

## The Stimulating Effect of Plant Extracts on Tomato Cultivation

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### ABSTRACT

As it might be known, organic products are increasingly in demand. Indeed, one of the most environmentally and friendly innovations are the bio stimulants. Their effect is exposed on the flowering, the plant growth, the fruits and the crop productivity and nutrient use efficiency. In this study we evaluate the effect of two aqueous plant extracts *Ocimum basilicum* and *Eucalyptus globulus* on the growth and the performances of tomato. The methodology consist on two parts of evaluation: the effect on the plant performances and the quality of the fruit. At the same time the composition of the two plant extracts have been necessary to evaluate the compound responsible on the effects. The results showed that both aqueous extracts had a positive effect on the plant performances. Indeed, *Ocimum basilicum*-treated seedling reached  $1.35 \pm 0.05$  cm at the high 50% concentration. Compared with  $1.02 \pm 0.16$  cm for the negative control. Same for *Eucalyptus globulus* that reached  $1.35 \pm 0.25$  cm at the same dose. For the circumference of the seedlings, the same extract reached  $8 \pm 0.93$ cm at the highest concentration. The number of flowers per fruits had a positive effect for two plant extracts. For the quality of the fruit, a positive effect had been evaluated on the fruit weight that reached  $121 \pm 24$  g with 8% of *Ocimum basilicum* and  $135 \pm 15$  g with *Eucalyptus globulus*. Same for circumference; PH; the titrable acidity and the total Carbohydrate. At the same time, it had a negative effect on the total juice that has decreased by the aggregate of the concentrations. It had a neutral effect on the coloration of the fruit that didn't change by the application of the plants extracts. The phytochemical screening of the plant extracts showed that both *Ocimum basilicum* and *Eucalyptus globulus* are high in polyphenols that might be the reason of these positive impacts. This study showed that it is possible to use the plant extracts of *Ocimum basilicum* and *Eucalyptus globulus* as biostimulant to have a better production and productivity of tomatoes.

**Keywords:** plant extract, stimulant effect, tomato, *Ocimum basilicum*, *Eucalyptus globulus*, medicinal, aromatic plants.

### INTRODUCTION

In recent decades, climate change has become a major challenge facing mankind. Droughts, floods, strong winds and the like are causing multiple fluctuations in the production of various crops, including tomatoes. The agricultural sector is also faced with increasing demand to cope with a growing world population. At the same time, this increase is aimed at protecting the environment and human health. Fertilizers and pesticides play an important role in ensuring continuous production under different conditions. Thanks to a large decrease in the usage of synthetic agrochemicals like fertilizers

and pesticides, a number of technological advancements have been proposed in recent decades to increase the sustainability of agricultural production systems. A potentially beneficial and eco-friendly advancement is the utilization of natural plant biostimulants, which enhance crop yield, blooming, plant growth, fruit set, and nutrient usage efficiency. (Youssef and Colla, 2020). The variety of international journals used has expanded, and there are now more scholarly papers on plant biostimulants (Colla and Rouphael, 2015).

Tomato is considered one of the eighth most precious agricultural products as mentioned by the Food and Agriculture Organization of the

United Nations (FAO). China, India, the United States, Turkey and Egypt are the top five tomato-producing countries. Tomatoes are a substantial source of income and one of Morocco's principal fresh product exports. Farmers are more interested into tomato's production than any other vegetable because of its multiple harvests, which is translated into a high profit per unit area. In addition to its use in a variety of dishes, tomato is also an important income-generating crop for small farmers and provides jobs in the production and processing industries (Fikreyesus et al., 2011) It has become the number one choice for many processed products. Indeed, processed tomato products have become an important part of the diet in both developed and developing countries (Muralidhar and Koteswara, 2016). It is also an important source of vitamins A and C as well as minerals (Fikreyesus et al., 2011). This diversity of uses makes the tomato an important vegetable in the country in particular and in the world in general.

The European Commission (stemming from European Regulation 2019/1009 published in the OJ in June 2019) has defined bio stimulants as elements that stimulate natural plant nutrition processes. Biostimulants aim to improve plant nutrient use efficiency, abiotic stress tolerance, and qualitative characteristics or increase the availability of nutrients confined in the soil or rhizosphere. They aim to optimize fertilizer efficiency and reduce nutrient input rates.

Biostimulants improve the effect of nutrients. Among other things, they improve the absorption of nutrients available in the soil. They also help plants to better tolerate abiotic stresses such as drought, extreme temperatures, salinity and flooding. These benefits help to improve yields and ensure better quality of production. Thus, the farmer's profitability. The effect of bio stimulants varies according to their type, soil, crop. ect. Nevertheless, yields can vary by 5 to 10% (EBIC, 2023).

