Vol.67: e24231031, 2024
https://doi.org/10.1590/1678-4324-2024231031
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Biological and Applied Sciences

Cytogenotoxicity of Basil (*Ocimum basilicum* 'Basilicão') by *Allium cepa* Test under Saline Stress Induction

Andrielle Wouters Kuhn[1]
https://orcid.org/0000-0002-9085-0038

Jéssica Mena Barreto de Freitas[2]
https://orcid.org/0000-0002-4374-2544

Nadine Lysyk Funk[3]
https://orcid.org/0000-0003-1341-3982

Lara Colles de Oliva Araujo[3]
https://orcid.org/0000-0002-3636-6763

Viviane Dal-Souto Frescura[2]
https://orcid.org/0000-0003-1520-6979

Thais Scotti do Canto-Dorow[4]
https://orcid.org/0000-0002-6282-7957

Cristiane de Bona da Silva[3]
https://orcid.org/0000-0002-5993-214X

Jerônimo Luiz Andriolo[5]
https://orcid.org/0000-0002-7439-2604

Carmine Aparecida Lenz Hister [2*]
https://orcid.org/0000-0003-1692-7698

Solange Bosio Tedesco[2]
https://orcid.org/0000-0002-9673-1996

[1]Universidade Federal de Santa Maria, Palmeira das Missões, Rio Grande do Sul, Brasil; [2]Universidade Federal de Santa Maria, Departamento de Biologia, Santa Maria, Rio Grande do Sul, Brasil; [3]Universidade Federal de Santa Maria, Departamento de Farmácia Industrial, Santa Maria, Rio Grande do Sul, Brasil; [4]Universidade Franciscana, Programa de Pós-Graduação em Ensino de Ciências e Matemática, Santa Maria, Rio Grande do Sul, Brasil; [5]Universidade Federal de Santa Maria, Departamento de Fitotecnia, Santa Maria, Rio Grande do Sul, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jane Manfron Budel

Received: 09-Oct-2023; Accepted: 04-Apr-2024

*Correspondence: carmine.hister@ufsm.br; Tel.: +55-55-997250531 (C.A.L.H.).

HIGHLIGHTS

- The induction of saline stress does not interfere with the cytogenetoxicity of basil.
- The aqueous extract of basil cultived in winter shows antiproliferative potential.
- The essential oil of basil does not have cytotoxic potential, but shows genotoxicity.

Abstract: Basil has aroused biological, pharmacological, agronomic and industrial interests, and stand out in the essential oil production. This study aimed to conduct cytogenetoxicity analysis through the *Allium cepa* L. test, of aqueous extracts and essential oil of basil (*Ocimum basilicum* L. "Basilicão") grown under the conditions of presence or absence of induction to saline stress. The basil was grown in a greenhouse in two seasons, summer and winter, in which some of the plants received induction to saline stress (solution of electrical conductivity of 5 dSm^{-1}) and the other part did not receive induction to saline stress (electric conductivity 1 dSm^{-1}). With the collected plants, was prepare the aqueous extracts of the leaves (5 e 10 gL^{-1}) and the extraction of essential oil of the leaves and inflorescences (0.10%) of basil, which were used for the cytogenetoxicity analysis by *A. cepa* test. Only the treatment with aqueous extract (5 gL^{-1}) of basil

grown under induction to saline stress and the treatment with aqueous extract (10 gL^{-1}) of basil grown without induction to saline stress (both during the winter) are shown to have antiproliferative potential. As for the genotoxic potential, only the treatment with essential oil (0.10%) of plants which received induction to saline stress during the summer has that effect. The other treatments have not cytogenotoxic potential.

Keywords: salinity; antiproliferative potential; aqueous extract; essential oil; genotoxic potential.

INTRODUCTION

The use of teas, essential oils and other derivatives of medicinal plants, such as basil, as an alternative to traditional medicines, has been gaining increased space in the treatment and cure of diseases [1,2]. However, coupled with this, there is concern about the safety and efficacy of such products [3], because it is known that each species, depending on cultivation conditions, may have a specific chemical composition, producing different biological properties [4].

Secondary compounds present themselves as an important part of this process, as they are responsible for many of the properties of interest observed in medicinal plants. These compounds have a complex structure and are present in low concentrations in the plant [5], being largely responsible for pollination and defense [6]. Thus, they arouse the interest of many researchers, not only for the pharmacological activities linked to these substances, but also for their importance in the food, agronomic and cosmetic areas [7].

The production of secondary compounds in the plant organism is dependent on the genetic control of the plant and the interaction genotype x environment. That is, this production can be influenced by changes in factors such as water, light, temperature, nutrients and other characteristics of the environment [8], also the vegetative cycle and organ of location in the plant [9]. The production of essential oils is stimulated especially in times of plant stress [10], such as increased salt. On the other hand, salinity can inhibit plant growth due to nutritional imbalance [11]. Thus, the growing environment in which the plant is found may be responsible for quantitative and/or qualitative changes in its chemical composition.

