease in fresh seedling weight (Figure 5).

▪ *Seedling length*

Seed pre-treatment appears to positively and significantly ( $P < 0.05$ ) affect tomato seedling length, but to varying degrees. Pretreatments with most extracts significantly increased the length of tomato plants (Figure 6).

The most notable effects were observed with WS1% (11.0 cm), RM50 (10.46 cm), RM100 (10.13 cm) and LC100 (9.8 cm).

Statistical analysis revealed a highly significant effect of pre-treatment on tomato seedling length growth ( $P < 0.001$ ).



**Figure 6. Seedling length of tomato (var. super strain B) treated with different plant extracts. Each value is the average of three repetition  $\pm$  SD.**

- ***Callus induction***

The main objective of this study is to evaluate the effect of extracts of three medicinal plants and a commercial seaweed extract on callus induction in tomato *S. lycopersicum* var. super strain B (see section Material & Methods).

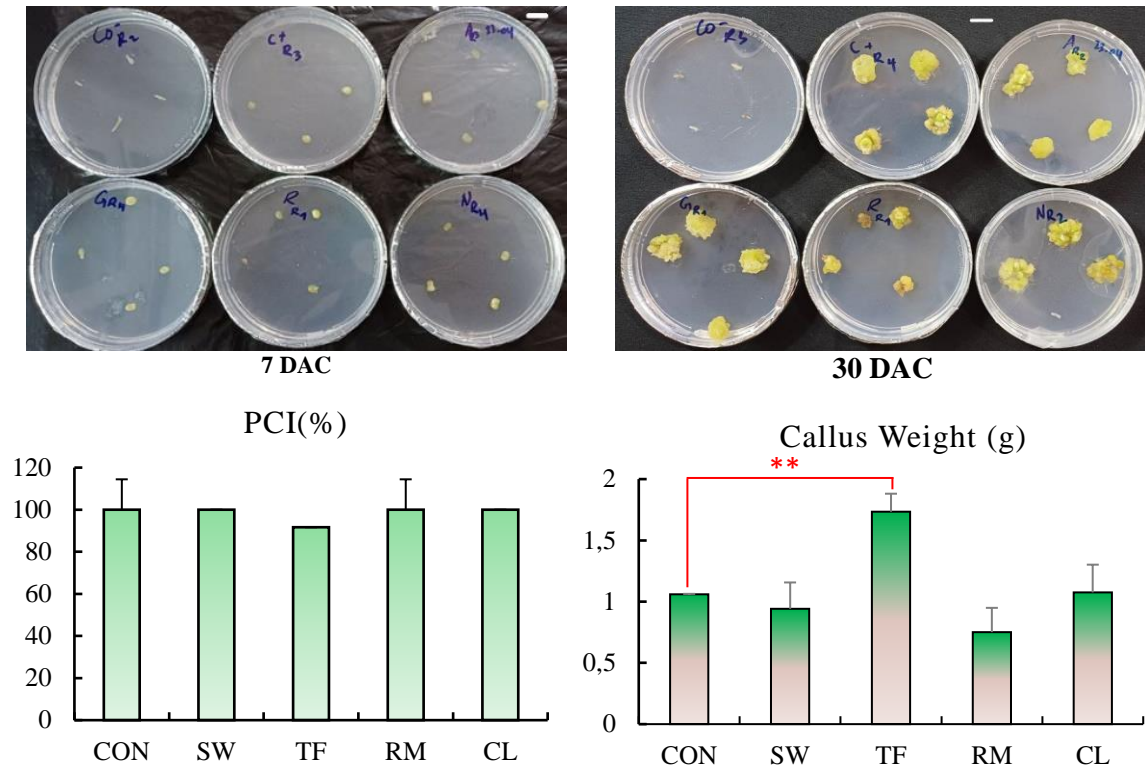
The two types of explants used in this experiment (hypocotyl and leaf) were chosen after a thorough study of the literature. A smaller size of the explants (0.5 to 1 cm) was chosen to minimize the risk of contamination, in addition to the fact that the larger explants show a total loss of morphogenic potential. The results show that the hypocotyl was the most effective explant, with rapid callus formation in 5 to 7 days, compared to 10 to 15 days for leaf explants, and produced larger calluses than those of leaf explants (Figure 7). However, it should be noted that the contamination rate was zero in the case of foliar explants and around 8.33% in the case of hypocotyl explants.

On the other hand, it is worth noticing that the age and the vigor of the explant (hypocotyl) influenced the callus induction (data not shown). The young and vigorous hypocotyls obtained through in vitro seed germination (first experiment) have given rise to rapid and big callus (Figure 7). Also, the green hypocotyl seems to be more susceptible for callusing than the white ones. Nonetheless, these findings (observations) require further validation in future studies.