Although the use of biostimulants in agriculture is on the increase, they are still not subject to scientific peer review. The most widely used biostimulants are microbial inoculants, humic acids, fulvic acids, protein hydrolysates, amino acids and seaweed extracts (Calvo, Nelson, and Kloepper 2014). These biostimulants have shown positive effects on crop growth, development and yields. Seaweed (*Ascophyllum nodosum*) increased lettuce yields and mitigated the adverse effects of potassium deficiency during plant growth and storage of processed produce. For

the plant extract *Moringa oleifera* leaf extract increased the nutrient content (N, P, K, Ca) of bean leaves (Chrysargyris et al., 2020). Nevertheless, plant extracts didn't had enough attention in the market of biostimulant and in the scientific researchers. Indeed some plant extracts such as moringa or seaweed that showed a very potential results in the market of biostimulants, many plants extracts can be studied to evaluate their effect on the development and the quality of plants.

One obstacle to the development of the biostimulants market is the lack of standardized regulations and definitions. Unlike conventional agricultural inputs such as fertilizers and pesticides, biostimulants often encompass a range of different products with different compositions and modes of action. This lack of uniformity makes it difficult for regulators to establish clear guidelines for product registration, labeling and use. What's more, the lack of standardized definitions can confuse farmers, sellers and consumers about the efficacy and proper use of biostimulant products. Thus, market growth can be hampered by regulatory uncertainty and ambiguity, which can pose barriers to market entry, limit investment and undermine opportunities, investment limitations and a loss of consumer confidence in biostimulant technologies (marketsandmarkets 2024).

At the same time factors influencing the efficacy of biostimulants include product formulation, timing of application, environmental conditions and crop genetics. This variability can lead to inconsistent results for farmers, and uncertainties about their economic and agronomic benefits. In addition, the absence of standardized test protocols and performance measurements makes it difficult to accurately evaluate and compare different biostimulant products (marketsandmarkets 2024).

Plant extracts are an alternative and a useful tool in the integrated control of foliar pathogens. Nevertheless, they are not widely used to improve crop yields. Nowadays, however, environmental and food safety issues are taken into account when choosing a method for stimulating production. These alternatives have the potential to be environmentally friendly.

This study focuses on the use of *Ocimum basilicum* and *Eucalyptus globulus* plant extracts for tomato growth stimulation and analyses their chemical composition. These two plant extracts had been chosen for their impact on many plants pathogens and especially on tomato fungal diseases (Abbad et al., 2023). Their composition intrigued

so that they can play a role as a biostimulant and a fungal treatment at the same time. The purpose of this study is to find a solution that can be easily found and applicable by farmers, doesn't cost much and be a solution that can develop the agriculture domain, the scientific studies and help farmers to obtain a better production in tomato fields, without creating a negative impact on the environment. We focused only on two plant extracts so that both *Eucalyptus globulus* and *Ocimum basilicum* are easily found in Morocco and so our study can be more determinate and concentrated.

## MATERIALS AND METHODS

### Aromatic and medicinal plants

In this study, we obtained the *Eucalyptus globulus* leaves from the Skhirat region in Morocco and we purchased the *Ocimum basilicum* leaves from the market. Both *Ocimum basilicum* leaves and *Eucalyptus globulus* leaves were containing dust that we rinsed with water and put into shade in room temperature to dry. Then grinding it with an electric grinder into a fine powder.

### Preparation of extracts

We opted for aqueous extraction by infusion in Ibn Tofail University, Faculty of Science. Laboratory of Natural Resources and Sustainable Development, Faculty of Science. As mentioned before, one of the objective of this study is to be able to have a product that is easily found or prepared and applied by farmers. The chosen concentrations are 4%, 8% and 50%. The two first concentrations are inspired by other products sold in the market and other studies such as (Fikreyesus et al., 2011). We also choose the 50% concentration as a high concentration to verify if there will be a negative effect or a better effect for higher concentrations.

### Technical management

The technical management of tomatoes followed a theoretical and practical plan. Following soil analysis and crop requirements.

Soil preparation began with a deep ploughing operation followed by a Cover Crop for a well-levelled seedbed. To avoid weeds, we opted for black plastic sheeting. Nutrient inputs combined soil analysis and crop requirements.

### Seedling preparation

Tomato seeds were purchased at the market. We opted for the "Maria" and "ALMAS" varieties, because of their abundant use by farmers.

Sowing was carried out in crates on wet peat. The crates were placed in a greenhouse for 15 days before being transplanted directly.

### Stimulating effect of plant extracts

Plant extracts were applied in a Split-Plot design over three applications: We opted for 20 plants with 4 repetitions per concentration of each extract with 2 positive control plants and two negative control plants. The positive control is a product authorized for organic farming, "Smartfoil". The first application of plant extracts was applied at sowing.

