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FOOD TECHNOLOGY

Productivity and antioxidant activity of mung bean sprouts (*Vigna radiata* L.) mediated by some elicitors

Marcelo Duarte[1] Verônica Letícia da Silva[1] Ana Claúdia Pacheco[1] Nelson Barbosa Machado Neto[1] Ceci Castilho Custódio[1*]

[1]Agricultural Science College, Universidade do Oeste Paulista (UNOESTE), 19067-175, Presidente Prudente, SP, Brasil. E-mail: ceci@unoeste.br. *Corresponding author.

ABSTRACT: *The production of bioactive food is one of the goals of agriculture. Sprouts used for human consumption are an efficient way to increase the intake of essential nutrients, such as minerals, vitamins, and other bioactive compounds. The use of elicitors can increase the nutritional value of these products. Biomass production, phenolic compound content, and antioxidant activity of mung bean sprouts (Vigna radiata L.) after elicitation were examined in this study. Mung bean sprouts were treated with salicylic acid (SA), ascorbic acid (AA), and tocopherol (TOC) at different concentrations and numbers of applications. Shoot and root lengths and dry mass were measured on five-day-old sprouts. Total phenol content and antioxidant activity were determined using the DPPH assay on dried ethanolic extracts. Total soluble protein content and superoxide dismutase activity were measured in frozen hypocotyls. All elicitors stimulated the antioxidant functions of sprouts and, at the highest concentrations, reduced the biometric parameters; therefore, lower concentrations were better. For the first time, a balance between elicitor concentration and application in instalments was achieved to maintain the production of sprouts with enhanced nutritional characteristics.*
Key words: *salicylic acid, ascorbic acid, tocopherol, bioregulation, functional food.*

Produtividade e atividade antioxidante de brotos de feijão (*Vigna radiata* L.) mediada por alguns elicitores

RESUMO: A produção de alimentos bioativos é um dos objetivos da agricultu*ra. O uso de brotos para consumo humano é uma forma eficiente de aumentar a ingestão de nutrientes essenciais, como minerais, vitaminas e outros compostos bioativos. O uso de elicitores pode aumentar o valor nutricional desses produtos. A produção de biomassa, o conteúdo de compostos fenólicos e a atividade antioxidante de brotos de feijão (Vigna radiata L.) após a elicitação foram examinados neste estudo. Brotos de feijão foram tratados com ácido salicílico (SA), ácido ascórbico (AA) e tocoferol (TOC) em diferentes concentrações e número de aplicações. O comprimento da parte aérea e da raiz e a massa seca foram medidos em brotos com cinco dias de idade. O teor de fenol total e a atividade antioxidante foram determinados usando o ensaio DPPH em extratos etanólicos secos. O conteúdo total de proteína solúvel e atividade da superóxido dismutase foram medidos em hipocótilos congelados. Todos os elicitores estimularam as funções antioxidantes dos brotos e, nas maiores concentrações, reduziram os parâmetros biométricos; assim, concentrações mais baixas foram mais eficientes. Pela primeira vez, foi alcançado um equilíbrio entre a concentração do elicitor e a aplicação em parcelas para incrementar a produção de brotos e aprimorar características nutricionais aprimoradas.*
Palavras-chave: *ácido salicílico, ácido ascórbico, tocoferol, biorregulação, alimentos funcionais.*

INTRODUCTION

The low incidence of diseases in some populations has drawn attention to their diet as is the case of the Eskimos whose diet is rich in fish polyunsaturated fatty acids (ELAGIZI et al., 2021), the French people who consume red wine (LIPPI et al., 2010) and Asians due to the soy consumption (MASKARINEC et al., 2017). In Asian and European countries, the high consumption of fresh fruits and vegetables also diminishes the risk of coronary heart disease and cancer, as proven by epidemiological data (FARIA & ANJO, 2004).

Pulse crops are rich in carbohydrates (55%–65% of weight), proteins (21%–26%), including essential amino acids, and low in fat (1%–4%) (IRITI; VARONI, 2017). They are also an excellent source of nutrients and components, with well-documented pro-health properties, such as fibre, phenolics and vitamins (IRITI; VARONI, 2017; TANG et al., 2014).

The use of sprouts of pulse seeds (green pea, lentil, alfalfa and mung bean), considering the general deficiency of high-protein foods, are excellent alternatives to improve the nutritional value and quality of readily available foods, which

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are an interesting research topic (LEITE et al., 2016; ŚWIECA & GAWLIK-DZIKI, 2015). Bean sprouts obtained from the germination of mung bean (*Vigna radiata* L.) seeds have high contents of ascorbic acid (AA) and total phenolics which are related to their antioxidant capacity and are highly consumed worldwide (GAN et al., 2016).

During seed germination, complex nutrients are converted to more digestible forms, and better absorbed by the gastrointestinal tract (IRITI & VARONI, 2017). Sprouts play an important role in health because they are rich in enzymes, vitamins, minerals and can also stimulate the defense systems, enabling a healthier life. These are functional foods and fall into several categories: pro and prebiotics, sulfur and nitrogen, pigments, vitamins, phenolic compounds, polyunsaturated fatty acids and fibres (LANG, 2007).

The dynamic changes in metabolites during the sprouting process in mung beans and the related biological activities were summarized by TANG et al. (2014) including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, antitumor, and lipid metabolism accommodation. The phyto nutrients level in mung bean sprouts compared to mature grains were determined and sprouts had a 2.7-fold higher vitamin C content than the mature grains (EBERT et al., 2017).