Basil (*Ocimum basilicum* L., Lamiaceae) is well known for its medicinal, aromatic, and spicy properties (Figure 1). The plant's tea is considered a digestive stimulant, gastric antispasmodic, and antirheumatic [12], as well as aiding in the treatment of respiratory problems [13]. The essential oil is used as a repellent, for its insecticidal properties [14] and in aromatherapy as a means of relieving anxiety and depression [15]. Antiseptic, antibacterial, anti-inflammatory, antimicrobial, and antioxidant properties are also attributed to the species [16]. Rosmarinic acid is the most abundant phenolic compound in basil aqueous extracts [17,18]. Linalool is the major compound in the essential oil of basil leaves and inflorescences, and has already demonstrated antimicrobial effects and induction of tumor cell apoptosis [19].

Figure 1. *Ocimum basilicum* ‘Basilicão’ grown in the conditions of absence and presence of saline stress induction, in the winter and summer seasons, respectively.

The possibility of such changes demonstrates the importance of knowing the plant product consumed, even when grown under different conditions for a given environmental factor. Thus, the need for tests with bioindicators of cytogenotoxicity of plant extracts is highlighted [20], such as the *Allium cepa* L. test, which allows the observation of the effects of the product through the cell cycle and the analysis of chromosomal changes [21]. Studies like these allow the use of medicinal plants in a safer way by the general population [22].

Considering the above, this work aimed to perform the cytogenotoxic analysis, through the *A. cepa* test, of aqueous extracts (AE) and essential oil (EO) of basil (*O. basilicum* 'Basilicão') grown under the conditions of presence or absence of induction to saline stress in two seasons of the year, summer and winter.

MATERIAL AND METHODS

Cultivation of *Ocimum basilicum* "Basilicão"

Basil (*Ocimum basilicum* 'Basilicão'), used in the preparation of AE and EO extraction, was cultivated in a greenhouse (29°43'24.6 "S 53°43'12.1 "W) in the Department of Plant Science of the Federal University of Santa Maria (UFSM), in two seasons: summer (starting in January 2016) and winter (starting in July 2016). A specimen of the species basil 'Basilicão' was deposited in the SMDB Herbarium of the Biology Department of UFSM, with the registration number 17.315, after identification performed by Profª Drª Thais Scotti do Canto-Dorow.

Basil seedlings were obtained in local commerce and planted in low density polyethylene greenhouse with a thickness of 100 µm. The adopted cultivation system was above ground, using four dm^3 polypropylene pots filled with commercial Plantmax® substrate, which were placed on fiber cement tile benches. A 300 L tank was installed on each bench to store the nutrient solution. The nutrient solution was supplied through a drip hose connected to a submerged pump inside the reservoir, activated by a timer. A drip tape was distributed to each pot row, so that there was a dripper for each pot. A drainage coefficient of no less than 30% was used in each fertirrigation, and the drained solution was collected and returned to the original reservoir in a closed system. Fertirrigation was done daily to replenish the volume of water transpired by the plants, estimated as described by Pardossi and coauthors [23].

For basil cultivation, the nutrient solution proposed by Frescura and coauthors [24] was used, with the following composition in $mmolL^{-1}$: 8,3 of NO_3^-; 1 of NH_4^+; 0,7 of $H_2PO_4^-$; 5 of K^+; 1,5 of Ca^{+2}; 1,25 of Mg^{+2} and 1,25 of SO_4^{-2} The micronutrients were supplied by a solution composed of ($mmolL^{-1}$): 0,03 of Mo; 0,26 of B; 0,06 of Cu; 0,50 of Mn; 0,22 of Zn by a stock solution and, the iron chelate, separately (1 mgL^{-1}). The macronutrients were supplied with potassium nitrate, monopotassium phosphate, calcium nitrate-Calcinit® and magnesium sulfate. This nutrient solution was used for growing rosemary (*Rosmarinus officinalis* L.), a plant also belonging to the Lamicaceae family, and adjusted for growing basil.

From this standard saline solution, the desired electrical conductivities (EC) were maintained for the cultivation of the plants. Those not induced to saline stress received a 1 dSm^{-1} solution, while those induced to saline stress received a 5 dSm^{-1} solution. The EC was kept close to the initial value, with a maximum deviation of 10%, using for possible corrections, water or aliquots of new nutrient solution, depending on the need. The pH was kept between 5.5 and 6.5, tolerating a deviation of 0.2 units, by adding NaOH or H_2SO_4 in 1M concentration, as needed. The nutrient solution was topped up whenever the volume was equal to or less than 50% of the original volume.