Three other applications were applied at a rate of 5 l/ha by root. The first at transplanting, the second 15 days after the first application and the third 15 days after the second application.

The observations were from the 15<sup>th</sup> day after the third application and focused on:

- plant length,
- stem circumference,
- number of flowers per plant.

The second observations were made at harvest, and focused on:

- average fruit weight,
- fruit size,
- fruit coloration,
- average juice content,
- hydrogen potential,
- titratable acidity,
- total carbohydrate content.

### Average fruit weight

Average fruit weight is determined by measuring the total weight generated by 10 fruits collected from plants treated with the same extract using an electric balance.

Fruit size – fruit size is determined by measuring the equatorial circumference of the fruit. Fruit coloration – coloration is determined visually (Loussert, 1989).

### Average juice content

Juice extraction is carried out using a rotating head extractor. The juice collected is filtered

through a 1mm mesh strainer and weighed (EAC-CE). It is expressed as a percentage by mass according to the following equation:

$$\text{Juice content} = \frac{\text{Total weight of juice (g)} \times 100}{\text{Total weight of fruit (g)}} \quad (1)$$

### Hydrogen potential (PH)

Hydrogen Potential was measured using a PH meter. This was calibrated with buffer solutions at known standard PH values. A sufficient volume of juice was immersed in the PH meter probe.

### Titratable acidity

The principle involves titration with a sodium hydroxide (NaOH) solution in the presence of Phenolphthalein as a colored indicator (French standard NF V 05-101). For juice acidity titration, 10 ml of decanted juice was used, to which three drops of phenolphthalein were added. The NaOH solution (0.1 M) was placed in a burette and poured in slowly. A magnetic stirrer was used to homogenize the solution during titration (Nielsen, 2010)

Titration ended with a persistent pinkish color. Titratable acidity was expressed by gram citric acid/liter. It is calculated by the following formula

$$\text{Acidity (g/l)} = \frac{\text{Titre} \times 0.0064}{10 \text{ ml juice}} \quad (2)$$

### Soluble sugar content (SSC)

Total carbohydrate content was determined using a refractometer.

### Phytochemical tests

We used the qualitative and the quantitative identification of the different plants extracts.

### Qualitative analysis

For the qualitative examination, we employed the tube characterization. This process relies on precipitation processes to form insoluble compounds.

### Phenolic compounds characterization

#### *Tannins characterization*

The indication of tannin was determined by mixing 1ml of the aqueous solution of  $\text{FeCl}_3$  at 1% and 1ml of the aqueous extract. The development of a blackish blue or greenish coloration indicates the presence of tannins.

Utilizing the Stiasny reaction, we were able to distinguish between Gallic and Catechic tannins. In a water bath set at 90 degrees for 15 minutes, 2 milliliters of the extract and 1 milliliter of the Stiasny reagent are mixed together. The precipitate's presence indicates the presence of catechic tannins. The resulting filtrate is then saturated with a small amount of sodium acetate and 1%  $\text{FeCl}_3$  to produce a blue-black color and release the Gallic tannins (Bruneton, 2009).

### Flavonoïds characterization

#### *Anthocyanins and leucoanthocyane*

With some magnesium cups, we combined one milliliter of isoamyl alcohol, one milliliter of hydrochloric alcohol, and one milliliter of the extract. The formation of a red hue at the isoamyl alcohol layer indicates the presence of flavones and flavonols. For fifteen minutes, we soaked the identical mixture in water without the magnesium cups. Leucoanthocyanins are indicated by the development of a cherry red or purplish coloration, while catechols are indicated by the presence of a reddish-brown tint (Bruneton, 2009).

### Saponins characterization

We diluted to half 1ml of aqueous extract that we shacked for 15 seconds. If the foam persists for at least 15 min it indicates the presence of saponins (Prashant et al., 2011)

### Mucilage characterization

A flaky precipitate that appears while mixing 5ml ethanol with 1ml of the extract indicates the mucilages (Paris et al., 1978).

### Characterization of lipoids

A heated plate is used to evaporate the mixture created by combining 1 g of plant powder with 7.5 ml of petroleum ether for 30 minutes. Three drops of  $\text{H}_2\text{SO}_4$  were combined with the greasy residue. The vivid green-purple hue suggests the existence of lipodes (Masumbuko, 1996).

### Characterization of iridoïds

1 ml of HCl combined with 1ml of the plant extract provides after heating a black precipitate that confirms the existence of iridoïdes (Paris et al, 1965).