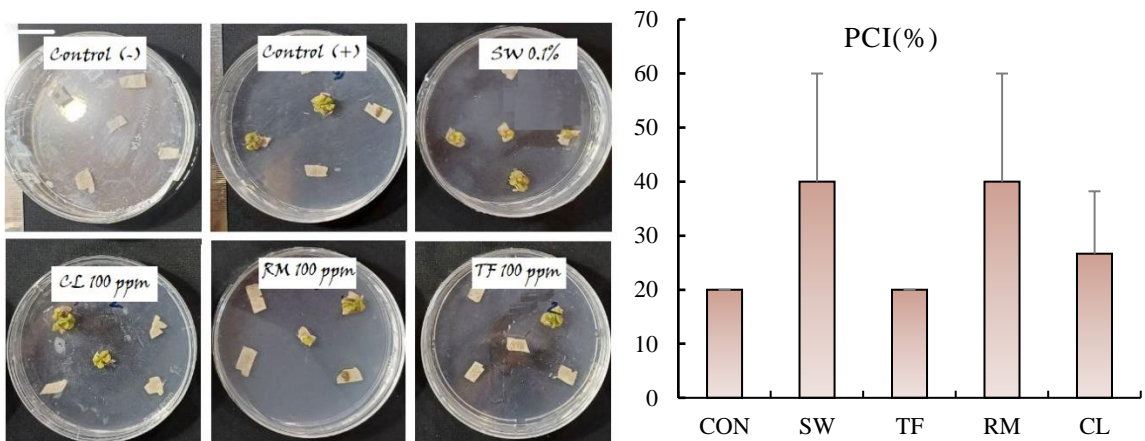
Regarding the use of medicinal plant extracts, we found that the addition of MPEs did not seem to affect callus induction except in the absence of PGRs. However, it should be noted that the extracts had more or less positive effects on callus growth/size. The best results were recorded for TF (64% increase in weight,  $P < 0.0026$ ) and CL (1% of increase) extracts (Figure 7). In contrast, RM extract have exhibited a negative effect on the callus growth (more than 43% of decrease in weight compared to control). This can be attributed to the difference in the chemical composition of the extracts (Appendix 1).

Callus initiation from leaf explants cultivated on MS medium supplemented with PGRs was not as significant as that obtained with hypocotyls. Callus induction rates did not exceed 40% in the best conditions (SW and RM) (Figure 8). During initiation the explants show any browning of tissues. In contrast, leaf explants culture on MS basal medium without any PGR-MPE supplementation show only slight swelling of explants that were not significant for callusing (Figure 9). This was possibly due to significant role of PGRs over

callusing. In the media supplemented with BAP and IAA, the leaf segments remain green for long period with very slow process of callus induction (Figure 8).