The respiratory process and many oxidative reactions occurring in aerobic cells lead to the production of reactive oxygen species (ROS), which are to a certain extent, beneficial, however, high amounts of those compounds may cause a cascade of damage, from molecular to body level (KRANNER et al., 2010), contributing to the onset of many diseases and accelerating the ageing process. Cells depend on certain antioxidant capacities to protect against the ROS harmful effects (SHUI & LEONG, 2006). The superoxide dismutase enzymes (SOD) is the first line of the enzymatic c antioxidant defense system (MORIYA et al., 2015) and plays a key role in the antioxidant defense system, protecting against damage generated by $O_2^{\bullet-}$, catalyzing the dismutation of $O2^{\bullet-}$ and the subsequent formation of H_2O_2 and O_2 (CHEN& ARORA, 2011).

Fresh vegetables, fruits, and nuts are usually rich in compounds with antioxidant properties. AA, glutathione, α-tocopherol, phenolic compounds and carotenoids are the main antioxidant substances present in vegetables (MITTLER, 2002). The use of elicitors can improve the biosynthesis and biological activity of secondary compounds, constituting a technology with great potential for the production of these foods. The use of elicitors in mung bean sprouts can stimulate the synthesis of phenolic compounds, providing greater antioxidant activity (GAN et al., 2016; RANDHIR et al., 2004; SADIQ et al., 2019).

Phytochemicals are among the main research topic in the field of functional foods. The development of efficient strategies to increase the levels of beneficial metabolites in edible plants is becoming increasingly relevant and significant (LEITE et al., 2016). Exogenous application of elicitors is a suitable strategy for the enrichment of bioactive compound concentrations in healthy foods via the activation of secondary metabolite pathways (BAENAS et al., 2015).

The present study evaluated different elicitors (salicylic acid [SA], ascorbic acid [AA], and tocopherol [TOC]) in green mung bean sprouts and verify the effects of doses and number of applications on biometric and biochemical aspects.

MATERIALS AND METHODS

Plant material and germination conditions

Vigna radiata L. seeds were obtained from bean sprout growers with a minimum germination rate of 80% and 12% water content. The seeds were sown in a plastic box (11 × 11 × 3 cm), 50 seeds per box, 1000 seeds per trial, and 12 mL of distilled water per box, and were transferred to a growth chamber in the dark at 25 °C. During the production of bean sprouts, water was changed every day until the harvesting at the fifth day, to maintain the sprouts clean and healthy.

After 24 hours, the box was analyzed, discarding non germinated seeds and sprouts with impaired development (late or early embryos, not adequate for commercialization) to obtain a box with 50 homogeneous sprouts.

Treatments with elicitors

As the water had to be changed daily, after the first day the elicitors SA (138.12 g mol^{-1})at concentrations of 0, 115.1, 230.2, and 575.5 mg L^{-1}; AA (176.12 g mol^{-1}) at concentrations of 0, 0.147, 1.468, and 14.680 g L^{-1} were applied at one or two applications of 12 mL or two applications of 6 mL per box with 50 sprouts (Table 1) on the second and third days after sowing (DAS). TOC (vitamin E, α-tocopherol, 772.74 g mol^{-1}) were applied at 0, 25, 50, and 100 mg L^{-1} in one, two, and three applications of 12 mL per box with 50 sprouts (Table 2). Distilled water was used as a control. For all treatments, sprouts were

Table 1 – Sprout selection and application of salicylic acid solutions (SA) or of ascorbic acid solutions (AA) and distilled water (W) in the different growth assays of bean sprouts from seed sowing to harvest.

Assays (A)	Days after sowing (DAS)			Sum SA applied (mL)
	2	3	5	
	Sprouts selection	-	-	
	Salicylic acid			
SA 1	+ 12 mL SA at different concentrations	+ 12 mL W	Harvest	12
SA 2	+ 12 mL SA at different concentrations	+ 12 mL SA at different concentrations	Harvest	24
SA 3	+ 6 mL SA at different concentrations + 6 mL W	+ 6 mL SA at different concentrations + 6 mL W	Harvest	12
SA 4*	+ 6 mL SA at different concentrations + 6 mL W	+ 12 mL W	Harvest	6
	Ascorbic Acid			
AA 1	+ 12 mL AA at different concentrations	+ 12 mL DW	Harvest	12
AA 2	+ 12 mL AA at different concentrations	+ 12 mL AA at different concentrations	Harvest	24
AA 3*	+ 6 mL AA at different concentrations + 6 mL W	+ 6 mL AA at different concentrations + 6 mL W	Harvest	12

*Sprouts from these treatments were used in biochemical analysis and for the Growth Indexes (TDMI and TDM.TL^{-1}I).

grown in a box without a lid in a growth chamber in the dark at 25 °C until harvesting at five DAS.

For the evaluation of the treatments, the following biometric variables were measured: length and mass production. The biochemical parameters, the content of soluble proteins, Superoxide Dismutase (SOD) activity, phenols and antioxidant capacity were measured in the treatments

Each treatment was composed of five repetitions (boxes) with 50 sprouts, four repetitions for the biometric measurements and one for the biochemistry. The whole experiment was repeated a second time to obtain enough material for the production of the hydroalcoholic extracts. The assays indicated with an "*" in Tables 1 and 2 were used for the biometric indexes and the biochemical ones.