## Highlighting of alkaloids

We mixed 5 ml of diluted  $H_2SO_4$  to 1/10 with 1g of plant powder, it was stirred and macerate for 24 hours at room temperature. Then, it was filtered. To get a brown precipitate, a mixture of a few drops of Wagner's reagent was made by combining 2 g of KI and 1.27 g of  $I_2$  solubilized in 100 ml of distilled water. This mixture was then added to the filtrate (Prashant et al., 2011).

## Characterization of reducing sugars

We put into evaporation in a water bath 5 ml of aqueous decoction until dry. Then, we added 1 ml of Fehling's reagent. A brick red precipitate indicates the presence of reducing sugar (Diallo, 2005).

## QUANTITATIVE ANALYSIS

### Determination of total polyphenols

0.5 ml of plant extract is mixed with 2.5 ml of Folin's reagent diluted 10 times. After being shacked we added 1ml of 7.5% (w/v) sodium carbonate solution. The absorbance was measured at 765 nm. Methanol was used to prepare blanks (Singleton and Rossi, 1965).

### Determination of total flavonoids

We mixed 1 ml of  $AlCl_3$  solution (2% in methanol) with 1ml of the plant extract. Methanol is used in place of the extract to prepare blanks. After 10 min of reaction the absorbance is read at 430 nm (Lamaison and Carnet, 1990).

### Determination of condensed tannins

To create 2.5 ml of vanillin reagent, equal parts 1% vanillin in methanol and 8% HCl in methanol were combined. This was then combined with 0.5 ml of plant extracts. 4% methanol-acid combination replaced the reagent in the blanks. After 20 minutes at 30 °C, the absorbance is measured at 500 nm (Hagerman, 2002).

## RESULTS

### Effect on tomato seedlings

In agriculture, it is important to study the effect of a product on the condition of seedlings.

Production quality is closely linked to plant development. Better plant development means better production. We have tried to study the effect of plant extracts on the length and diameter of seedlings, as well as on the number of flowers per plant.

### Seedling length and diameter

The two studied extracts showed a stimulating effect on the development and growth of tomato seedlings. Indeed, as shown on Table 1 the aqueous extracts of *Ocimum basilicum* and *Eucalyptus globulus* had an effect on the length and circumference of seedlings to different degrees and at different doses. After 15 days from the last application of the aqueous extracts, an initial observation showed the stimulating effect. The length of the *Ocimum basilicum*-treated seedling was  $1.23 \pm 0.07$  cm at the 4% dose, increasing to  $1.29 \pm 0.07$  cm at the 8% dose and reaching  $1.35 \pm 0.05$  cm at the high 50% dose. Compared with  $1.02 \pm 0.16$  cm for the negative control, which underwent no treatment, and  $1.40 \pm 0.14$  cm from treatment with a commercial stimulant product. For *Eucalyptus globulus*, seedling length reached  $1.1 \pm 0.31$  cm at the 4% dose,  $1.25 \pm 0.42$  cm at the 8% dose and  $1.35 \pm 0.25$  cm at the high 50% dose. The latter is the same as that obtained by *Ocimum basilicum* at the same dose, and close to that obtained by the positive control.

Similarly, the effect of the two extracts on the circumference of the seedlings studied was observed. The untreated control measured only  $6.43 \pm 1.2$  cm, whereas the two extracts showed circumferences of  $7.2 \pm 0.50$  cm at the 8% dose of *Ocimum basilicum* and  $10 \pm 0.35$  cm at the high dose of 50% of the same extract. For *Eucalyptus globulus* we obtained a circumference of  $6.8 \pm 0.86$  cm at the 8% dose and  $8 \pm 0.93$  cm at the 50% dose. These figures show the positive effect of the two aqueous extracts of *Ocimum basilicum* and *Eucalyptus globulus*, which increases as the dose of extract applied increases. These numbers are very similar to those of the positive control, which was treated with a commercial product and reached a length of  $8.2 \pm 1.16$  cm.

Measurements of the seedlings studied at harvest showed the same stimulating effect on seedling length, which obviously evolved over time. As shown on Table 2 for *Ocimum basilicum*, the length of the tomato seedling reached  $1.89 \pm 0.15$  cm at the 4% dose, and  $1.97 \pm 0.09$  cm at the 8% dose. With the 50% dose of the same extract, the

average length was the same as that of the positive control with the commercial product. The same thing was observed for *Eucalyptus globulus*. At the 4% dose we obtained a length of  $1.87 \pm 0.05$  cm, at 8% we reached  $1.9 \pm 0.15$  cm and the 50% dose approaches the positive control with  $1.97 \pm 0.20$  cm. The diameter of the seedlings did not change over time. The seedlings retained the same circumference at harvest and therefore the same diameter. We can conclude from these figures that it is possible to obtain the same effect with the plant extract and the commercial product. At the same time, the higher the dose of extract, the greater the effect of the extract and consequently, the better the results (Table 1 and 2).

