Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online

ZINC AS PROMOTER OF GROWTH AND BIOCHEMICAL ACTIVITY IN BASIL CULTIVARS UNDER *in vitro* CONDITIONS

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SAP 21909 Received: 12/03/2019 Accepted: 12/05/2019
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 18, n. 4, oct./dec., p. 342-350, 2019

ABSTRACT - *In vitro* cultivation of basil allows the manipulation of the concentration of certain micronutrients, commonly neglected by the micropropagation protocols. It is a plant of great economic importance for the cosmetic and pharmaceutical industry, due to the components present in its essential oil. In view of the above, the objective of this study was to evaluate zinc (Zn) concentrations in the micropropagation of basil, in addition to antioxidant activity and total phenolic compounds. Basil seeds, cultivars Manolo and Grecco Palla were oxygenated for 4 h, passed through asepsis and placed in test tubes with MS medium supplemented with 30 g L^{-1} sucrose and 6.5 g L^{-1} agar and pH adjusted to 5.8. The treatments were composed by the addition or not of 25 µM of zinc sulfate ($ZnSO_4$) and arranged in a completely randomized design. The tubes containing the seeds and the culture medium were kept in a growth chamber for 90 days. The cultivar Manolo was more sensitive to the addition of $ZnSO_4$ due to the increase in the number of leaves and in the antioxidant activity, however, the addition of this component in the culture medium did not influence the production of phenolic compounds or the activity of the antioxidant enzymes SOD, CAT and APX.
Keywords: *Ocimum basilicum* L.,antioxidant, superoxide dismutase, catalase, ascorbate peroxidase.

ZINCO COMO PROMOTOR DE CRESCIMENTO E ATIVIDADE BIOQUÍMICA DE CULTIVARES DE MANJERICÃO SOB CONDIÇÕES in vitro

RESUMO - O cultivo *in vitro* de manjericão permite a manipulação da concentração de determinados micronutrientes, comumente negligenciados pelos protocolos de micropropagação. É uma planta com grande importância econômica para a indústria cosmética e farmacêutica, devido aos componentes presentes em seu óleo essencial. Diante do exposto, objetivou-se com o presente trabalho avaliar concentrações de zinco (Zn) na micropropagação de manjericão, além da atividade antioxidante e compostos fenólicos totais. Sementes de manjericão, cultivares Manolo e Grecco Palla foram oxigenadas por 4 h, passaram pela assepsia e foram colocadas em tubos de ensaio com meio MS suplementado com 30 g L^{-1}de sacarose e 6,5 g L^{-1} de ágar e pH ajustado para 5,8. Os tratamentos foram compostos pela adição ou não de 25 µM de sulfato de zinco($ZnSO_4$) e dispostos em delineamento inteiramente casualizado. Os tubos contendo as sementes e o meio de cultivo foram mantidos em câmara de crescimento por 90 dias. A cultivar Manolo se mostrou mais sensível à adição de $ZnSO_4$ devido ao aumento no número de folhas e na atividade antioxidante, porém, a adição deste componente no meio de cultura não influenciou na produção de compostos fenólicos ou atividade das enzimas antioxidantes SOD, CAT e APX.
Palavras-chave: *Ocimum basilicum* L., antioxidante, superóxido dismutase, catalase, ascorbato peroxidase.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a plant belonging to the Lamiaceae family (LIBER et al., 2011), native to tropical Asia (VERMA et al., 2016) and Africa (BERTOLI et al., 2013). Although it is used as a seasoning and condiment, it can have its essential oil extracted from the leaves, as it contains chemical compounds of industrial interest (LIBER et al., 2011), with antiallergic, antiseptic and anticancer effect (HAKKIM et al., 2007). In addition to the functions described, it has antioxidant potential, making the species highly valued in the cosmetic and pharmaceutical industry (KWEE and NIEMEYER, 2011; FLANIGAN and NIEMEYER, 2014).