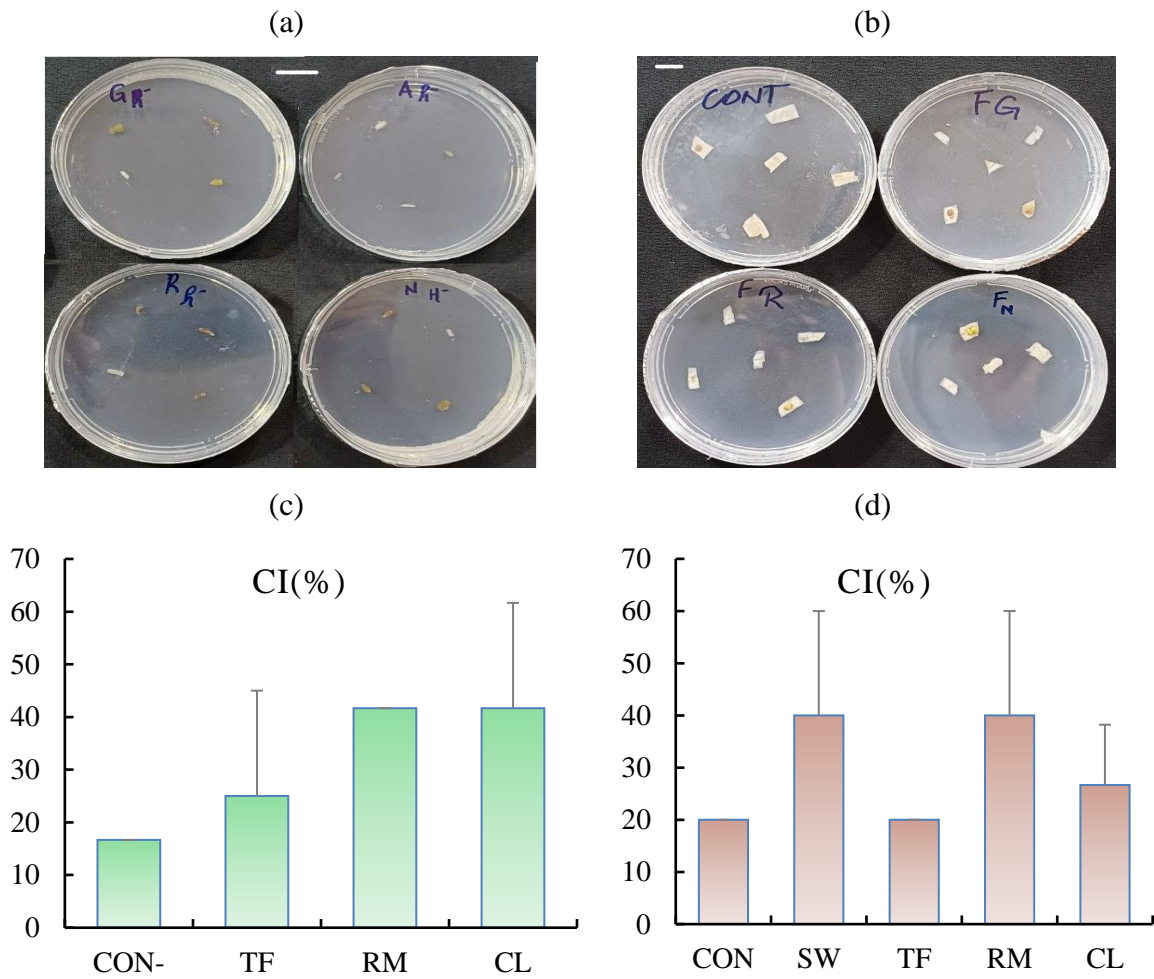


**Figure 7. Effect of medicinal plant and seaweed extracts on callus formation from tomato hypocotyl explants cultivated on MS medium supplemented with PGRs (NAA 0.2 ppm and BAP 2 ppm).**



**Figure 8. a) Effect of medicinal plant and seaweed extracts on callus formation from tomato leaf explants cultivated on MS medium supplemented with PGRs (NAA 1 ppm; BAP 3 ppm). b) Callus induction percentage in different culture mediums calculated based on three repetitions.**

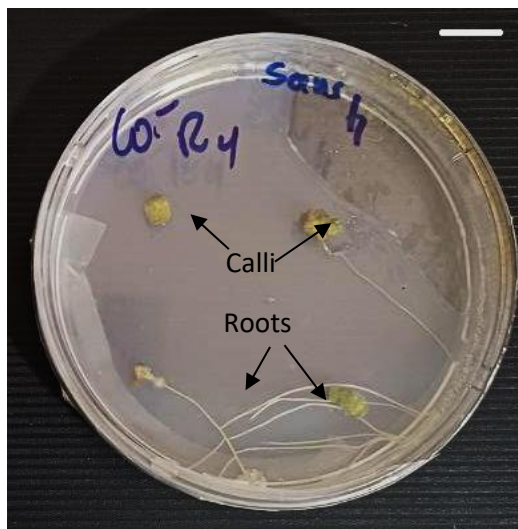
On the other hand, the culture of hypocotyls/leaves on basic MS medium without PGRs supplementation but to which small amounts of the tested extracts are added (200  $\mu$ L per medium), showed a small but positive effect of the MPEs on the callus induction, especially by using Tafgha, Rhamnus and Cloves extracts, were the CI% was 66.7%, 58% and 58% respectively (Figure 9).



**Figure 9. Effect of medicinal plant extracts on callus formation from tomato (a) hypocotyl or (b) leaf explants cultivated on MS medium without PGRs supplementation. (c) Callus induction percentage (%) form leaf explants. (d) Callus induction percentage (%) form hypocotyl explants. Explants are obtained from seedlings issued form pretreated seeds.**

On the other hand, it should be noted that we have observed the depletion (consumption) of the MS medium in about 4 to 6 weeks of culture, which implies the transfer to new MS media in order to ensure the continuity of the growth of the calls or their transformation.

Surprisingly, we achieved root regeneration on one of the Petri dishes filled with MS medium without PGR supplements, a phenomenon that remains unexplained (Figure 10).



**Figure 10. Root regeneration in MS medium without any supplement of PGRs. 4 weeks after culture.**

Successful seed germination, which is crucial for the production of the next generation of plants and their subsequent growth and survival, involves various processes such as imbibition, protein synthesis, and phytohormone production. These processes can be influenced by external factors, including the application of biostimulants (**Makhaye et al., 2021**).

The effect of biostimulants often depends on their type, concentration, and the plant species being treated. Certain seaweed extracts, humic substances, and microbial inoculants can influence hormonal metabolism, resulting in an increased germination rate (**Makhaye et al., 2021**).