### Number of flowers per plant

The number of flowers per plant had the same effect as that of seedling length and diameter. In fact, the negative control recorded a number of flowers per plant of  $6 \pm 2$  only as shown on Table 3, whereas with *Ocimum basilicum* at 4% the number of flowers was  $10 \pm 1.97$ , it also reached  $14 \pm 2.56$  flowers with the 8% dose of the same extract and  $20 \pm 2.30$  with a high dose. Numbers that are close to that of the positive control, which had  $22 \pm 5.5$  flowers per plant. This allows us to say that with a high dose of *Ocimum basilicum* extract we can achieve the effect of the commercial product.

**Table 1.** Seedling length and circumference after 15 days

Extract	Concentration	Seedling length (cm)	Seedling circumference (cm)
Negative control		$1.02 \pm 0.16$	$6.43 \pm 1.2$
Positive control		$1.40 \pm 0.14$	$8.2 \pm 1.16$
<i>Ocimum basilicum</i>	4%	$1.23 \pm 0.07$	$6.86 \pm 0.65$
	8%	$1.29 \pm 0.07$	$7.2 \pm 0.50$
	50%	$1.35 \pm 0.05$	$10 \pm 0.35$
<i>Eucalyptus globulus</i>	4%	$1.1 \pm 0.31$	$6.8 \pm 0.86$
	8%	$1.25 \pm 0.42$	$7.5 \pm 1.32$
	50%	$1.35 \pm 0.25$	$8 \pm 0.93$

**Table 2.** Seedling length at harvest

Extract	Concentration	Seedling length (cm)
Negative control		$1.80 \pm 0.07$
Positive control		$2 \pm 0.03$
<i>Ocimum basilicum</i>	4%	$1.89 \pm 0.15$
	8%	$1.97 \pm 0.09$
	50%	$2 \pm 0.06$
<i>Eucalyptus globulus</i>	4%	$1.87 \pm 0.05$
	8%	$1.9 \pm 0.15$
	50%	$1.97 \pm 0.20$

**Table 3.** Number of flowers per plant

Extract	Concentration	Number of flowers per plant
Negative control		$6 \pm 2$
Positive control		$22 \pm 5.5$
<i>Ocimum basilicum</i>	4%	$10 \pm 1.97$
	8%	$14 \pm 2.56$
	50%	$20 \pm 2.30$
<i>Eucalyptus globulus</i>	4%	$8 \pm 0.33$
	8%	$10 \pm 3$
	50%	$18 \pm 0.2$

On the other hand, *Eucalyptus globulus* had an effect similar to that of *Ocimum basilicum*. With the 8% dose, the number of flowers per plant reached  $10 \pm 3$ , while with the 50% dose it reached  $18 \pm 0.2$  flowers.

### Fruit quality

It is essential to study the effect of *Ocimum basilicum* and *Eucalyptus globulus* plant extracts on fruit quality. The effect on seedlings will ensure better production, while the effect on fruit quality will ensure better market insertion and a better ability to compete with tomatoes treated with a commercial product. This study focused on the essential elements considered by the consumer, the processing companies and the retailers. Fruit quality was analyzed in terms of weight and size, color, juice content, PH, acidity and total carbohydrate content.

### Fruit coloration

Fruit coloration was not greatly affected by the plant extracts. Indeed, we did not notice any difference between the coloration of the positive, the negative control and the fruit treated with *Ocimum basilicum* and *Eucalyptus globulus* plant extracts.

### Fruit weight and circumference

Table 4 showed that *Ocimum basilicum* and *Eucalyptus globulus* plant extracts also had a positive effect on fruit weight and circumference. In fact, at the 8% dose of *Ocimum basilicum*, the weight of the fruit reached  $121 \pm 24$  g and at the 50% dose it reached  $135 \pm 29$  g. This was well above the negative control that reached only  $75 \pm 23$  g and close to the positive control that reached  $145 \pm 50$  g. Similarly, for *Eucalyptus globulus*, at a dose of just 4% we achieved an

average weight of the tomato fruit around  $126 \pm 6$  g, and at the high dose of 50% we achieved an average weight around  $140 \pm 50$  g.

The same extracts also had an effect on the circumference of tomato fruits (Table 4). At the 8% dose of *Ocimum basilicum*, we achieved a circumference of  $22 \pm 1.59$  cm compared to the negative control that reached  $20 \pm 0.70$  cm. For *Eucalyptus globulus* the 4% dose gave an average fruit circumference around  $21.07 \pm 0.93$  cm. For 8% it reached  $22 \pm 1$  cm and at the 50% dose we were able to reach  $23.02 \pm 0.98$  cm compared with  $24.02 \pm 0.98$  cm for the positive control treated with the commercial product.