The conventional propagation of medicinal plants is sexually via seeds (LUZ et al., 2015), but micropropagation has shown itself to be a promising

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alternative. Through this biotechnological technique, it is possible to increase the multiplication index, development speed and improve seedling health (MORAIS et al., 2012). In addition, it allows the obtainment of some secondary metabolites of industrial interest. For the chemical and pharmaceutical industry, the use of micropropagated plants is important, due to the limitations that conventional planting brings, for example, changing the chemical composition of the plant in the face of climatic interference, pest attacks, diseases and cultural treatments (AHSAN et al., 2013; TRETTEL et al., 2018). However, the industry needs standardization, homogeneity and speed in the production of chemical compounds. Thus, cultivation *in vitro* can contribute to mitigate this problem.

The proper development of plants using the tissue culture technique is dependent on some factors, such as photoperiod, temperature, composition and supplementation of the culture medium. The inadequate amount of essential elements in the culture medium can inhibit the biochemical processes that depend on the interaction between nutrients and the species studied (GRAHAM et al., 2001). Therefore, to determine a micropropagation protocol, studies of the specific factors for each plant are necessary.

In tissue culture, it is possible to manipulate the nutrients in the medium, thus allowing to explore the function of some micronutrients, essential requirements in the growth and balanced development of plants (GRAHAM et al., 2001). An example is the element zinc (Zn), present in a concentration of 1720 mg L^{-1} in the MS culture medium, proposed by Murashige and Skoog (1962). Although it is a micronutrient, when present in inadequate conditions it can interfere with the growth and biochemical activity of plant species. Zn participates in many physiological and metabolic processes in plants (RAMESH et al., 2004), being a cofactor and activator of enzymatic reactions, in addition to participating in redox reactions, electron transfer and structural functions in nucleic acid metabolism (NAGAJYOTI et al., 2010).

Much of the work related to tissue culture, attempts have been made to demonstrate how the composition of the culture medium interferes with the growth of plant species, without, however, being concerned with biochemical analyzes of the plant. Medicinal plants are producers of secondary metabolites par excellence (LIBER et al., 2011), having antioxidant compounds, phenolic and enzymes, which can be quickly changed, which can trigger some oxidative stresses (KWEE and NIEMEYER, 2011). An example are antioxidant compounds responsible for the natural defense of organisms against the accumulation of reactive oxygen species (ROSs). ROSs are formed constantly during the physiological processes of plants, such as cellular respiration, for example. They are considered to be very unstable free radicals, where their production occurs in a disordered manner, thus being able to harm cellular metabolism (BALEN et al., 2011). As an antioxidant mechanism, the first line of defense of plants is the activation of antioxidant enzymes, such as superoxide dismutase (SOD). This enzyme uses some metals as cofactors, among them, Zn (BARBOSA et al., 2014).

Thus, knowing how the change in the concentration of micronutrients present in the medium is capable of interfering in the growth and biochemical activity of plant genotypes can assist during the processes of determination of micropropagation protocols, aiming at inducing the biosynthesis of hormonal precursors, in order to stimulate cell stretching and division (HERTEL, 1983), the activation of antioxidant enzymes, as a way of controlling plant stress, the production of phenolic compounds (DERAKHSHANI, 2011) or even the activation of secondary metabolism routes , seeking to increase the production of essential oils (TRETTEL et al., 2017).

In view of the above, the objective of this study was to evaluate zinc (Zn) concentrations in the micropropagation of basil, in addition to antioxidant activity and total phenolic compounds.

MATERIAL AND METHODS

Obtaining plant material and asepsis

The experiment was carried out at the Molecular Biology and Plant Tissue Culture Laboratories belonging to the Universidade Paranaense (UNIPAR), *Campus* Umuarama (PR). Basil seeds, cultivars Manolo and Grecco Palla, were purchased commercially (Feltrin®). Before starting the tests, the seeds were kept under oxygenation, in a refrigerator, for 4 h at a constant temperature of 6°C to overcome dormancy. Then, the seeds were treated with 70% ethanol for 2 min. and immersed in 2% sodium hypochlorite solution, for 15 min., with stirring. Subsequently, they were rinsed four times in a row with distilled water.

Conditions *in vitro*

The MS culture medium, in its full strength, was supplemented with 30 g L^{-1} of sucrose; 6.5 g L^{-1} agar (Kasvi®) and pH adjusted to 5.8. Zinc was added to the medium in the form of sulfate ($ZnSO_4$), in a concentration of 25 µM. The culture medium was poured into 50 ml test tubes, in the amount of 10 ml and subjected to autoclaving at 121°C for 20 min.