Several previous studies have showed the potential of seaweed (i.e., *Ascophyllum nodosum*) extracts to promote seed germination and seedling growth of tomato (**Hernández-Herrera et al., 2022**). Similarly, several studies on seed treatment with plant extracts are showed the potential of plant extracts to promote the germination and the seedling growth of crops (**Silva et al., 2022; Mansangwa et al., 2017; Aiyaz et al., 2015**).

Consistent with these findings, our results demonstrated that MP and SW extracts used in this study increased the germination rate and seedling growth of tomato (var. Super Strain) suggesting the potential use of MPE to improve callus induction in tomatoes, which was tested in the second experiment.

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium (auxins and cytokinins) (**Ozyigit et al., 2007**). The specific concentration of plant regulators needed to induce callus, varies from species to species and even depends on the source of explant (**Charriere et al., 1999**).

It has been demonstrated in many cases that the combination of NAA and BAP is usually the choice of auxin/cytokinin for callus induction and subculture of tomato (**Abd El-Hameid, 2021**). Minimal cytokinins and auxins in culture media would avoid somaclonal variation and efficiently produce true to type plantlets (**Ozyigit et al., 2007**). Our results confirmed these findings, since both concentration (NAA 0.2 ppm & BAP 2 ppm) and (NAA

1 ppm & BAP 3 ppm) have given rise to normal callus formation from hypocotyl and leaf respectively.

Similarly, the concentration of PGRs seems to have an impact on callusing. For instance, in the case of leaf explants, it was found that explants grown on medium supplemented with NAA 1 ppm and BAP 3 ppm performed well callusing than explants grown on medium supplemented with NAA 0.2 ppm and PAB 2 ppm (data not shown). These results are consistent with those of many previous studies (**Shah *et al.*, 2015**; **Yaroshko *et al.*, 2023**).

The success of micropropagation largely relies on the selection of suitable plant part, which is to be used as the starting material for the experiment. In the present experiment hypocotyl explants was best fit for purposes. The best callusing was observed in media having BAP: IAA in concentration ratio of (2:0,2 ppm). Thus, the PGR have significant impact on callusing. This is basically due to endogenous level of growth regulators.

In the media supplemented with only MPE the callus induction was significant (Figure 9). This remains in accordance with previous reported works (**Mathur *et al.*, 1987**).

The development of a reliable in vitro protocol is of great importance for producing plant material and for conservation of rare plant species, and offset the pressure on the natural populations as well as plant medicinal purposes.

The present study describes a reliable protocol of Tomato callogenesis from hypocotyl/leaf explants with much higher rate of multiplication. However, this protocol needs to be further investigated before to be used as a basic tool for cultivation of tomato plant.

In line with our results, **Jalal *et al.* (2015)** reported that plant extracts can be used as an effective alternative to phytohormones in tissue culture, since plant extract has the promising potential for the induction of callus and direct organogenesis (**Setiaji *et al.*, 2020**). Nonetheless, the impact of plant extracts on callogenesis depend, among others, on its chemical composition.

## CONCLUSION

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This research aimed to examine the impact of extracts (50 and 100 ppm) from three medicinal plants (*Syzygium aromaticum*, *Rhaponticum acaule*, *Rhamnus lycioides*) and marine algae (0.1-0.5%, Dalgin) on germination and callus formation in tomato plants (Super Strain B).

The findings revealed that pre-treating seeds with these extracts positively influenced seed germination and the in vitro growth of tomato seedlings. Additionally, callus culture studies showed that growing hypocotyls with a combination of auxins and cytokinins (0.2 ppm NAA & 2 ppm BAP) produced better results than using leaves.

The extracts, including the commercial marine algae extract, had varying but generally positive effects, particularly in the absence of hormones. Notably, the extract from the Tafgha plant (*Rhaponticum acaule*) had a more positive impact on hypocotyl growth compared to the other extracts, with the highest growth rate observed at 66.67%, despite the absence of hormones in the medium.

These beneficial effects are likely due to the chemical composition of the extracts, such as those found in the algae extract. Further studies and detailed chemical analyses are needed to confirm these effects and to explore the mechanisms by which plant extracts enhance growth and callus induction in tomatoes and other crops.