The cultivation of the plants, both in summer and winter, was conducted in two stands, each containing 22 plants. In the initial growth phase, all plants received only standard nutrient solution without induction of saline stress (1 dSm^{-1}). In the summer season, this procedure was performed during the first 15 days after planting (DAP). In the winter season, we waited for the plants to reach the same physiological age, measured by thermal sum, that the plants reached during the first 15 DAP in the summer growing season. After this initial phase of cultivation, the application of saline stress induction (5 dSm^{-1}) was started in one of the stands, while the other remained in a 1 dSm^{-1} solution, i.e., not receiving saline stress induction during the entire cultivation (Table 1).

Table 1. Description of the basil (*Ocimum basilicum* L. 'Basilicão') crop.

Basil cultivation	
Platform	**Summer**
1	Plants without induced saline stress
2	Plants under induced saline stress
	Winter
1	Plants without induced saline stress
2	Plants under induced saline stress

The daily temperature sum (STd ºC day) was calculated by the method described by Gilmore and Rogers [25] and Arnold [26], where: STd= Tmed - Tb. 1 day; if Tmed< Tb, then Tmed = Tb. For this, it was taken into consideration that: Tb = basal temperature (ºC) for the species, which was established at 10.9 ºC according to Chang and coauthors [27].

Tmed = average daily air temperature (ºC) calculated by the arithmetic mean between the minimum and maximum daily air temperatures, according to the conventional station of INMET/8º DISME, located approximately 500 meters from the experimental area.

After the beginning of the application of saline stress induction in one of the stands, the cultivation remained for another 30 days. At the end of 45 days after planting (DAP), in summer, and 89 DAP, in winter, six plants per stand were collected for the extraction of EO and preparation of AE, which were used in cytogenotoxic analysis using the *Allium cepa*.

Analysis of basil 'Basilicão' by the *Allium cepa* test.

The AE were prepared by infusion of fresh leaves, at concentrations of 5 gL^{-1} and 10 gL^{-1}. The lower concentration was established considering the amount of plant usually used by the population in the preparation of medicinal teas [28]. The extracts were prepared in the Laboratory of Plant Cytogenetics and Genotoxicity (LABCITOGEN) of the Department of Biology (UFSM). The infusions were obtained by boiling distilled water at 100ºC and pouring it over the chopped plant material to facilitate its action. Afterwards, the container containing the material was covered with a lid for 10 minutes. The extracts were strained and, when they were already at room temperature, used in the *A. cepa* test.

The extraction of the EO was performed in the Laboratory of Pharmacotechnical Development in the Department of Industrial Pharmacy (UFSM) using fresh basil leaves and inflorescences by means of hydro distillation in a Clevenger apparatus for three hours [29] for each 50 g sample of leaves and inflorescences and 250 mL of distilled water. The oil was dried over anhydrous sodium sulfate and, after filtration, stored at 4 ºC.

The *A. cepa* test was conducted at LABCITOGEN. For this, we considered concentrations of 5 and 10 gL^{-1} of aqueous extract and essential oil diluted, in ethanol, in one concentration (0.10 %), both extracted from plants grown without and with induction to saline stress, both from summer and winter crops. Thus, totaling 12 treatments, plus three controls (Table 2).

Table 2. Description of controls and treatments of *Ocimum basilicum* L. 'Basilicão' analysis by *Allium cepa* test.

Treatment	Description
T1	Negative control in distilled water
T2	Positive control in 1% glyphosate
T3	AE 5 gL^{-1} - without saline stress - S
T4	AE 5 gL^{-1} - with saline stress - S
T5	AE 10 gL^{-1} - without saline stress - S
T6	AE 10 gL^{-1} - with saline stress - S
T7	AE 5 gL^{-1} - without saline stressed - W
T8	AE 5 gL^{-1} - with saline stress - W
T9	AE 10 gL^{-1} - without saline stressed - W
T10	AE 10 gL^{-1} - with saline stress - W
T11	Negative control in ethanol
T12	EO 0.10 % - without saline stress - S
T13	EO 0.10 % - with saline stress - S
T14	EO 0.10 % - without saline stress - W
T15	EO 0.10 % - with saline stress - W

AE= aqueous extracts; EO = essential oil; S= summer-grown plants; W= winter-grown plants.

Distilled water was used as a negative control because this makes it possible to observe whether the results obtained with the aqueous extracts, which were prepared by infusion in distilled water, can be attributed exclusively to the plant components. The same is true for ethanol, which was used as a negative control for comparison with the results found for the essential oil, because it was diluted in ethanol. Glyphosate was used as a positive control because it has the ability to cause chromosomal changes in the genetic material of cells [30], making it possible, when compared to the treatments, to say whether or not they have the same ability to induce chromosomal changes.