In a laminar flow room, one seed was inoculated per tube. Then, it was closed with a plastic cover and sealed with polyvinylpyrrolidone film (PVP). The tubes + seeds were kept in a growth room, with 24 h of light, 2°C and luminous intensity of 2000 lux (with luxmeter®), obtained with white LED lamps (Blumenau brand®, LED T8 10W 6,000K, 100-240V-50/60 Hz and power factor (PF) ≥ 0.92 (high PF) (TRETTEL et al., 2018).

After 90 days in the growth room, the percentage of germination was evaluated, being considered germinated those that presented root protrusion and leaf primordia, number of leaves and length of the main sprout (mm), using a digital caliper.

Evaluation of total phenolic compounds

To obtain the extract, 1 g of fresh leaves obtained after 90 days of cultivation *in vitro* were weighed. These leaves were macerated in liquid nitrogen and then transferred to a 25 mL falcon tube and 20 mL of 50% methyl alcohol was added. This mixture was homogenized with the aid of the vortex equipment (Kasvi®) and left to stand for 1 h, at room temperature and in the absence of light. After this period, it was centrifuged in a refrigerated centrifuge at 12,000 rpm, for 15 min. at 4°C. The collected supernatant was placed in falcon tubes (25 mL capacity) and 20 mL of 70% acetone was added to the residue. The mixture was again homogenized and left to stand for 1 h, in the absence of light and under refrigeration at 6°C. Subsequently, the residue was centrifuged again at 12,000 rpm, for 15 min. The supernatants were transferred to a 50 ml volumetric flask and the volume was made up with distilled water (WATERHOUSE, 2002).

The determination of total phenolic compounds was by the method proposed by Kuskoski (2005), using the Folin-Ciocalteu solution (Sigma Aldrich Chemical®). In 0.5 mL of extract from each sample, 2.5 mL of 10% (v/v) solution and 2 mL of 4% (v/v) sodium carbonate solution were added. The tubes were shaken with the aid of a vortex (Kasvi®) and left to stand on a bench for 2 h in the absence of light. The readings of the solution were performed on a 700 Plus spectrophotometer (Femto®), with a wavelength of 750 nm, containing three biological replicates and three techniques, totaling 9 replicates per sample. The calculation of total phenolic compounds was performed using the calibration curve for gallic acid (Equation 1):

$$y = 0{,}011x + 0{,}021 \text{ (Equation 1)}$$

Where:

x = concentration of total phenolic compounds (mg EAG 100 g^{-1})

y = absorbance

R^2 = 0.99, considering the amount of sample in the extract.

Evaluation of antioxidant activity

From the extract was determined the antioxidant activity based on the extinction of the absorption of the radical 2,2-diphenyl-1-picryl hydrazyl (DPPH 60 µM) proposed by Rufino et al. (2009). For the determination, an aliquot of 0.1 mL of the obtained extract was used, transferred to a test tube, protected from light and added 3.9 mL of the DPPH radical (0.06 mM) and homogenized in a tube shaker. The readings taken on the (Beckman 640 B) spectrophotometer (515 nm) and monitored every 30 min. for a total of four readings, where the absorbance reduction was observed until its stabilization. The results were expressed as percentage of free radical sequestration (% SRL), according to the equation 2:

$$AA(\%) = \left[\frac{(Ca-As)}{Ca}\right].100 \text{ (Equation 2)}$$

Where:

AA = antioxidant activity (%)

Ca = control absorbance;

As = absorbance of the sample.

Enzymatic evaluation

To obtain the enzymatic extract, 200 mg of leaf tissue were macerated in liquid nitrogen and homogenized in 1.5 mL of extraction buffer, composed of 400 mM of potassium phosphate (pH = 7.8), 1.0 mM of EDTA and 200 mM ascorbic acid. The homogenate was centrifuged at 12,000 rpm, for 15 min at 4ºC and then the supernatant was collected (BONACINA et al., 2017). Enzymatic activities were expressed in enzymatic unit.