These results allow us to deduce that there is not a great difference between a high dose of the extract (50%) and a fairly low dose of 8%. Nevertheless, it allows to obtain a better results that are closer to the positive control treated with the commercial product.

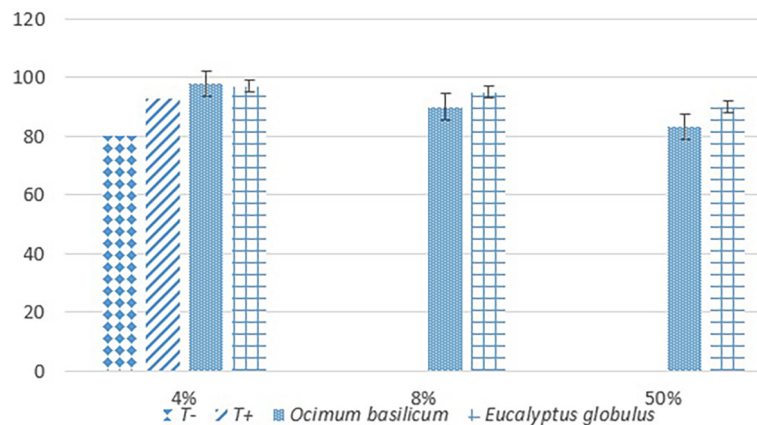
### Juice content

The appearance and consistency of tomato fruit do not give a clear idea of the amount of juice that can be extracted. Oranges seem to be juicier than apples. On the other hand, the amount of liquid extracted from apples is higher than oranges. In some cases, if production is destined for extraction, the amount of juice can play a decisive role in the selling price. The juice content of ripe tomatoes is generally between 93 and 95%, compared with 5 to 7% dry matter.

The two aqueous extracts have a positive effect on juice content as shown on the Figure 1. The juice content of the negative control was only 80%, while that of the two extracts exceeded 80%. Nevertheless, the juice rate decreased while the concentration for both extracts increased. For *Ocimum basilicum*, the juice yield at the 4% concentration was 98%, compared with 83% at

**Table 4.** Plant extracts effects n fruit weight and circumference

Extract	Concentration	Fruit weight (g)	Fruit circumference (cm)
T-		$75 \pm 23$	$20 \pm 0.70$
T+		$145 \pm 50$	$24.02 \pm 0.98$
<i>Ocimum basilicum</i>	4%	$89 \pm 8$	$20.98 \pm 0.70$
	8%	$121 \pm 24$	$22 \pm 1.59$
	50%	$135 \pm 29$	$23 \pm 1.73$
<i>Eucalyptus globulus</i>	4%	$126 \pm 6$	$21.07 \pm 0.93$
	8%	$135 \pm 15$	$22 \pm 1$
	50%	$140 \pm 50$	$23.02 \pm 0.98$



**Figure 1.** Juice content variation at different concentrations of the two aqueous extracts of *Ocimum basilicum* and *Eucalyptus globulus*, as well as the two positive and negative controls

the high concentration of 50%. For *Eucalyptus globulus* it fell from 97% at the 4% concentration to 90% at the 50% concentration. However, these results do not reach the negative control, which in this case was only 80%.

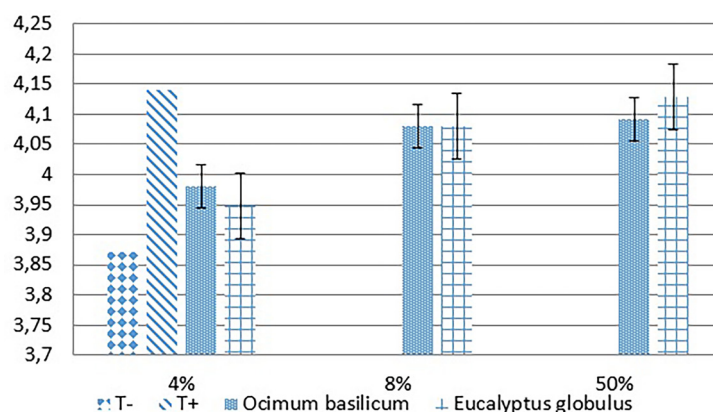
### Hydrogen potential (PH) and titratable acidity

Tomatoe’s Hydrogen Potential defines their acidity. It changes with ripening and affects texture, flavor and color. In fact, the lower the tomato’s PH, the more sour and acidic it becomes.