To set up the experiment, the bulbs were rooted in distilled water and when the rootlets were already emitted and had an average size of 1.5 cm, they were placed in contact with the respective controls and treatments (Table 2), remaining there for 24 hours. The experiment consisted of 15 groups of five onion bulbs each, the groups representing the different controls and treatments analyzed, and the bulbs, each of their replicates. Then the rootlets were collected and fixed in ethanol: acetic acid (3:1) also for 24 hours and, at the end, they were stored in ethanol 70 %, where they remained in refrigerator until the slides were prepared. This process was performed by crushing the meristematic region of the rootlets, as proposed by Guerra e Souza [31], with modifications [32], using the 2 % acetic orcein dye.

The analysis and counting of cells were done under a microscope at 40X magnification, considering the cell cycle phase in which each cell was (interphase, prophase, metaphase, anaphase or telophase), and the occurrence of chromosomal changes. The mitotic index (MI) was then calculated for each treatment and control (referring to the percentage of cells in division), and the percentage of chromosomal abnormalities (CA). In total 1000 cells were analyzed per bulb, or 5000 cells per control/treatment.

The experiment was conducted using an entirely randomized design (15 treatments and five replications). The data were subjected to analysis of variance and compared by the Scott-Knott test, both at 5% probability of error, using the SISVAR 5.6 software [33].

RESULTS

The MI and CA results obtained by the *A. cepa* test for the AE and EO of basil 'Basilicão' grown in the conditions of absence and presence of saline stress induction, in the summer and winter seasons in the year 2016, are presented in Table 3.

Table 3. Mitotic Index (MI) (%) of controls and treatments analyzed by *Allium cepa* test.

	Description	Cells in interphase	Cells in division	Irregular cells	MI (%)	CA (%)
T1	Negative control in distilled water	4681	319	0	6,38[a]*	0[b]*
T2	Positive control in 1% glyphosate	4938	62	22	1,24[c]	0,44[a]
T3	AE 5 gL^{-1} - without saline stress - S	4729	271	1	5,42 [a]	0,02 [b]
T4	AE 5 gL^{-1} - with saline stress - S	4728	272	8	5,44 [a]	0,16 [b]
T5	AE 10 gL^{-1} - without saline stress - S	4746	254	5	5,08 [a]	0,10 [b]
T6	AE 10 gL^{-1} - with saline stress - S	4741	259	7	5,18 [a]	0,14 [b]
T7	AE 5 gL^{-1} - without saline stressed - W	4739	261	3	5,22 [a]	0,06 [b]
T8	AE 5 gL^{-1} - with saline stress - W	4806	194	6	3,88[b]	0,12 [b]
T9	AE 10 gL^{-1} - without saline stressed - W	4836	164	8	3,28[b]	0,16 [b]
T10	AE 10 gL^{-1} - with saline stress - W	4775	225	3	4,50 [a]	0,06 [b]
T11	Negative control in ethanol	4747	253	6	5,06 [a]	0,12 [b]
T12	EO 0.10 % - without saline stress - S	4711	289	5	5,78 [a]	0,08 [b]
T13	EO 0.10 % - with saline stress - S	4682	318	25	6,36 [a]	0,50[a]
T14	EO 0.10 % - without saline stress - W	4616	384	11	7,68 [a]	0,22 [b]
T15	EO 0.10 % -with saline stress - W	4679	321	3	6,42 [a]	0,06 [b]

AE= aqueous extracts; EO = essential oil; MI = mitotic index; CA = chromosomal abnormalities; S= summer-grown plants; W= winter-grown plants. *Values followed by different letters differ statistically by the Scott-Knott test ($p < 0.05$).

It can be observed that most of the treatments with AE (T3, T4, T5, T6, T7 and T10) and EO (T12, T13, T14 and T15) showed a similar behavior to the negative controls (distilled water and ethanol), not inducing an increase or decrease in cell proliferation in the meristematic cells of onion roots. For these, it is clear that no antiproliferative potential was found.

Differently, treatments T8 (AE 5 gL^{-1} - with saline stress - W) and T9 (AE 10 gL^{-1} - without saline stress - W) showed antiproliferative potential, since, when compared to the negative control in distilled water (T1), they statistically reduced the mitotic index of *A. cepa* cells (Table 3). Thus, these two treatments showed antiproliferative potential.

Only T13 (EO 0.10% - with saline stress - S) showed a genotoxic effect. differing significantly from the negative control (T1).

DISCUSSION

From a medicinal standpoint, the absence of cytogenotoxic activity in AEs is of great importance, as it confirms that the consumption of this extract by the general population, in moderation, is safe. Studies conducted by Nadeen and coauthors [18] have demonstrated that basil leaf extracts pose no toxicity potential and serve as an excellent source of natural antioxidants.