Biomass evaluation

For each elicitor treatment, seedlings obtained at five DAS were measured for shoot length (SL), root length (RL), and total seedling length (TL), randomly taking 20 units per repetition, and the measurements were expressed as cm. seedling^{-1}. The already segmented shoot and root parts were placed in correctly labelled paper bags and were oven-dried at 60 °C for 48 h to obtain shoot dry mass (SDM) and

Table 2 – Sprout selection and application of tocopherol solutions (TOC) and distilled water (W) in the different growth assays of bean sprouts from seed sowing to harvest.

Assays (A)	Days after sowing (DAS)				Sum TOC applied (mL)
	2	3	4	5	
	Sprouts selection	-	-	-	
A 1	+ 12 mL TOC at different concentrations	+ 12 mL W	+ 12 mL W	Harvest	12
A 2	+ 12 mL TOC at different concentrations	+ 12 mL TOC at different concentrations	+ 12 mL W	Harvest	24
A 3*	+ 12 mL TOC at different concentrations	+ 12 mL TOC at different concentrations	+ 12 mL TOC at different concentrations	Harvest	36

*Sprouts obtained from A 3 were used in biochemical analysis and for the Growth Indexes (TDMI and TDM.TL^{-1}I).

root dry mass (RDM) expressed as g. 20 seedlings^{-1}. The total seedling dry mass (TDM) of each repetition was calculated as the sum of the parts (SDM + RDM). The length ratio of root/shoot (R_RS) was obtained by dividing the RL by the SL. The TDM index (TDMI) was calculated by dividing the TDM of each treatment by the TDM of the control of the same assay. The Total dry mass per total length index (TDM. TL^{-1}I) was obtained by dividing the TDM. TL^{-1} of each treatment by the TDM. TL^{-1} of the control in each assay.

Biochemical analysis

A total of 50 seedlings per treatment, obtained as described above, were frozen at −80 °C to determine the soluble protein content and SOD activity. The protein extract was obtained using three repetitions of 200 mg of hypocotyls, which were macerated in liquid nitrogen. Later, 2 mL of 0.1 M phosphate buffer (pH 7.8) was added and the mix was placed in tubes, which were shaken and centrifuged at 9500 rpm for 10 min. The pellet was re-extracted as described above, the supernatants were mixed, stirred and stored at −80 °C until quantitation. Proteins (mg g FW^{-1}) were quantified according to the method described by Bradford (1976).

SOD activity (EC.1.15.11) was measured using the method described by MORIYA et al. (2015) adding 50 µL of hypocotyl extract to 4.95 mL of 0.1 M phosphate buffer (pH 7.8) containing 13 mM methionine and 63 µM nitro blue tetrazolium (NBT), with 13 µM riboflavin. The tubes were incubated at 25 °C for 15 min under fluorescent light, centrifuged at 10000 rpm for 5 min, and the absorbance was read at 560 nm. Tubes containing the same medium without extract and light exposure were used as the controls. One unit of SOD per mg protein was defined as the enzyme activity capable of inhibiting NBT-formazan photoreduction by 50%. SOD activity results (mg protein^{-1}) were normalized to soluble proteins, as determined by the Bradford (1976) method.

Extraction and determination of phenols and antioxidant capacity

The above experiments were repeated (production of five DAS sprouts) to obtain the hydroalcoholic extracts for the analysis of total polyphenols and antioxidant activity in three replicates per treatment. The fresh sprouts (including cotyledons and roots) were washed in water and placed in a blender for 5 min, mixed with ethanol: water (70:30, v/v) at a ratio of 1:1 (mass/volume). The extract was then kept on ice for another 5 min, vacuum filtered, distributed in Petri dishes with 1 mL of acetone, kept in the exhaust cabinet for 1 h, and then oven-dried at 40 °C until reaching the grease point. The materials were placed in plastic containers with a lid and stored in the dark.

The total polyphenol content present in sprouts (µg mL^{-1}) was determined by the Folin–Ciocalteu method (JAYAPRAKASHA et al., 2003) using gallic acid to construct the standard curve.

The antioxidant activity was assayed by the DPPH free-radical method (BLOIS, 1958) and expressed as IC_{50} (µg mL^{-1}). The hydroalcoholic extracts of each treatment were diluted to a final concentration of 1000µg mL^{-1}. The DPPH ethanol solution (0.25 mL) was added to 0.05 mL of sample plus ethanol (1.25 mL) and acetate buffer (1 mL) and allowed to react at room temperature in the dark. After 30 min, the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity (AA) using the following formula:

$AA\% = \left(Abs\ control - \frac{Abs\ sample}{Abs\ control}\right) x\ 100$; where, Abs control = ethanol +acetate buffer + DPPH solution and Abs sample = ethanol + acetate buffer + DPPH solution + sample.

From the DPPH inhibition percentages, by linear regression, the inhibitory concentration (IC_{50}) was calculated, that is, the extract concentration capable of reducing 50% of the initial DPPH concentration.

Statistical analysis

The assays were conducted in a completely randomized design with four replicates per treatment (biometric growth) and three (biochemical) replicates per treatment. Each assay was considered independent during the biometric assays. The biochemical analysis was conducted together, and each combination of elicitors and doses was considered as one treatment. The results were submitted to analysis of variance (One Way ANOVA) and the means of the treatments compared by Tukey's HSD test ($P \leq 0.05$) for both biometric growth and biochemical parameters. SISVAR software was used for the analysis (FERREIRA, 2011).