SOD (EC 1.15.1.1)

The activity of the enzyme superoxide dismutase (SOD) was measured by its ability to inhibit photoreduction of nitroblue tetrazolium (NBT) as described by Giannopolitis and Ries (1977). The reaction (200 µL) consisted of 100 mM buffer (pH = 7.8), 100 mM KPO_4 (pH = 7.8), 1mM EDTA, 120 mL - methionine, 750 µM NBT, 20 µM of riboflavin and 20 µL of the crude extract of the samples. The reading was taken at 560 nm, in which one unit of SOD (U) activity was defined as the amount of enzyme required to inhibit 50% reduction in NBT. SOD activity was expressed as U SOD mg^{-1} MF min^{-1}.

CAT (EC 1.11.1.6)

Catalase enzyme activity (CAT) was performed according to the methodology proposed by Havir and McHale (1987). The reaction, which totals 200 µL, consisted of 100mM KPO_4 buffer (pH = 7.0), 125 mM hydrogen peroxide, H_2O autoclaved and 20 µL of crude extract from the samples. The activity was determined by the degradation of hydrogen peroxide in the range of 1 min. at 260 nm. Enzyme activity was quantified using the molar extinction coefficient 36 m^{-1} cm^{-1} (ANDERSON et al., 1995) being expressed in mmol H_2O_2 g^{-1} MF $min^{-1.}$

APX (EC 1.11.1.11)

The activity of ascorbate peroxidase enzyme (APX) was performed according to the methodology proposed by Nakano and Asada (1981). The reaction, which totals 200 µL, consisted of 200 mM KPO_4 buffer (pH = 7.0), 5mM ascorbic acid,H_2O_2, H_2O autoclaved and 20 µL of the crude extract of the samples. Activity was determined by the degradation of H_2O_2 in 1mM the range of 1 minute at 290 nm. Enzyme activity was quantified using the molar extinction coefficient 2,8 mM^{-1} cm^{-1}(NAKANO and ASADA, 1981). APX activity was expressed as mmol ascorbic acid g^{-1} MF min^{-1}.

All enzymes were evaluated using 96-well flat bottomed elisa plates. In all enzymatic assays three technical repetitions and three biological repetitions were used. The equipment used was the UV-VIS spectrophotometer, Espectra Max Plus with SoftMax Pro 6.5.1 program.

Treatments and statistical analysis

The treatments were composed by: T1 = cultivate Manolo without addition of $ZnSO_4$, T2 = cultivate Manolo with addition of $ZnSO_4$, T3 = cultivate Grecco Palla without addition of $ZnSO_4$ and T4 = cultivate Grecco Palla with addition of $ZnSO_4$. The experiment was conducted in a completely randomized design (CRD), in a simple scheme, with and without the addition of Zn, in two cultivars of basil, with 4 repetitions of 10 test tubes for each cultivar. The measurements of biometric characteristics, phenolic compounds and antioxidant activity were subjected to the normality test, according to Shapiro-Wilk. Then, they were subjected to analysis of variance (ANOVA) and the means compared by the Tukey test, at 5% probability of error, using the statistical program SISVAR 5.6 (FERREIRA, 2011).

RESULTS AND DISCUSSION

Growth of the two basil cultivars

For all morphological characteristics evaluated (germination, number of leaves and length of main shoots). The differences found occurred both between cultivars and between the addition or not of zinc sulfate ($ZnSO_4$). The cultivar Manolo showed 90% germination of the seeds, while the cultivar Grecco Palla, 72%. The difference in the germination percentage was dependent on the genotype, since the means of the isolated cultivars, when zinc (Zn) was added and in the control treatment remained similar (Table 1). This result demonstrated that the cultivar Manolo presented seeds more tolerant to the addition of Zn, different from that observed for the other cultivar.

TABLE 1 -Morphological characteristics of basil grown *in vitro*, cultivars Manolo and Grecco Palla, with and without added $ZnSO_4$.