The different EA concentrations (5 or 10 gL^{-1}) of basil 'Basilicão' tested, with or without saline stress, did not impact the MI of *A. cepa*. This is in contrast to Frescura [34], where the concentration of the AE of *R. officinalis* inhibited cell division in a manner directly proportional to the concentration, although electrical conductivity did not interfere with the results. Thus, it can be concluded that the variation in salinity does not affect the cytotoxicity of basil. However, concerning genotoxicity, both rosemary EA and basil EA were found to be non-genotoxic under all studied conditions.

What appears to have slightly influenced cytotoxicity were the seasons, as two treatments of basil 'Basilicão' plants grown in winter – treatments T8 and T9 – exhibited antiproliferative potential. Antiproliferative activity is a biological property widely sought in medicinal plants and has been observed in other species belonging to the Lamiaceae family, such as *Mentha pulegium* L. [35], *Rosmarinus officinalis* [36], *Stachys byzantina* C. Koch. [37], and *Peltodon longipes* Kunth ex Benth. [38].

Previous studies indicate that rosmarinic acid is the most abundant phenolic compound in basil AE [17,18]. This compound is renowned for its antioxidant, anti-inflammatory, and antibacterial properties [18,39,40] and its potential protective effect against cellular injuries caused by chemotherapy and radiotherapy [17]. Additionally, it has been suggested to play a role in the prevention and treatment of cancer [40,41], inducing cell cycle arrest and apoptosis [42].

It is possible to note the essential oil (0.10 %) of the plants that received induction to saline stress during the summer (T13) was the only treatment that showed genotoxic potential, resembling, statistically, the positive control. The primary compound in basil essential oil, linalool, exhibited an increase in concentration under saline stress, irrespective of the growing season [43]. This elevation in linalool concentration under saline stress might possibly account for the genotoxicity observed in this treatment (T13). Castronuovo et al. [44] discovered elevated levels of phenolic compounds and increased antioxidant activity in basil plants exposed to higher air temperatures and greater total irradiance, conditions characteristic of summer. This observation, coupled with the heightened concentration of linalool in the essential oil under saline stress [43], led to the conclusion that these conditions of saline stress and higher temperature enhance the genotoxic potential of basil EO.

The other treatments involving basil EO did not exhibit cytogenotoxicity, consistent with the findings of [45], in their analysis of the EO of *R. officinalis* (0.20%) cultivated under salinity conditions (EC 5 dSm^{-1}), no genotoxic activity was observed, and there was no significant antiproliferative effect.

Chromosomal alterations were found and accounted for in all treatments and controls, except the control in distilled water, but, for most of them, in minimal amounts, not conferring to them genotoxic activity, since they did not differ significantly from the values presented by the negative control in water. The types of alterations found in the genetic material were micronuclei in interphases, lost chromosomes in metaphases and chromosome bridges in anaphases, which are shown in Figure 2.

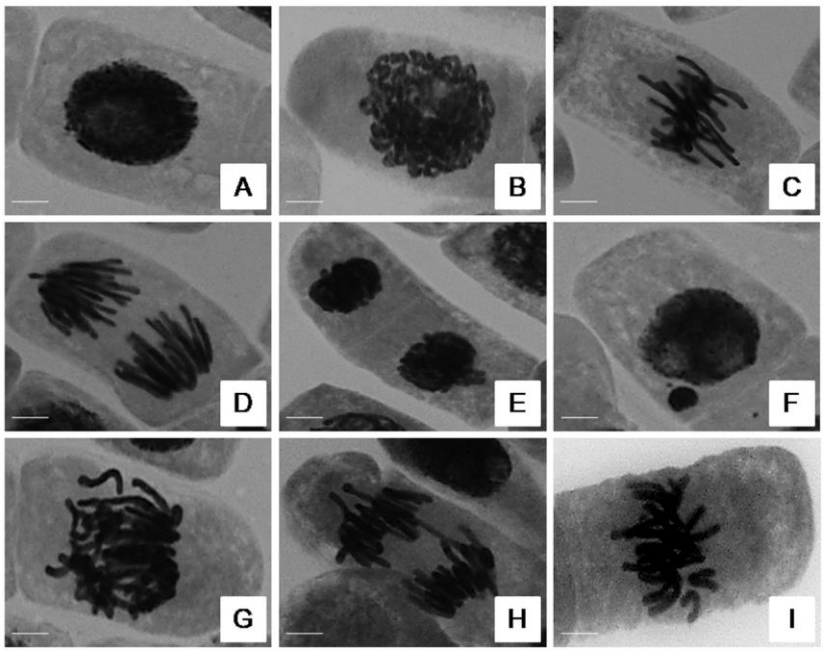

Figure 2. Cells of *Allium cepa* under the treatments with aqueous extracts and essential oil of *Ocimum basilicum* 'Basilicão' grown in the conditions of absence and presence of saline stress induction, in the summer and winter seasons. Cells with normal cell division: (A) Interphase; (B) Prophase; (C) Metaphase; (D) Anaphase; (E) Telophase. Cells with chromosomal irregularities: (F) Micronucleus; (G) Disorganized metaphase and lost chromosome; (H) Anaphasic bridge; (I) Lost chromosomes. Scale: 10 µm.