RESULTS AND DISCUSSION

Salicylic acid (SA)

At concentrations of 230.2 and 575.5 mg L^{-1} in the 12 mL applications, SA differed from the control in most of the evaluated parameters. RL was decreased by 60.15% at the highest SA concentration, and the total SL was decreased by 38.69% (Table 3).

Table 3 – Salicylic acid application, at different doses, over the biometrical variables of *Vigna radiata* sprouts: Shoot (SL), root (RL) and total length (TL), ratio root / shoot (R_RS), shoot (SDM), root (RDM) and total dry mass (TDM).

Concentration	SL	RL	TL	R_RS	SDM	RDM	TDM
(mg L^{-1})	----------cm. seedling^{-1}----------			-	----------g. 20 seedlings^{-1}----------		
----------Assay 1 - 1 application of 12 mL.50 sprouts^{-1}----------							
0.0	8.18 a$^{£}$	5.27 a	13.44 a	0.64 a	0.29 a	0.05 a	0.34 a
115.1	7.68 a	4.23 b	11.90 ab	0.55 c	0.30 a	0.04 ab	0.34 a
230.2	7.00 ab	3.03 c	10.03 bc	0.43 b	0.25 b	0.03 bc	0.28 b
575.5	6.15 b	2.1 d	8.24 c	0.34 d	0.24 b	0.03 c	0.27 b
F	8.4*	41.6**	22.9**	44.5**	12.5**	13.9**	13.5**
CV	0.083	0.117	0.086	0.081	0.058	0.117	0.062
----------Assay 2 - 2 applications of 12 mL.50 sprouts^{-1}----------							
0.0	8.17 a	5.27 a	13.44 a	0.64 a	0.29 a	0.05 a	0.34 a
115.1	7.00 b	3.03 b	10.03 b	0.43 b	0.25 bc	0.03 b	0.28 bc
230.2	7.01 b	2.53 b	9.61 b	0.36 c	0.26 b	0.04 b	0.29 b
575.5	5.87 c	1.52 c	7.40 c	0.26 d	0.22 c	0.02 b	0.25 c
F	22.5**	60.5**	45.9**	83.6**	17.2**	13.4**	19.4**
CV	0.056	0.132	0.073	0.084	0.052	0.143	0.057
----------Assay 3 - 2 applications of 6 mL.50 sprouts^{-1}----------							
0.0	6.46	4.16 ab	10.62 a	0.65 a	0.26	0.05 a	0.30
115.1	6.51	4.28 a	10.79 a	0.66 a	0.27	0.04 b	0.30
230.2	5.51	3.10 bc	8.62 b	0.57 a	0.23	0.04 b	0.37
575.5	5.44	2.09 c	7.53 b	0.39 b	0.18	0.03 c	0.21
F	3.9^{ns}	16.1**	11.6**	11.3**	2.3^{ns}	18.8**	2.9^{ns}
CV	0.098	0.150	0.99	0.134	0.217	0.072	0.188
----------Assay 4 - 1 application of 6 mL.50 sprouts^{-1}----------							
0.0	8.01 a	4.70 a	12.70 a	0.59 a	0.29 a	0.05 a	0.34 a
115.1	7.15 a	3.34 b	10.49 b	0.47 b	0.28 ab	0.05 a	0.32 ab
230.2	7.21 a	4.08 a	11.29 b	0.57 a	0.30 a	0.05 a	0.35 a
575.5	6.24 b	2.52 c	8.76 c	0.41 b	0.26 b	0.04 b	0.30 b
F	11.8**	36.2**	32.3**	12.9**	7.0**	10.8**	7.8**
CV	0.059	0.085	0.053	0.094	0.043	0.065	0.044

* Means followed by equal letters do not differ by Tukey's HSD test ($P < 0.05$). F test significant $^{*}(P < 0.05)$ $^{**}(P < 0.01)$

Sprouts treated with 12 mL of SA for two consecutive days (Table 3) exhibited inferior results to the control for all evaluated biometric parameters, with greater impairment in growth. When the sprouts were treated with 6 mL of SA solution for two consecutive days, there was no interference on growth. However, at 575.5 mg L^{-1}, the other parameters indicated a reduction in shoot growth, with the TL decreased by 29% (Table 3).

The application of 6 mL of SA solution in a single dose resulted in lower impairment of shoot growth. SL, SDM, RDM, and TDM did not differ from the control at lower SA concentrations (115.1 and 230.2 mg L^{-1}), whereas the TL was reduced by approximately 31% at a concentration of 575.5 mg L^{-1}.

In the present study, SA concentrations ranged from 0.83 to 4.16 mM. The application of low SA concentrations relieves the abiotic stress sensitivity in plants corroborating the data exhibited by NAWAZ et al. (2021), whereas high concentrations (usually more than 1 mM) induced high levels of oxidative stress, leading to decreased abiotic stress tolerance and reduced growth (CHANDRA et al., 2007; MACHADO-NETO; DURÃES, 2006; MIURA; TADA, 2014).

Ascorbic acid (AA)

The 12 mL application of AA at concentrations of 0.147 and 1.468 g L^{-1} did not significantly differ from the control for all biometric

variables analyzed. However, the higher AA concentration resulted in a 77% decrease in the TL than the control, causing decreases in the other parameters (Table 4).