The difference between the PH of the control tomato and the one treated with plants extracts is clearly visible as shown on Figure 2. The PH of the negative control was 3.87, while that of the tomato treated with *Ocimum basilicum* at 4% reached 3.68, and 4.092 at 50%. Similarly, for *Eucalyptus globulus*, the PH at the 8% dose was 4.08 and at the 50% dose it reached 4.13. These numbers are close to the positive control that is

around 4.14. Therefore, *Ocimum basilicum* and *Eucalyptus globulus* plant extracts have a positive effect on the Hydrogen Potential of tomatoes. This effect may be translated into a demand for the product on the market, particularly the processing plants, for whom an acid and sour tomato may give a product that is easily perishable and less in demand for its taste.

Titratable acidity is the sum of free mineral and organic acids in the fruit juice. Depending on the concentration and type of aqueous extract applied, titratable acidity changed. As we can see on Table 5 the acidity of the negative control is 0.77%, while the positive control is 0.44%. Increasing the dose of extracts decrease the acidity. At the 4% dose of *Ocimum basilicum*, acidity is 0.59%, decreasing to 0.56% at the 8% dose and reaching 0.51% at the high dose of 50%. Similarly for *Eucalyptus globulus*, at the high dose of 50%, the acidity is 0.50%. Whereas at the 4% dose we obtained a value of 0.69%.



**Figure 2.** Variation in pH levels according to the different concentrations of the two aqueous extracts of *Ocimum basilicum* and *Eucalyptus globulus* and the two positive and negative controls



**Table 5.** Impact of extracts on fruit acidity

Extract	Concentration	Titration acidity
Negative control		0.77%
Positive control		0.45%
<i>Ocimum basilicum</i>	4%	0.59%
	8%	0.56%
	50%	0.51%
<i>Eucalyptus globulus</i>	4%	0.69%
	8%	0.64%
	50%	0.50%

### Total carbohydrate determination

Carbohydrates are the most important group of organic substances in the chemical composition of plants. Glucose, fructose and sucrose are the main sugars in fruit. Fruit's flavor is typically associated with the quantities of sugars and acids. The greatest tomatoes are those with high sugar and acid concentrations.

Sugar content increased with increasing applied dose of the aqueous extract of the two plants *Ocimum basilicum* and *Eucalyptus globulus*. The sugar level of the negative control is around 22.68 mg/ml while the positive control is around 37.02 mg/ml. For the 4% dose of *Ocimum basilicum*, we recorded a sugar level of 27.09 mg/ml, at the 8% dose it's around 58.54 mg/ml and 69.02 mg/ml at 50%. For *Eucalyptus globulus*, the sugar content reached 55.78 mg/ml at the 4% dose, 73.43 mg/ml at the 8% dose and 88.88 mg/ml at the 50% dose.

### Phytochemical screening

The phytochemical makeup of our extracts is displayed in Table 6. The two extracts include tannins. *Eucalyptus globulus* has a medium concentration of tannins as well as high concentrations of catechic and gallic tannins. Tanins and Gallic tannins are present in *Ocimum basilicum* in small amounts together with significant concentrations of catechic tannins.

*Ocimum basilicum* has a moderate amount of cyanidins. Then flavones, flavonoles and Leucoanthocyanins are moderately present in both extracts. Saponosides are also weakly present in both extracts, while alkaloids are only present in *Ocimum basilicum*. Iridoids are strongly present in the same extract.

Table 7 shows that *Ocimum basilicum* recorded the highest values for total flavonoids, total polyphenols and condensed tannins, with values of  $0.027 \pm$

**Table 6.** Phytochemical screening of test plants

Parameter	<i>Ocimum basilicum</i>	<i>Eucalyptus globulus</i>
Tanins	+	++
Catechic tannins	+++	+++
Gallic tannins	+	+++
Cyanidins	++	-
Flavones	++	-
Flavonoles	++	-
Anthocyanins	-	-
leucoanthocyanins	++	++
Alkaloids	+	-
Saponosides	+	+
mucilages	-	-
Reducing sugars	-	-
Lipoids	--	-
Iridoids	+++	-

**Note:** - : negative test ; + : positive test; ++ : moderately positive test; +++ : very positive test

**Table 7.** Flavonoid, total polyphenol and condensed tannin content

Plant extract	Total flavonoids (mg GA eq./g MS)	Total polyphénols (mg GA eq./g MS)	Condensed tanins (mg GA eq./g MS)
<i>Ocimum basilicum</i>	0.027±0.01	0.39±0.025	0.375±0.035
<i>Eucalyptus globulus</i>	0.017±0.04	0.15±0.05	0.0575±0.048

0.01 mg GA eq/g MS,  $0.39 \pm 0.025$  mg GA eq/g MS and  $0.375 \pm 0.035$  mg GA eq/g MS respectively.

*Eucalyptus globulus* recorded lower values than *Ocimum basilicum*, but still significant. For total flavonoids we recorded a value of  $0.017 \pm 0.04$  mg GA eq/g MS and for condensed tannins a value of  $0.0575 \pm 0.048$  mg GA eq/g MS.