The absence of genotoxic potential is a positive point for plants that are widely consumed by the population, such as basil. The *A. cepa* test has been used numerous times to monitor plant extracts and essential oils and often showed that this unwanted effect was present in these plants, as reported by Lubini and coauthors [46], Tedesco and coauthors [47], Dias and coauthors [48], Pereira and coauthors [32], among others. This serves as a warning and demonstrates how much studies are still needed aimed at more specific knowledge of medicinal and aromatic plants for their use in a safe manner.

Regarding the use of saline stress in the cultivation of plants used for the preparation of extracts and essential oils, no data was found to confirm the alteration of the effects on the genetic material through biomonitoring tests. However, it is known that external factors, such as salinity in the plant environment, can interfere with the chemical composition of the plant [8,49] and, consequently, modify its effects on the target organism. The lack of studies on this issue underscores the necessity for further research on cultivation conditions, not only for basil, but also for other medicinal species that may be cultivated under these conditions.

CONCLUSION

Observing the results obtained, it is concluded that there is no cytotoxic potential in basil 'Basilicão' EO or AE when grown in the summer. Only two treatments involving basil plants cultivated in winter (AE of 5 gL^{-1} with saline stress induction and AE of 10 gL^{-1} without saline stress induction) demonstrate antiproliferative potential. EO and EA do not present genotoxic potential, except the treatment with EO (0.10%) from plants that received induction of saline stress during summer showed this effect.

In general, it can be said that the induction to saline stress on basil 'Basilicão' does not interfere with the effects of aqueous extracts and essential oil of plants, when it comes to cell proliferation and genotoxic potential on the organism *A. cepa*, because the results obtained in this experiment do not demonstrate this relationship when comparing all treatments. Therefore, additional studies focusing on investigating factors that affect plant growth, such as the type, intensity, and duration of salinity, are necessary to prove their action.