Two 12 mL applications of AA, at the same concentrations for 2 days, decreased shoot growth at the highest concentration (14.680 g L^{-1}), whereas the concentration of 0.147 g L^{-1} did not differ statistically from the control, except for SL, which presented the highest statistical result. Higher concentrations caused a 78% decrease in total shoot growth compared to the control.

The 6 mL application of AA for two consecutive days resulted in better values of SL, SDM, and TDM at a concentration of 0.147 g L^{-1} compared to the control.

The higher AA concentration caused a decrease in the total growth of approximately 72% compared to the control. AA participates actively in mitosis, cellular elongation, senescence, and cell death and acts as a stabilizer of enzymes with prosthetic metallic ions (FRANCESCHI & TARLYN, 2002). EMAM et al. (2011) reported that AA significantly increased flax plant (*Linum usitatissimum* L.) production components regarding plant height, fiber, fruit, and seed yield, and the weight of 1000 seeds compared to the control, unlike what occurred in the present study.

AA is one of the most essential antioxidant metabolites in plant cells because it can interact with different ROS, neutralize their toxic effects, and act as an electron donor in enzymatic reactions, leading to a decrease in H_2O_2 content via ascorbate peroxidase activity (GARA et al., 2003; MÜLLER-MOULÉ et al., 2003). According to BRILHANTE et al. (2013), exogenous application of 0.85 mM AA to cowpea seeds [*Vigna unguiculata* (L.) Walp] 'Epace 10' after artificial ageing attenuated the deleterious effects on membrane integrity caused by artificial ageing and favoured the physiological quality of seeds. Similarly, *Vigna radiata* seeds submitted to treatment with AA (NAWAZ et al., 2021; ROYCHOUDHURY et al., 2016) increased their tolerance to environmental stress caused by salinity and cadmium toxicity.

Table 4 – Ascorbic acid application, at different doses, over the biometrical variables of *Vigna radiata* sprouts: Shoot (SL), root (RL) and total length (TL), ratio root / shoot (R_RS), shoot (SDM), root (RDM) and total dry mass (TDM).

Concentration	SL	RL	TL	R_RS	SDM	RDM	TDM
(g L^{-1})	----------cm. seedling^{-1}----------				----------g. 20 seedlings^{-1}----------		
----------Assay 1 - 1 application of 12 mL.50 sprouts^{-1}----------							
0.00	8.40 a	4.61 a	13.02 a	0.55 a	0.31 a	0.06 a	0.37 a
0.147	8.49 a	5.14 a	13.63 a	0.61 a	0.31 a	0.05 a	0.36 a
1.468	8.72 a	4.78 a	13.51 a	0.55 a	0.32 a	0.06 a	0.38 a
14.680	2.16 b	0.87 b	3.03 b	0.41 b	0.13 b	0.03 b	0.16 b
F	71.83**	35.13**	60.87**	7.36**	114.13**	61.28**	142.41**
CV	0.1084	0.1751	0.1231	0.1195	0.0647	0.0745	0.0563
----------Assay 2 - 2 applications of 12 mL.50 sprouts^{-1}----------							
0.00	8.41 b*	4.61 ab	13.02 ab	0.55	0.31 a	0.06 a	0.37 a
0.147	9.81 a	5.16 a	14.97 a	0.53	0.31 a	0.06 a	0.37 a
1.468	7.52 b	3.69 b	11.20 b	0.49	0.28 a	0.06 a	0.34 b
14.680	1.90 c	1.02 c	2.92 c	0.55	0.12 b	0.02 b	0.14 c
F	126.02**	27.44**	74.22**	0.226^{ns}	188.70**	60.73**	259.58**
CV	0.0895	0.1939	0.1170	0.2168	0.0527	0.0888	0.0446
----------Assay 3 - 2 applications of 6 mL.50 sprouts^{-1}----------							
0.00	6.55 b	4.84 a	11.38 ab	0.75	0.25 b	0.05 a	0.31 b
0.147	7.49 a	4.89 a	12.38 a	0.66	0.29 a	0.05 a	0.34 a
1.468	6.39 b	3.96 a	10.34 b	0.62	0.24 b	0.05 a	0.29 b
14.680	1.98 c	1.62 b	3.14 c	0.61	0.11 c	0.03 b	0.14 c
F	129.63**	59.00**	159.02**	1.13^{ns}	124.01**	42.75**	175.59**
CV	0.0772	0.1230	0.0715	0.1778	0.0624	0.0795	0.0501

*Means followed by equal letters do not differ by Tukey's HSD test ($P < 0.05$) ** F test significant at ($P < 0.01$)

AA treatment did not produce a significant increase in total phenolic content (Figure 1). FAROOQ et al. (2013) reported that the exogenous application of AA to seeds improved drought resistance in wheat by increasing endogenous levels of AA, antioxidant capacity, and osmotic adjustment. They further stated that the manipulation of endogenous levels of AA by genetic or biotechnological means might result in the development of drought resistance in wheat.

Tocopherol (TOC)

A single application of TOC (12 mL of solution) had no significant effecton SL, RL, TL, RDM, and TDM (Table 5). There was a decrease in RAT_RS when the highest TOC concentration was used (100 mg L^{-1}), similar to SDM with 50 mg L^{-1}. In contrast, SOLTANI et al. (2012) reported improvements in vegetative growth and flowering parameters of *Calendula officinalis* after exogenous application of TOC (0, 50, and 100 mg L^{-1}). According to these authors, TOC at 100 mg L^{-1} increased leaf area (9.48%) and the fresh and dry weight of the aerial parts (19.58% and 22.24%, respectively). TOCs (α-, β-, γ-, and δ-TOC) are reported in plastids, associated with thylakoid membranes, and possess antioxidant activity (SOARES et al. 2019).