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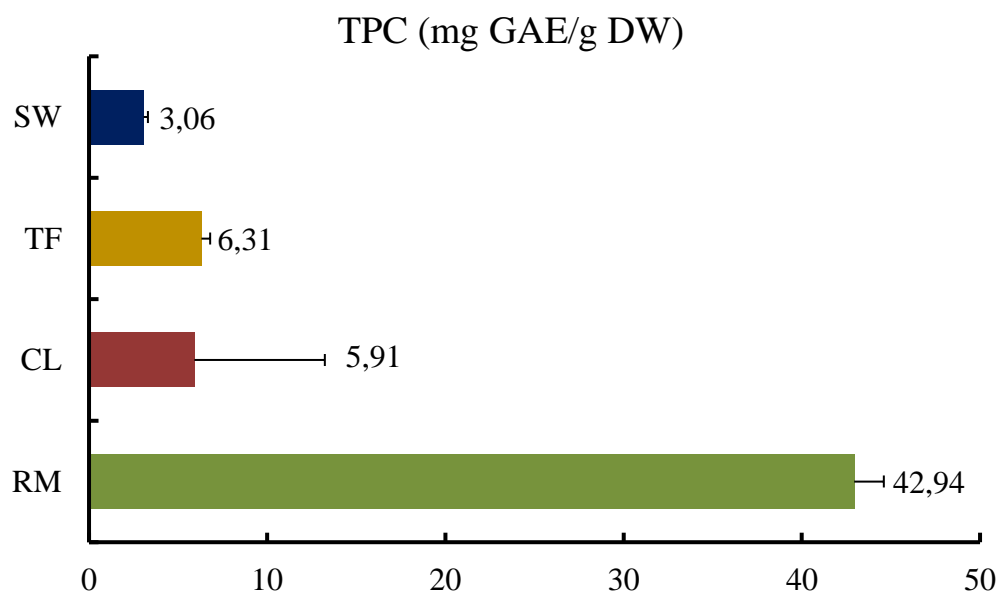
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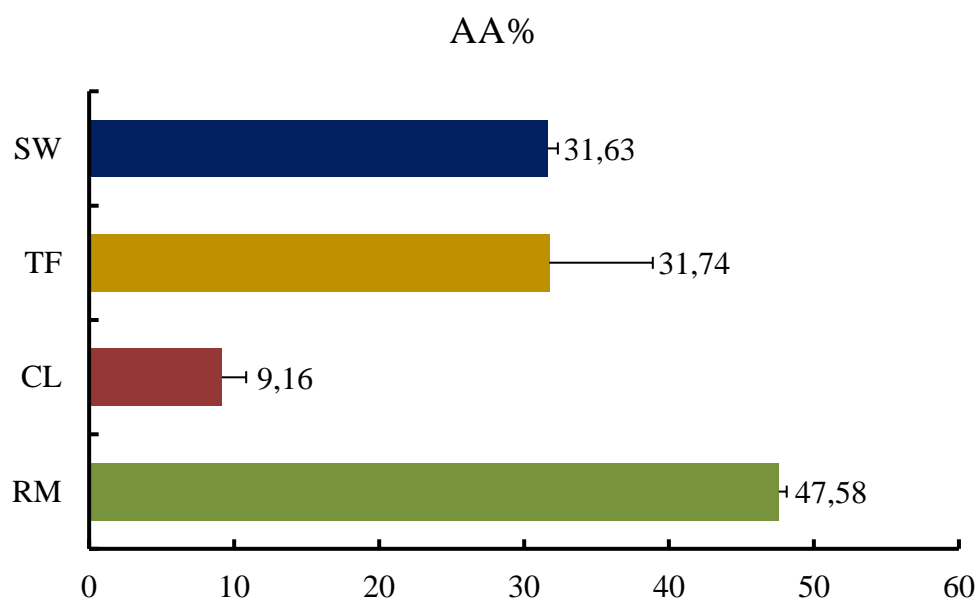
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**Appendix 1**

**Total polyphenol content of the different extracts used in the present study.**



**Antioxidant activity of the different extracts used in the present study.**



## Appendix 2

### Statistical analyses

ANOVA Table for FGP (%) by Treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,116155	8	0,0145194	3,47	0,0070
Within groups	0,112876	27	0,0041806		
Total (Corr.)	0,229031	35			

Multiple Range Tests for FGP by Treatment

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
CL100	4	0,756368	A
CON	4	0,863592	B
RM50	4	0,902814	BC
TF50	4	0,907113	BC
TF100	4	0,919943	BC
SW 1%	4	0,920677	BC
CL50	4	0,934384	BC
RM100	4	0,935119	BC
SW0,5%	4	0,961512	C