Funding: This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

1. Silva TB, Miranda MLD. Medicinal plant trade as a business alternative in Araguari, MG, Brazil. Rev. Gestão, Inovação Negócios. 2019;5(2):52–60.
2. Bones UA, Flach KA, Da Rosa GM, da Costa Junior JA. Comparative evaluation between empirical and scientific knowledge about the use of medicinal plants and their compounds. Rev. Gestão Soc. Ambient. 2022;16(2):1–17.
3. Cheikhyoussef A, Shapi M, Matengu K, Mu Ashekele H. Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. J. Ethnobiol. Ethnomed. Mar 2011; 7(10):1–11.
4. De Sousa DP, Damasceno ROS, Amorati R, Elshabrawy HA, de Castro RD, Bezerra DP, et al. Essential Oils: Chemistry and Pharmacological Activities. Biomolecules. 2023;13(7):1144.
5. Berg JM, Tymoczko JL, Stryer L. [Biochemistry]. 6th ed. Rio de Janeiro, RJ: Guanabara Koogan; 2008. 545 p.
6. Rodrigues GBC, Fernandes CC, Marcionilio SML de O, Martins CHG, Pedroso R dos S, Santiago MB, et al. Bioprospecting the Cerrado's aromatic flora: Chemical and biological studies of three essential oils. Braz. Arch. Biol. Technol. 2023;66:e23220034.
7. Simões CMO, Schenkel EP, Mello JC, Mentz LA, Petrovick PR. [Pharmacognosy: from natural product to medicine]. Porto Alegre, RS: Artmed; 2017. 502 p.
8. Klein T, Longhini R, Bruschi ML, Mello JCP. [Phytotherapeutics: a promising market]. Rev. Ciênc. Farm. Básica Apl. 2009;30(3):241–8.
9. Heinzmann BM, Spitzer V, Simões CMO. [Volatile oils]. In: Simões CMO, Schenkel EP, Mello JCP, Mentz LA, Petrovick PR, organizadores. [Pharmacognosy: from natural product to medicine]. Porto Alegre, RS: Artmed; 2017. p. 167–84.
10. De Azevedo CD, de Moura MA. [Growing medicinal plants: practical guide 27]. Niterói, RJ: Programa Rio Rural; 2010. 19 p.
11. Neves ALR, Lacerda CF de, Guimarães FVA, Hernandez FFF, Silva FB da, Prisco JT, et al. [Biomass accumulation and nutrient extraction by cowpea plants irrigated with saline water at different stages of development.]. Ciênc. Rural. 2009;39(3):758–65.
12. Lorenzi H, Matos FJ de A. [Medicinal plants in Brazil: native and exotic]. 3rd ed. Nova Odessa, SP: Instituto Plantarum; 2021. 576 p.
13. Favorito PA, Echer MM, Offemann LC, Schlindwein MD, Colombare LF, Schineider RP, et al. [Productive characteristics of basil (*Ocimum basilicum* L.) depending on the spacing between plants and between rows]. Rev. Bras. Plantas Med. 2011;13:582–6.
14. Costa CMGR, Santos MS, Barros HMM, Agra PFM, Farias MAA. [Inhibitory effect of basil essential oil on the *in vitro* growth of *Erwinia carotovora*]. Tecno. Ciênc. Agropecu. 2009;3(3):35–8.
15. Gradinariu V, Cioanca O, Hritcu L, Trifan A, Gille E, Hancianu M. Comparative efficacy of *Ocimum sanctum* L. and *Ocimum basilicum* L. essential oils against amyloid beta (1–42)-induced anxiety and depression in laboratory rats. Phytochem. Rev. 2015;14(4):567–75.
16. Ávila LC. [Phytotherapeutic therapeutic index]. 1st ed. Petrópolis, RJ: Publicações Biomédicas; 2008. 328 p.
17. Ibrahim RYM, Mansour SM. Phytochemical profile and protective effect of *Ocimum basilicum* aqueous extract in doxorubicin/irradiation-induced testicular injury. J. Pharm. Pharmacol. 2020;72(1):101–10.
18. Nadeem HR, Akhtar S, Sestili P, Ismail T, Neugart S, Qamar M. Toxicity, antioxidant activity, and phytochemicals of basil (*Ocimum basilicum* L.) leaves cultivated in Southern Punjab, Pakistan. Foods. 2022;11:1239.
19. Camargo SB, De Vasconcelos DFSA. [Biological activities of Linalool: current concepts and future possibilities of this monoterpene]. Rev. Ciênc. Méd. Biol. 2014;13(3):381–7.
20. Carmo LR do, Leal LS, Ribeiro LR. [*Allium cepa* and micronucleus test as bioindicators of cytogenotoxicity in aqueous extracts of medicinal plants]. Braz. J. Dev. 2020;6(10):82419–30.
21. Tedesco SB, Laughinghouse IV HD. Bioindicator of Genotoxicity: The *Allium cepa* Test. In: Srivastava J, organizador. Environmental Contamination [Internet]. InTech; 2012. p. 137–53. Available in: http://dx.doi.org/10.5772/31371.
22. Sturbelle RT, Pinho DS, Restani RG, Oliveira GR, Garcias G de L, Martino-Roth M da G. [Evaluation of the mutagenic and antimutagenic activity of *Aloe vera* in the *Allium cepa* test and micronucleus test in binucleated human lymphocytes]. Rev. Bras. Farmacogn. 2010;20(3):409–15.
23. Pardossi A, Carmassi G, Diara C, Incrocci G, Maggini R, Massa D. Fertigation and substrate management in closed soilless culture. Pisa: University of Pisa; 2011. 63 p.
24. Frescura VD, Schmitt OJ, Trapp KC, Andriolo JL, Lopes SJ, Tedesco SB. [Influence of nitrogen and collection periodicity on the production of rosemary leaves and essential oil (*Rosmarinus officinalis*)]. Rev. Bras. Plantas Med. 2021;23(4):179–85.
25. Gilmore EC, Rogers JS. Heat Units as a Method of Measuring Maturity in Corn. Agron. J. 1958;50(10):611–5.
26. Arnold CY. Maximum-minimum temperatures as a basis for computing heat units. Proceedings Am. Soc. Hortic. Sci. 1960;76:682–92.
27. Chang X, Alderson PG, Wright CJ. Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum* L.). J. Hortic. Sci. Biotechnol. 2005;80(5):593–8.
28. Martins ER, Castro DM, Castellani DC, Dias JE. [Medicinal Plants]. Editora UFV, organizador. Viçosa, MG; 2000. 220 p.