The most abundant isomer is α-TOC (90% of the foliar total TOC content), which is particularly active due to its interaction with $O_2^{\bullet-}$, OH, and several lipid radicals derived from the oxidation of polyunsaturated fatty acids, preserving the integrity of membranes and their permeability (MAEDA & DELLAPENNA, 2007).

SADAK et al., (2010) demonstrated that the application of α- TOC in sunflower plants led to the accumulation of total carbohydrates, stimulation of protein synthesis, and senescence delay in sunflower plants.

Many studies have shown that exogenous TOC application has an effective protective effect against different abiotic stresses (DELONG & STEFFEN, 1998; ESPINOZA et al., 2013; SKŁODOWSKA et al., 2009). This result is partly because TOC, being a lipophilic molecule and a membrane component, has a proven role in membrane stabilization (WANG; QUINN, 2000) and contributes to redox homeostasis (MAEDA & DELLAPENNA, 2007; SHAO et al., 2008). KUMAR et al. (2013) reported that TOC levels governed the heat sensitivity of wheat, and its exogenous application might partially alleviate the adverse effects of stress because of its protective role on membranes, photosynthetic function, and antioxidant activity.

TOC play a role in various physiological phenomena, including plant growth and development, senescence, prevention of lipid peroxidation, and interaction with the main components of the signal transduction pathways in the cellular environment (BAFEEL & IBRAHIM, 2008). In our results, there was no increment in plant development, which was practically the same as that of the control (Table 5).

Total dry mass index and Total dry mass per total length index

Considering the total dry mass index (TDMI Figure 1 A) there were reductions in sprouts

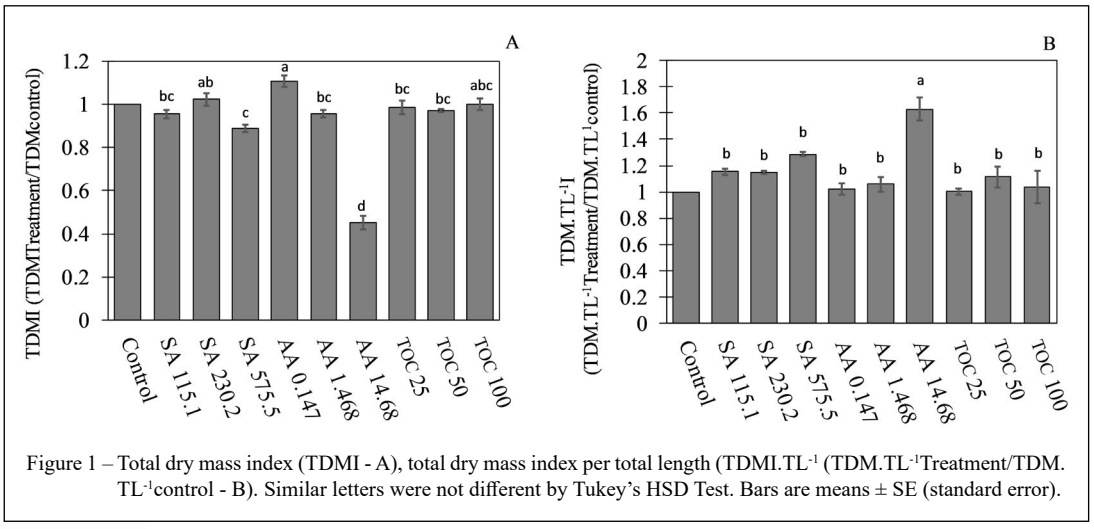

Figure 1 – Total dry mass index (TDMI - A), total dry mass index per total length (TDMI.TL^{-1} ($TDM.TL^{-1}$Treatment/TDM.TL^{-1}control - B). Similar letters were not different by Tukey's HSD Test. Bars are means ± SE (standard error).

Table 5 – Tocopherol application, at different doses, over the biometrical variables of *Vigna radiata* sprouts: shoot (SL), root (RL) and total length (TL), ratio root / shoot (R_RS), shoot (SDM), root (RDM) and total dry mass (TDM).

Concentration	SL	RL	TL	R_RS	SDM	RDM	TDM
(mg L^{-1})	----------cm. $seedling^{-1}$----------				----------g.20 $seedlings^{-1}$----------		
Assay 1 - 1 application of 12 mL.50 $sprouts^{-1}$							
0	9.08	5.31	14.39	0.59 ab	0.31 ab	0.05	0.36
25	8.62	4.77	13.38	0.55 ab	0.32 ab	0.06	0.39
50	9.03	5.46	14.48	0.61 a	0.30 b	0.06	0.36
100	9.65	5.10	14.75	0.53 b	0.33 a	0.06	0.39
F	2.25 ^{ns}	2.19 ^{ns}	1.79 ^{ns}	4.43 *	35.35 *	0.40 ^{ns}	4.536 ^{ns}
CV	0.0624	0.0787	0.0627	0.0558	0.0369	0.0561	0.0356
----------Assay 2 - 2 applications of 12 mL.50 $sprouts^{-1}$----------							
0	8.17	4.85	13.02	0.59	0.31	0.05	0.37
25	8.65	5.20	13.85	0.60	0.32	0.05	0.37
50	9.30	5.97	15.27	0.64	0.34	0.06	0.40
100	9.37	6.18	15.55	0.66	0.32	0.06	0.38
F	2.60 ^{ns}	2.01 ^{ns}	2.56 ^{ns}	0.83 ^{ns}	2.30 ^{ns}	2.78 ^{ns}	2.55 ^{ns}
CV	0.0796	0.1603	0.1038	0.1158	0.0498	0.0876	0.0532
----------Assay 3 - 3 applications of 12 mL.50 $sprouts^{-1}$----------							
0	9.49	5.09	14.58	0.53	0.33	0.07	0.40
25	9.30	5.03	14.33	0.54	0.33	0.06	0.39
50	8.62	4.30	12.92	0.49	0.32	0.07	0.39
100	9.28	5.09	14.36	0.54	0.34	0.06	0.40
F	1.48 ^{ns}	0.59 ^{ns}	0.94 ^{ns}	0.37 ^{ns}	1.33 ^{ns}	4.23 ^{ns}	0.40 ^{ns}
CV	0.0685	0.2043	0.1113	0.1508	0.0461	0.0703	0.0449