Basil Cultivars	$ZnSO_4$ (µM)	Germination (%)	Number of leaves	Shoot length (mm)
Manolo	0	90±1.22a*	48.07±10.20b	41.28±4.03b
	25	92±0.83a	92.80±2.69a	42.82±4.85b
Grecco Palla	0	72±1.64ab	15.33±2.68c	60.64±5.36a
	25	66±1.14b	14.20±3.05c	67.07±5.71a
CV(%)		15.56	23.78	17.94

*Means followed by the same letter in the column do not differ statistically from each other, by the Tukey test, at 5% probability of error.

Liu et al. (2013) reported that the expression of transcription factors linked to Zn is able to promote tolerance to abiotic stress during the germination of *Arabidopsis*. This probably occurs because plants whose development occurs under conditions of adequate Zn concentration, tend to express these transcription factors, originating seeds that are more tolerant to abiotic stress and with greater germination potential (TANG et al., 2005). In addition to the genotypic characteristics of the cultivars, the conditions in which the seeds develop are decisive for their germination.

For number of leaves, the cultivar Manolo produced an average of 48 leaves per plant, representing 3.2 times more leaves than Grecco Palla, which produced an average of 14 leaves. For this characteristic, it can be observed that the addition of $ZnSO_4$ to the medium favored the increment of the leaves. The cultivar Manolo produced approximately 50% more leaves, when compared to the medium not supplemented with Zn. For the number of leaves of the cultivar Grecco Palla, no statistical difference was verified in relation to the addition of Zn to the culture medium.

The production of leaves is one of the main characteristics in the production of medicinal plants *in vitro*, since these organs concentrate glands that produce essential oil. An increment in the quantity of leaves is therefore directly proportional to the yield of this oil (SANGWAN et al., 2001). As for the influence of Zn, authors such as Takaki and Kushizaki (1970) and Hertel (1983) suggest that this element may be involved in the biosynthesis of indole-3-acetic acid (IAA) and inducing the synthesis of tryptophan, which is a precursor of this auxin. This, in turn, influences plant growth, especially in cell stretching and division (DERAKHSHANI et al., 2011). Probably, the cultivar Manolo when exposed to the highest concentration of Zn more easily induced the metabolic pathway that synthesizes tryptophan. Therefore, the largest leaf formation in this genotype, as observed by Saa et al. (2015), in his work with *Prunus dulcis*.

Shoot length was independent of the addition of Zn. The differences in this characteristic were shown to be linked to the genotype. Regarding the length of shoots to cultivar Manolo, the length was 41.28 mm and Grecco Palla, 60.64 mm. In this way, the cultivar Manolo produced a greater number of leaves and shorter sprout length in opposition to Grecco Palla, which produced longer sprouts, but with fewer leaves. This probably occurred only as a function of the genotype, indicating that the concentration of Zn did not prevail in the genotypic characteristics of the cultivars in relation to the length of the main sprout.

According to Verma et al. (2016), $ZnSO_4$ concentrations are not able to cause morphological changes in cultivated basil *in vitro*. In the presence of Zn in the medium, oxidation of explants was observed,

suggesting that the stress caused by this nutrient can prevent seedling growth.

Response to biochemical activity of plants

There was a significant difference ($p>0.05$) between antioxidant activity (%) and concentration of total phenolic compounds in the cultivars. The cultivar Grecco Palla showed levels of phenolic compounds of 1.32 mg 100 g^{-1}, higher than the cultivar Manolo. The addition of Zn to the medium did not interfere the concentration of phenolic compounds in the cultivars (Figure 1). Studies have shown that, among phenolic compounds, rosmarinic, shikimic, caffeic, chicory and anthocyanins acid are among the main groups present in basil, with proven antioxidant and medicinal capacity (KWEE and NIEMEYER, 2011; FLANIGAN and NIEMEYER, 2014).