## DISCUSSION

Typically, plant biostimulants are used on high-value crops such ornamentals, field vegetables, fruit trees, greenhouse crops, and flowers. The aim of their use is to ensure higher yields and better production quality. It used to be applied only for organic farming, to be generalized to conventional agriculture in order to meet economic and sustainability trenches (Colla and Rouphael, 2015).

The aqueous extract of *Ocimum basilicum* showed an effect on the germination speed index of tomato seeds and the root growth of lettuce and lemon balm. Conversely, the essential oil of the same plant showed an inhibitory effect on lettuce, tomato and lemon balm seeds, affecting germination speed index, germination percentage and root length of seedlings (Rosado et al., 2009).

Foliar application of an eco-product containing the essential oils of rosemary and eucalyptus had an effect on the length, number of leaves, biomass, weight of dry matter and yield of tomatoes. This effect was not the same between a single application and 3 applications of the product. Indeed, with the exception of the effect on tomato yield, the effect on the various components studied decreased between one and 3 applications of the eco-product (Chrysargyris et al., 2020).

An opposite effect was demonstrated with the application of *Eucalyptus camaldulensis*. Tomato plant germination, root and shoot elongation were significantly inhibited ( $P < 0.01$ ) by aqueous extracts of the fruit, leaves, roots, and bark. The strongest inhibitory effect was permitted at the highest concentration (5–10%), and the inhibitory effect was proportionate to extract concentrations. Additionally, the results showed that

radicle length and germination efficiency were more strongly affected by the inhibitory effect than plumule length (Fikreyesus et al., 2011).

The stimulating effect of plant extracts may be due to their richness in phenolic compounds, flavonoids and antioxidants. Indeed, antioxidant compounds improve cell growth and even seed germination by reducing stress conditions. This explains the increase in stem length by 15%, internode distance by 20% and stem diameter by extracts of *Taraxacum officinale*, *Concharpus erectus*, *Allium jesdianum*, *Rheum ribes*, *Dorema aucheri* and *Juniperus sabina* on tomato (Sohrab et al., 2022).

Flavonoids are phenolic compounds that contain over 8.000 individual compounds (Pietta, 2000) and have different metabolic functions in plants. they attract pollinators, control auxin transport, offer defense against UV-B radiation, infections and herbivores and alter reactive oxygen species and plant fertility (Falcone et al., 2012). they are divided into subgroups that include anthocyanins, flavonols, flavanones, flavones, flavanols, isoflavones, chalcones, catechins, and aurones (Panche, 2016). Additionally, they support the mediation of allelopathy, chelation, and soil metal reduction (Cesco et al., 2012; Hassan and Mathesius, 2012; Weston and Mathesius, 2013).

Many plant species include saponins, a structurally varied class of chemicals. They possess a variety of qualities, such as sweetness and bitterness (Vincken et al., 2007).

Particularly those derived from extracts of plant residue, polyphenols have the potential to have allelochemical effects. The development of more sustainable agriculture, which includes weed and pest control through crop rotation, residue management, and a range of bio-control techniques, may be impacted by this allelochemical effect. These allelochemicals can also be used as growth promoters, herbicides, and insecticides. They enable the manipulation of allelochemical production through crop genome modification and improve our understanding of the chemical interactions among ecosystem components (Popa et al., 2008). The stimulating effect of polyphenols has also been demonstrated

on vine callus formation. Indeed, the number of callus and suckers in treated vines is much higher than in vines treated with a commercial bio-stimulant (Colvit) (Tudose and Popa, 2000). Likewise, with regard to callus production alone, the polyphenolic extract's biostimulant effect is more noticeable; in this instance, there are about 20% more vines with circular calluses than in the reference (Popa et al., 2008).

## CONCLUSIONS

To ensure the sustainability and longevity of production systems, it is advisable to find effective, long-lasting solutions that respect the environment and improve production. As it might be known, the aqueous extract of *Ocimum basilicum* and *Eucalyptus globulus* are good alternatives against pathogens and especially on tomato fields. The objective of this study was to evaluate if the same plant extracts can be used also as biostimulant. Indeed, *Ocimum basilicum* and *Eucalyptus globulus* plants extracts showed a positive impact on tomato's production and productivity: the seedling and the fruit quality. The Aqueous extracts of *Ocimum basilicum* and *Eucalyptus globulus* are proving to be good alternatives to conventional stimulants since it is easily prepared and found and without a negative impact on the environment. Since both extracts are high into polyphenols, it can be the reason of their positive impact. This study showed that it is possible to use the two plants extracts as biostimulant, and put into consideration for the next research to ensure the real element responsible for the biostimulant effect in the composition of both extracts.

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