*Means followed by equal letters do not differ by Tukey's HSD test ($P < 0.05$).

dry mass in almost all elicitors, but for SA (230.2 mg L^{-1}), AA (0.147 g L^{-1}) and TOC (100 mg L^{-1}) which presented 2, 10 and zero % of the increase concerning the control, respectively.

For the TDM.TL^{-1}I there were no differences among elicitors compared with the control, (Figure 1 B) except for the AA (14.68 g L^{-1}) which exhibited an increase of 63%. This means that seedlings submitted to this condition did not present a strong development being the sprouts the shortest and heavier than the others did, not reachING quality for marketing. Because of that, this treatment was excluded from the biochemical analysis.

Soluble protein content and SOD activity

Biochemical analysis was performed using plants from assays that induced lower impairment in the biometric measurements: a single application of SA (6 mL), two applications of AA (6 mL), and three applications of TOC (12 mL), all of which were applied per 50 sprouts. The TDMI of the sprouts (Figure 1 A) showed that the maximum impairment was due to the higher AA concentration; therefore, this treatment was not included in the biochemical analysis because as a product, it will be discarded and never reach the market. The elicitor treatments induced variable amounts of soluble protein (Figure 2A) and SOD activity (Figure 2 B) in the sprouts; however, the differences were not statistically significant. In terms of absolute values, there was more SOD activity in the TOC applications. The defense of living cells against ROS are diverse and starts with SOD acting to decrease the amount of superoxide ion, then peroxidases (MORIYA et al., 2015), and finally the non-enzymatic defenses (ascorbic acid, glutathione and many others) which can capture or neutralize the ROS.

The elicitors such as SA, AA and TOC may alter this, either by increasing the response as the synthesis of proteins or by increasing the metabolic pathways of phenolics and other molecules. However, the effect wanted is to increase the content of antioxidants to increase the nutritional value of the food. So, the primary antioxidant defense against