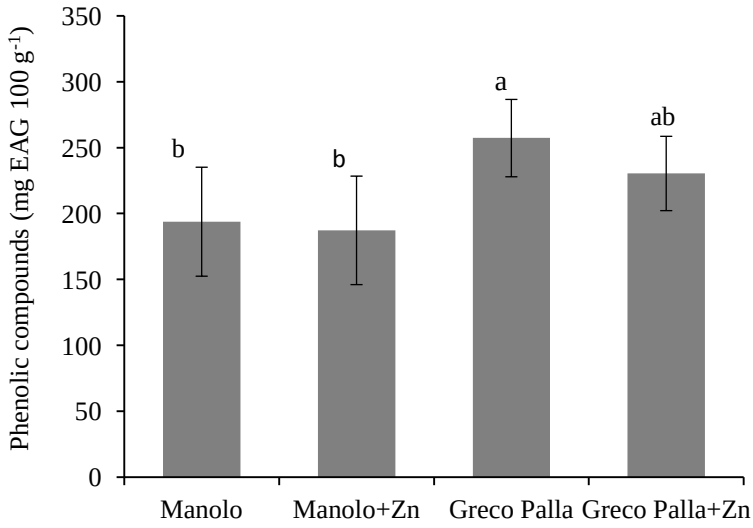

FIGURE 1 - Total phenolic compounds (mg EAG100g g^{-1}) present in the cultivars Manolo and Grecco Palla of basil kept *in vitro*, with and without the addition of $ZnSO_4$.

The cultivar Grecco Palla showed greater antioxidant potential, with 9.28 µg of trolox g^{-1}. The addition of $ZnSO_4$ to the culture medium for this cultivar did not alter its antioxidant activity. In contrast, in the cultivar Manolo, the addition of Zn to the medium increased the antioxidant activity by approximately 67% (Figure 2).

The results obtained suggest that the cultivar Grecco Palla may have been more sensitive to the damages caused by the addition of Zn, when compared to the cultivar Manolo, presenting greater production of phenolic compounds and antioxidant activity. That cultivar may have mobilized part of its energies and resources to produce these compounds, in order to avoid oxidative damage caused by the probable increase in free radicals in its metabolism (GILL and TUTEJA, 2010; NOCTOR et al., 2018). This reflected in the number of leaves, which was 15.3% less than the other cultivar studied. Free radicals are highly reactive molecules such as singlet oxygen (• O_2), hydroperoxyl radical (HO_2•), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH •), which cause destruction of lipids, proteins, membranes and DNA (NOCTOR et al., 2018). If not fought in the plant organism, they delay and limit the growth of seedlings, where, in more severe cases, lead to death.

On the other hand, the cultivar Manolo showed to be favored by Zn, presenting antioxidant activity and production of phenolic compounds statistically smaller than the second cultivar studied. This cultivar probably directed its resources towards the production of phenolic compounds, such as phenylpropanoids, not to reduce oxidative stress, but to produce precursor compounds for lignin (MOURA et al. 2010; LIU, 2012). Similar responses to this test were also verified for basil cultivar basilicão, which had a high number of leaves, when supplemented with 25 µM of $CuSO_4$ (TRETTEL et al., 2017; TRETTEL et al., 2018).

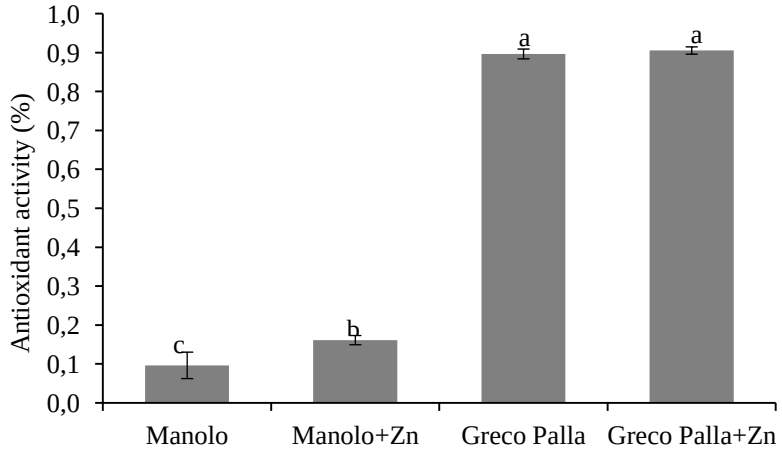

FIGURE 2 - Antioxidant activity (%) in cultivars Manolo and Grecco Palla of basil kept *in vitro*, with and without the addition of $ZnSO_4$.