Contrast	Sig.	Difference	+/- Limits
CL100 - CL50	*	-0,178017	0,0938095
CL100 - CON	*	-0,107224	0,0938095
CL100 - RM100	*	-0,178752	0,0938095
CL100 - RM50	*	-0,146447	0,0938095
CL100 - SW 1%	*	-0,16431	0,0938095
CL100 - SW0,5%	*	-0,205145	0,0938095
CL100 - TF100	*	-0,163575	0,0938095
CL100 - TF50	*	-0,150746	0,0938095
CL50 - CON		0,0707926	0,0938095
CL50 - RM100		-0,000734851	0,0938095
CL50 - RM50		0,0315702	0,0938095
CL50 - SW 1%		0,0137069	0,0938095
CL50 - SW0,5%		-0,0271281	0,0938095
CL50 - TF100		0,0144418	0,0938095
CL50 - TF50		0,027271	0,0938095
CON - RM100		-0,0715275	0,0938095
CON - RM50		-0,0392224	0,0938095
CON - SW 1%		-0,0570857	0,0938095
CON - SW0,5%	*	-0,0979207	0,0938095
CON - TF100		-0,0563508	0,0938095
CON - TF50		-0,0435217	0,0938095
RM100 - RM50		0,0323051	0,0938095
RM100 - SW 1%		0,0144418	0,0938095
RM100 - SW0,5%		-0,0263932	0,0938095
RM100 - TF100		0,0151766	0,0938095
RM100 - TF50		0,0280058	0,0938095
RM50 - SW 1%		-0,0178633	0,0938095
RM50 - SW0,5%		-0,0586983	0,0938095
RM50 - TF100		-0,0171285	0,0938095
RM50 - TF50		-0,00429928	0,0938095
SW 1% - SW0,5%		-0,040835	0,0938095
SW 1% - TF100		0,000734851	0,0938095
SW 1% - TF50		0,013564	0,0938095
SW0,5% - TF100		0,0415698	0,0938095
SW0,5% - TF50		0,054399	0,0938095
TF100 - TF50		0,0128292	0,0938095

\* denotes a statistically significant difference.

**ANOVA Table for FW by Treatment**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0,000137027	8	0,0000171283	2,82	0,0324
Within groups	0,000109473	18	0,00000608185		
Total (Corr.)	0,0002465	26			

**Multiple Range Tests for FW by Treatment**

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
SW0,5%	3	0,00486667	A
SW 1%	3	0,00533333	A
RM50	3	0,0065	AB
CON	3	0,00676667	AB
RM100	3	0,00696667	AB
CL100	3	0,0081	ABC
CL50	3	0,0099	BC
TF100	3	0,01	BC
TF50	3	0,0120667	C

Contrast	Sig.	Difference	+/- Limits
CL100 - CL50		-0,0018	0,00423042
CL100 - CON		0,00133333	0,00423042
CL100 - RM100		0,00113333	0,00423042
CL100 - RM50		0,0016	0,00423042
CL100 - SW 1%		0,00276667	0,00423042
CL100 - SW0,5%		0,00323333	0,00423042
CL100 - TF100		-0,0019	0,00423042
CL100 - TF50		-0,00396667	0,00423042
CL50 - CON		0,00313333	0,00423042
CL50 - RM100		0,00293333	0,00423042
CL50 - RM50		0,0034	0,00423042
CL50 - SW 1%	*	0,00456667	0,00423042
CL50 - SW0,5%	*	0,00503333	0,00423042
CL50 - TF100		-0,0001	0,00423042
CL50 - TF50		-0,00216667	0,00423042
CON - RM100		-0,0002	0,00423042
CON - RM50		0,000266667	0,00423042
CON - SW 1%		0,00143333	0,00423042
CON - SW0,5%		0,0019	0,00423042
CON - TF100		-0,00323333	0,00423042
CON - TF50	*	-0,0053	0,00423042
RM100 - RM50		0,000466667	0,00423042
RM100 - SW 1%		0,00163333	0,00423042
RM100 - SW0,5%		0,0021	0,00423042
RM100 - TF100		-0,00303333	0,00423042
RM100 - TF50	*	-0,0051	0,00423042
RM50 - SW 1%		0,00116667	0,00423042
RM50 - SW0,5%		0,00163333	0,00423042
RM50 - TF100		-0,0035	0,00423042
RM50 - TF50	*	-0,00556667	0,00423042
SW 1% - SW0,5%		0,000466667	0,00423042
SW 1% - TF100	*	-0,00466667	0,00423042
SW 1% - TF50	*	-0,00673333	0,00423042
SW0,5% - TF100	*	-0,00513333	0,00423042
SW0,5% - TF50	*	-0,0072	0,00423042
TF100 - TF50		-0,00206667	0,00423042

\* denotes a statistically significant difference.