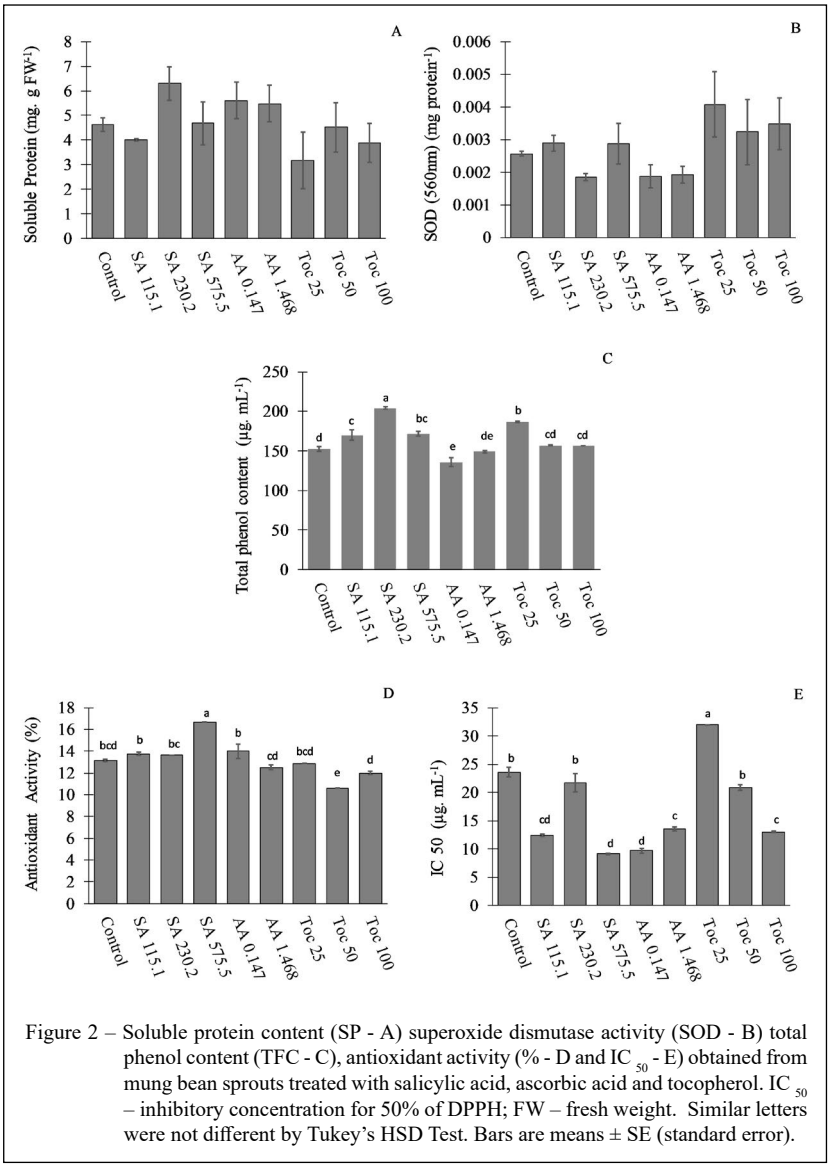

Figure 2 – Soluble protein content (SP - A) superoxide dismutase activity (SOD - B) total phenol content (TFC - C), antioxidant activity (% - D and IC_{50} - E) obtained from mung bean sprouts treated with salicylic acid, ascorbic acid and tocopherol. IC_{50} – inhibitory concentration for 50% of DPPH; FW – fresh weight. Similar letters were not different by Tukey's HSD Test. Bars are means ± SE (standard error).

ROS – SOD activity is not interesting as it may decrease the amounts of the other antioxidants. SA is an inductor of the polyphenol pathway (NAWAZ, 2021) and it may decrease the synthesis of protein in legumes (DUTRA et al., 2017; MACHADO-NETO & DURÃES, 2006) and as well as it may increase the AA content (ROYCHOUDHURY et al., 2016). Conversely, sprouts subjected to the lower dose of AA had the higher growth and resulted in a decrease in IC50 IC50 (Figure 1) meaning that as it acts directly as an antioxidant, since it alleviates the needs of the plant for the needs of the plant for expending resources in protein/enzymes synthesis (YOSHIMURA & ISHIKAWA, 2018). In this research, TOC increases the SOD activity, similarly to DUTRA et al. (2017) and SADIQ et al. (2018, 2019).

Phenolic content and antioxidant capacity

SA (230.2 mg L^{-1}) increased by 34% and 2.2%, respectively, the total phenol content of the sprouts (Figure 2C) and TDMI produced (Figure 1 A) compared to the control. However, the $TDMI.TL^{-1}$ index even not differing from the control increased by15% sprouts weight by length, which means that the tissue becomes a bit harder. This could be explained because SA is a precursor of the phenylpropanoid pathway, that produce lignin as a final product,

turning cellular division more difficult (MURO-VILLANUEVA et al. 2019). PÉREZ-BALIBREA et al., (2011) showed those applications of 200 and 300 µM SA induced increases, by 26% and 18%, respectively, in ascorbic acid content in 5-day-old broccoli sprouts. In addition, flavonoid concentration was increased by 33% after 100 µM SA treatment in 7-day-old broccoli sprouts.

Fruits and vegetables are natural sources of antioxidants, such as phenols, flavonoids, anthocyanins, and AA, which protect against the harmful effects of ROS, and are associated with lower incidence and mortality rates of cancer and heart disease, and other health benefits (SHUI & LEONG, 2006).

Other authors also reported positive effects of SA application on common beans, such as the higher activity of antioxidant enzymes and ROS supression. Pre-soaking bean seeds (*Phaseolus vulgaris* L.) in SA (1 mM), and subsequent, growth in the absence or presence of salt stress (NaCl) increased the activity of all antioxidant enzymes and the concentration of non-enzymatic antioxidants and osmoprotectants (RADY & MOHAMED, 2015). Pre-imbibition of cowpea seeds (Vigna unguiculata (L.) Walp) with SA (0.01 mM) increased antioxidant activity (SOD, CAT [catalase], and APX [ascorbate peroxidase] activity) (DUTRA et al., 2017). TOC application (25 mg L^{-1}) also increased the total phenol content of the sprouts by 23% (Figure 2C), with 98.7% of the total mass production compared to the control (Figure 1 A).

The antioxidant power of the studied extracts, measured by antioxidant activity in percentage, was higher with 575.5 mg L^{-1} of SA (Fig. 2 D). However, that measured by the IC_{50} (which is inversely related to antioxidant activity) was higher in the extracts obtained at concentrations of 575.5 mg L^{-1} of SA (62% more than control). The 0.147 g L^{-1} concentration of AA (59% more), and 100 mg L^{-1} of TOC (45% more) (Figure 2 E), confirmed that the treatments promoted secondary metabolism with a total mass production equivalent of 0.88, 1.10, and 0.99 of the TDMI produced by the control (Fig. 1 A), respectively. There were also increments in the IC_{50} of the sprouts treated with the lower concentration of SA (115.1 mg L^{-1}) (Figure 2 E) that presented an equivalent 0.95 of the TDMI production (Figure 1 A).

CONCLUSION

All elicitors stimulated antioxidant functions in mung bean sprouts and reduced the biometric parameters at higher concentrations.SA increased the antioxidant activity and decreased the mass of the sprouts, which is not beneficial to producers. However, AA applications at lower concentrations (0.148 gL^{-1}) had the highest sprout growth and increased the antioxidant activity by more than half, wich can be recommended for the improvement of the functional properties of the food. TOC application (100mg L^{-1}) is also recommended as it increased the antioxidant activity and did not reduced the growth.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization: CCC and ACP. Data acquisition: MD and VLS. Design of methodology and data analysis: CCC, ACP and NBMN. MD, ACP, NBMN and CCC prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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