Although the production of phenolic compounds was above 200 mg EAG 100^{-1} g for both cultivars, not all compounds produced have antioxidant potential. For a compound to have antioxidant action in the cell, it is necessary to neutralize free radicals (GILL and TUTEJA, 2010; NOCTOR et al., 2018). As the antioxidant activity was lower in cv. Manolo assumes that not all compounds produced by it have this purpose. The same was suggested by Abraham et al. (2011) who found in *Curcuma amada* (*Curcuma mangga*), kept *in vitro* and added yeast extract concentrations (3.5 mg L^{-1}), reported an increment in the production of total phenolic compounds above the double the control (500 mg EAG 100 g^{-1}). However, the same authors did not find a correlation between the total phenolic content and the elimination of free radicals using the DPPH method. Likewise, Kwee and Niemeyer (2011) reported that simple phenolic compounds found in basil, which can react with the Folin-Ciocalteu reagent, are often not effective antioxidants, which can lead to a low degree of correlation between the total of phenolic compounds verified in the plant and the DPPH method.

Regarding the enzymatic methods of antioxidant evaluation, cultivars Manolo and Grecco Palla showed an average of 18.8 and 24.87 mMol of APX per g of leaves between treatments with or without the addition of $ZnSO_4$, respectively. This indicates that, regardless of the addition of Zn to the medium, the second cultivar naturally has greater enzymatic activity (Figure 3). For CAT, cultivars Manolo and Grecco Palla showed an average of 2.29 and 2.52 mMol g^{-1} of leaves. The results indicated that, regardless of the addition of $ZnSO_4$, the activity of this enzyme was more evident in the second cultivar (Figure 4).

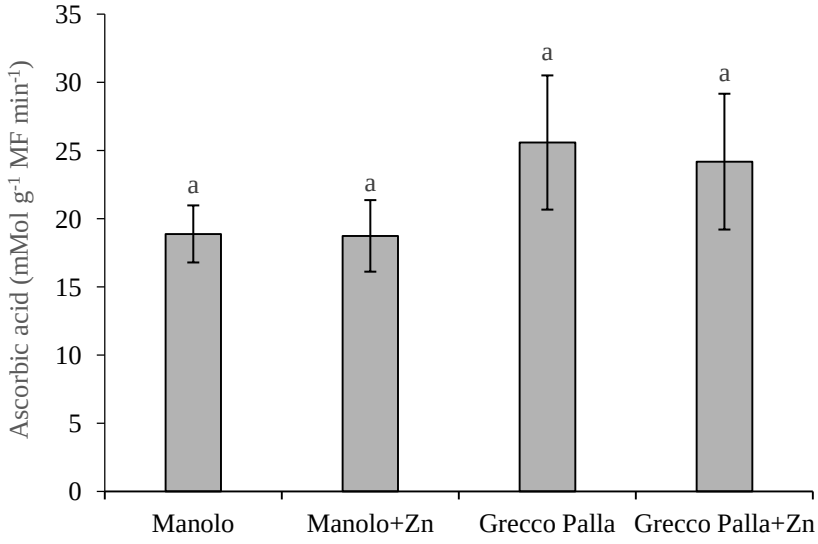

FIGURE 3 - APX enzyme activity in cultivars Manolo and Grecco Palla of basil kept *in vitro*, with and without the addition of $ZnSO_4$.