**ANOVA Table for PL by Treatment**

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	36,885	8	4,61063	2,72	0,0373
Within groups	30,5575	18	1,69764		
Total (Corr.)	67,4425	26			

**Multiple Range Tests for PL by Treatment**

Method: 95,0 percent LSD

Treatment	Count	Mean	Homogeneous Groups
CON	3	7,1	A
CL50	3	8,125	AB
SW0,5%	3	8,33333	ABC
TF100	3	8,875	ABCD
CL100	3	9,08333	ABCD
TF50	3	9,625	BCD
RM100	3	10,125	BCD
RM50	3	10,4583	CD
SW 1%	3	11,0	D

Contrast	Sig.	Difference	+/- Limits
CL100 - CL50		0,958333	2,23505
CL100 - CON		1,98333	2,23505
CL100 - RM100		-1,04167	2,23505
CL100 - RM50		-1,375	2,23505
CL100 - SW 1%		-1,91667	2,23505
CL100 - SW0,5%		0,75	2,23505
CL100 - TF100		0,208333	2,23505
CL100 - TF50		-0,541667	2,23505
CL50 - CON		1,025	2,23505
CL50 - RM100		-2,0	2,23505
CL50 - RM50	*	-2,33333	2,23505
CL50 - SW 1%	*	-2,875	2,23505
CL50 - SW0,5%		-0,208333	2,23505
CL50 - TF100		-0,75	2,23505
CL50 - TF50		-1,5	2,23505
CON - RM100	*	-3,025	2,23505
CON - RM50	*	-3,35833	2,23505
CON - SW 1%	*	-3,9	2,23505
CON - SW0,5%		-1,23333	2,23505
CON - TF100		-1,775	2,23505
CON - TF50	*	-2,525	2,23505
RM100 - RM50		-0,333333	2,23505
RM100 - SW 1%		-0,875	2,23505
RM100 - SW0,5%		1,79167	2,23505
RM100 - TF100		1,25	2,23505
RM100 - TF50		0,5	2,23505
RM50 - SW 1%		-0,541667	2,23505
RM50 - SW0,5%		2,125	2,23505
RM50 - TF100		1,58333	2,23505
RM50 - TF50		0,833333	2,23505
SW 1% - SW0,5%	*	2,66667	2,23505
SW 1% - TF100		2,125	2,23505
SW 1% - TF50		1,375	2,23505
SW0,5% - TF100		-0,541667	2,23505
SW0,5% - TF50		-1,29167	2,23505
TF100 - TF50		-0,75	2,23505

\* denotes a statistically significant difference.

**ANOVA Table for Callus Weight by Treatment**

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
Between groups	1,65161	4	0,412902	8,84	0,0026
Within groups	0,467271	10	0,0467271		
Total (Corr.)	2,11888	14			

**Multiple Range Tests for CW by Treatment**

Method: 95,0 percent LSD

<i>Treatment</i>	<i>Count</i>	<i>Mean</i>	<i>Homogeneous Groups</i>
RM	3	0,750867	A
SW	3	0,941433	A
CON	3	1,05987	A
CL	3	1,07537	A
TF	3	1,73407	B

<i>Contrast</i>	<i>Sig.</i>	<i>Difference</i>	<i>+/- Limits</i>
CL - CON		0,0155	0,393262
CL - RM		0,3245	0,393262
CL - SW		0,133933	0,393262
CL - TF	*	-0,6587	0,393262
CON - RM		0,309	0,393262
CON - SW		0,118433	0,393262
CON - TF	*	-0,6742	0,393262
RM - SW		-0,190567	0,393262
RM - TF	*	-0,9832	0,393262
SW - TF	*	-0,792633	0,393262

\* denotes a statistically significant difference.

## **Abstract**

This research aimed to investigate the impact of extracts from three medicinal plants (*Syzygium aromaticum*, *Rhaponticum acaule*, *Rhamnus lycioides*) and seaweed extract (Dalgin) on the germination and callus formation in tomato plants (Super strain B). The findings demonstrated that pre-treating seeds with these plant extracts positively influenced seed germination and the growth of tomato seedlings *in vitro*. Additionally, callus culture studies revealed that growing callus with a combination of auxins and cytokinins (0.2 ppm NAA & 2 ppm BAP) yielded better results compared to using leaves. The extracts, including commercial seaweed extract, had varying but generally positive effects, especially in the absence of hormones. However, the Tafgha plant extract exhibited the most significant effect on the speed and mass of callus formation when hormones were present. These beneficial effects are likely due to the chemical composition of the extracts, such as those found in the seaweed extract. Further studies and detailed chemical analyses are needed to confirm these effects and to explore the mechanisms by which plant extracts enhance growth and callus induction in tomatoes and other crops.