29. Qasem A, Assaggaf H, Mrabti HN, Minshawi F, Rajab BS, Attar AA, et al. Determination of chemical composition and investigation of biological activities of *Ocimum basilicum* L. Molecules. 2023;28(2):614.
30. De Souza LFB, Laughinghouse IV HD, Pastori T, Tedesco M, Kuhn AW, do Canto-Dorow TS, et al. Genotoxic potential of aqueous extracts of *Artemisia verlotorum* on the cell cycle of *Allium cepa*. Int. J. Environ. Sci. 2010;67(6):871–7.
31. Guerra M, De Souza MJ. [How to observe chromosomes: a guide to plant, animal and human cytogenetics techniques]. Ribeirão Preto, SP: FUNPEC; 2002. 132 p.
32. Pereira J de S, Hister CAL, Ubessi C, Tedesco SB. Genotoxicity, cytotoxicity and phenolic compounds from aqueous extracts of *Phyllanthus tenellus* Roxb. cultivated under different light conditions. Pakistan J. Biol. Sci. 2022;25(7):575–85.
33. Ferreira DF. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. Ciên. Agrotec. 2014; 38(2):109–12.
34. Frescura VD-S. [Phytochemical, genotoxic and growth parameters of rosemary (*Rosmarinus officinalis* L.) in different salinities and nitrogen doses]. Universidade Federal de Santa Maria; 2014.
35. Tedesco M, Kuhn AW, Aguiar AR de, Silva ACF da, Tedesco SB. [Antiproliferative potential of aqueous extracts of *Mentha pulegium* L. by the *Allium cepa* L. test]. Encicl. Biosf. 2012;8(15):1913–9.
36. Cardoso G, Dantas E, Sousa F, Peron A. Cytotoxicity of aqueous extracts of *Rosmarinus officinalis* L. (Labiatae) in plant test system. Braz. J. Biol. 2014;74(4):886–9.
37. Louvatel K, Zaions MIM, Arenhart AR. [Evaluation of the cytotoxicity and genotoxicity of extracts of *Stachys byzantina* C . KOCH . (Pulmonária) e *Tropaeolum majus* L. (Capuchinha), using the *Allium cepa* test system]. Unoesc Ciênc - ACBS. 2014; 12 (special edition):29–34.
38. Kuhn AW, Tedesco M, Boligon AA, Frescura VDS, Athayde ML, Tedesco SB. Genotoxic and chromatographic analyses of aqueous extracts of *Peltodon longipes* Kunth ex Benth. (hortelã-do-campo). Braz. J. Pharm. Sci. 2015;51(3):533–40.
39. Raina P, Deepak M, Chandrasekaran C V., Agarwal A, Wagh N, Kaul-Ghanekar R. Comparative analysis of anti-inflammatory activity of aqueous and methanolic extracts of *Ocimum basilicum* (basil) in RAW264.7, SW1353 and human primary chondrocytes in respect of the management of osteoarthritis. J. Herb. Med. 2016;6(1):28–36.
40. Nadeem M, Imran M, Gondal TA, Imran A, Shahbaz M, Amir RM, et al. Therapeutic potential of rosmarinic acid: A comprehensive review. Appl. Sci. 2019;9(15):3139.
41. Noor S, Mohammad T, Rub MA, Raza A, Azum N, Yadav DK, et al. Biomedical features and therapeutic potential of rosmarinic acid. Arch. Pharm. Res. 2022;45(4):205–28.
42. Zhao J, Xu L, Jin D, Xin Y, Tian L, Wang T, et al. Rosmarinic acid and related dietary supplements: Potential applications in the prevention and treatment of cancer. Biomolecules. 2022;12:1410.
43. Kuhn AW, Remuzzi SL, Boligon AA, Laughinghouse IV HD, de Campos MMA, Frescura VD-S, et al. Phytomass and essential oil of basil (cv. Basilicão) under periods of induced salt stress. J. Plant Nutr. 2023;46(20):4710–9.
44. Castronuovo D, Russo D, Libonati R, Faraone I, Candido V, Picuno P, et al. Influence of shading treatment on yield, morphological traits and phenolic profile of sweet basil (*Ocimum basilicum* L.). Sci. Hortic. (Amsterdam). 2019;254:91–8.
45. Pinheiro SMG, Mambrí AP de S, Frescura VD-S, Funk NL, Kuhn AW, Trapp KC, et al. [Phytochemical composition, antiproliferative effect and genotoxicity of rosemary essential oil (*Rosmarinus officinalis*) cultivated under different periods of salinity]. Rev. Bras. Plantas Med. 2020;22:17–24.
46. Lubini G, Fachinetto JM, Laughinghouse IV HD, Paranhos JT, Silva ACF, Tedesco SB. Extracts affecting mitotic division in root-tip meristematic cells. Biol. 2008;63(5):647–51.
47. Tedesco M, Kuhn AW, Boligon AA, Laughinghouse IV HD, Athayde ML, da Silva ACF, et al. Chromatographic analisys, antiproliferative effect and genotoxicity of aqueous extracts of *Citrus sinensis* (L .) OSBECK on the *Allium cepa* L . test system. Biosci. J. 2015;31(4):1213–21.
48. Dias WLF, do Vale Junior EP, das Dores Alves de Oliveira M, Barbosa YLP, do Nascimento Silva J, da Costa Júnior JS, et al. Cytogenotoxic effect, phytochemical screening and antioxidant potential of *Jatropha mollissima* (Pohl) Baill leaves. South African J. Bot. 2019;123:30–5.
49. Andrade FMC, Casali VWD. [Medicinal and aromatic plants: relationship with the environment, harvest and secondary metabolism]. Viçosa, MG: UFV; 1999. 139 p.