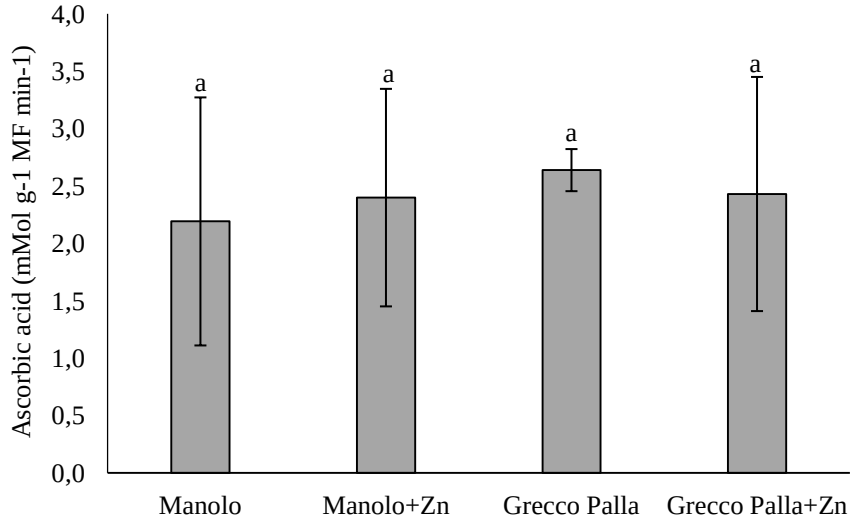

FIGURE 4 - CAT enzyme activity in cultivars Manolo and Grecco Palla of basil kept *in vitro*, with and without the addition of $ZnSO_4$.

The cultivar Manolo showed an average of 306.6 SOD per mg of leaf, while cv. Grecco Palla showed an average of 278.47 (Figure 5). Differently from that observed in the other antioxidant enzymes, the SOD activity did not present great differences between the cultivars, although their values were much higher when compared to the others evaluated. This was possibly because SOD is the first line of defense against radicals in the cell (SCHIEBER and CHANDEL, 2014). Initially singlet oxygen $\bullet O_2$is reduced by this enzyme or is dismutated in H_2O_2 by enzymes such as APX and peroxiredoxins (PRX) (NOCTOR et al., 2018). Subsequently, the catalase acts by oxidizing the peroxide H_2O_2 in water and an oxidized radical, thus completing the cell cleaning cycle. A single molecule of this enzyme is capable of acting on thousands of H_2O_2 (GILL and TUTEJA, 2010).

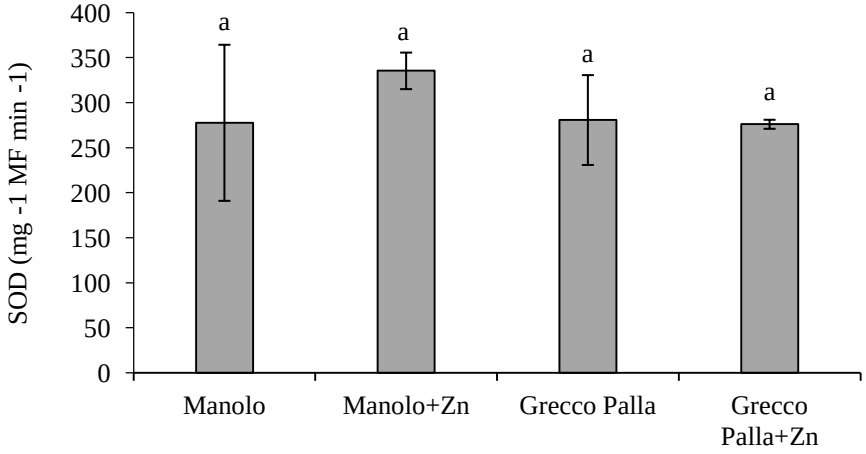

FIGURE 5 - SOD enzyme activity in cultivars Manolo and Grecco Palla of basil kept *in vitro*, with and without the addition of $ZnSO_4$.

Thus, the addition of Zn to the culture medium triggered different morphological and biochemical responses, depending on the basil genotypes. It was found in cv. Manolo an increase in leaf production and in cv. Grecco Palla an increase in the length of shoots. Regarding the biochemical activity, the antioxidant activity and the production of phenolic compounds were dependent on the concentration of Zn and the cultivars studied. The next tests should clarify which compounds and chemical groups increase their synthesis when the plant is exposed to $ZnSO_4$ and verify its relationship with antioxidant activity. As mentioned, not all phenolic compounds have this purpose of promoting the cleaning of free radicals from the cell.

Thus, the addition of Zn to the culture medium can contribute to the growth of basil cultivars. It is also assumed that, due to the stress caused by the addition of this element, both cultivars show an increase in antioxidant activity, synthesis of phenolic compounds and activity of antioxidant enzymes.

CONCLUSIONS

The cultivar Manolo was more sensitive to the addition of $ZnSO_4$ due to the increase in the number of leaves and in the antioxidant activity, however, the addition of this component in the culture medium did not influence the production of phenolic compounds or activity of the enzymes SOD, CAT and APX.

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