**Keywords:** *In vitro* culture, tomatoes, calluses, germination, plant extracts, seaweed.

## **Résumé :**

Cette recherche a été menée dans le but d'étudier l'effet d'extraits de trois plantes médicinales (*Syzygium aromaticum*, *Rhaponticum acaule*, *Rhamnus lycioides*) et d'extrait d'algues marines (Dalgin) sur la germination et la formation de callosités dans le plant de tomate (Super strain B). Les résultats ont montré un effet positif du prétraitement des graines par des extraits de plantes sur la germination des graines et la croissance des plants de tomates *in vitro*. L'étude de la culture de callosités a également montré que la culture de l'hypocotyle avec un mélange d'auxines et de cytokines donne un meilleur résultat par rapport aux feuilles. Les résultats ont également montré que les extraits de plantes tels que l'extrait d'algues marines commerciales ont des effets variables, mais la plupart d'entre eux sont positifs, surtout en l'absence d'hormones, mais dans une mesure limitée. En présence d'hormones, le traitement à l'extrait de Tafgha a montré le meilleur effet en termes de vitesse de formation de cal ou de sa biomasse. Ces effets positifs sont attribués à la teneur chimique des extraits utilisés tels que l'extrait d'algues. Les résultats obtenus nécessitent des études supplémentaires et des analyses chimiques approfondies pour confirmer l'effet et tenter d'expliquer l'impact positif des extraits de plantes sur la croissance et la formation de callosités dans les tomates et autres cultures vivrières.

## **Mots-clés :**

Culture *in vitro*, tomates, callosités, germination, extraits de plantes, algues marines.

## الملخص

أجري هذا البحث بهدف دراسة تأثير مستخلصات ثلاثة نباتات طبية ( *Syzygium aromaticum, Rhaponticum* ) و *acaule, Rhamnus lycioides* ) و مستخلص الطحالب البحرية (دالجن) على الانبات و تشكل الكالس عند نبات الطماطم (صنف- سوبر سترين ب).

أظهرت النتائج التأثير الإيجابي للمعاملة المسبقة للبذور بواسطة المستخلصات النباتية على انبات البذور و نمو بادرات الطماطم في الزجاج. كما أظهرت دراسة زراعة الكالس، أن زراعة الكولليبيتيل مع مزيج من الاكسينات و السيوكينات (0.2 ppm NAA & 2 ppm BAP) أعطى نتائج أفضل مقارنة لعينة الأوراق. كما دلت النتائج أن للمستخلصات النباتية على غرار مستخلص الطحالب البحرية التجاري تأثيرات متفاوتة لكن في معظمها إيجابية خصوصا عند غياب الهرمونات و لكن بصورة محدودة. في وجود الهرمونات أظهرت المعاملة بواسطة مستخلص نبات التافعة أو النافعة أفضل تأثير من حيث سرعة تشكل الكالس و كتلته. تعزى هذه التأثيرات الإيجابية الى المحتوى الكيميائي للمستخلصات المستعملة على غرار مستخلص الطحالب.

تحتاج النتائج المحصل عليها الى دراسات إضافية و تحاليل كيميائية معمقة لتأكيد التأثير و كذا محاولة تفسير التأثير الإيجابي للمستخلصات النباتية على نمو و تشكل الكالس عند الطماطم و باقي المحاصيل الغذائية.

## الكلمات المفتاحية:

الزراعة في الزجاج، الطماطم، الكالس، الانبات، المستخلصات النباتية، الطحالب البحرية.

الأطروحة : استخدام مستخلص الطحالب البحرية والمستخلصات النباتية لتحسين تحريض الكالس في الطماطم  
الإسم و اللقب : بلمكي أميرة & علاوي ريمة  
المؤطر : د. فرشة عزالدين

#### ملخص :

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الكلمات المفتاحية : الزراعة في الزجاج، الطماطم، الكالس، الانبات، المستخلصات النباتية، الطحالب البحرية.

#### Thesis : USE OF SEAWEED AND PLANT EXTRACTS TO IMPROVE CALLUS INDUCTION IN TOMATO

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Directed by : Dr. FERCHA Azzedine

#### Abstract:

This research aimed to investigate the impact of extracts from three medicinal plants (*Syzygium aromaticum*, *Rhaponticum acaule*, *Rhamnus lycioides*) and seaweed extract (Dalgin) on the germination and callus formation in tomato plants (Super strain B). The findings demonstrated that pre-treating seeds with these plant extracts positively influenced seed germination and the growth of tomato seedlings in vitro. Additionally, callus culture studies revealed that growing callus with a combination of auxins and cytokinins (0.2 ppm NAA & 2 ppm BAP) yielded better results compared to using leaves. The extracts, including commercial seaweed extract, had varying but generally positive effects, especially in the absence of hormones. However, the Tafgha plant extract exhibited the most significant effect on the speed and mass of callus formation when hormones were present. These beneficial effects are likely due to the chemical composition of the extracts, such as those found in the seaweed extract. Further studies and detailed chemical analyses are needed to confirm these effects and to explore the mechanisms by which plant extracts enhance growth and callus induction in tomatoes and other crops.

**Key words:** In vitro culture, tomatoes, calluses, germination, plant extracts, seaweed.

