

Action of nitric oxide in sesame seeds (*Sesamum indicum* L.) submitted to stress by cadmium¹

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ABSTRACT - The objective of this paper was to evaluate the effect of nitric oxide (NO) as a protecting agent of sesame seeds submitted to different concentrations of cadmium. The treatments were: water (control), water increased by sodium nitroprusside (SNP) and other treatments regarding the concentrations of cadmium increased by SNP. The following determinations were done: germination, first count of germination, germination speed index, length of hypocotyl and radicle and dry matter of hypocotyl and radicle, besides quantification of enzyme activities, superoxide dismutase, catalase, ascorbate peroxidase and total peroxidases. The statistical design was entirely randomized with five replicates. The data was submitted to a variance analysis and the averages obtained for the treatments were compared by the Tukey test at 5% significance. The averages obtained in the treatments with and without SNP were compared by the F test at 5% probability. The NO due to the application of SNP was beneficial, providing an increase in germination, vigor and growth of seedlings. There was a progressive increase of the antioxidant enzymes activity in the period of 0 to 24 hours, showing an organization of the antioxidant system in the sesame seeds throughout germination time.

Index terms: SNP, vigor, germination, antioxidant system.

Ação protetora do óxido nítrico em sementes de gergelim (*Sesamum indicum* L.) submetidas ao estresse por cádmio

RESUMO - Objetivou-se avaliar o efeito do óxido nítrico (ON) como agente protetor em sementes de gergelim submetidas a diferentes concentrações de cádmio. Os tratamentos foram: água (controle), água acrescida de nitroprussiato de sódio (SNP) e os demais tratamentos referentes às concentrações de cádmio e cádmio acrescido de SNP. Foram feitas as seguintes determinações: germinação, primeira contagem de germinação, índice de velocidade de germinação, comprimento de hipocótilo e radícula e massa seca de hipocótilo e radícula, além da quantificação da atividade das enzimas, superóxido dismutase, catalase, ascorbato peroxidase e peroxidases totais. O delineamento estatístico utilizado foi o inteiramente casualizado com cinco repetições. Os dados foram submetidos à análise de variância e as médias obtidas para os tratamentos foram comparadas pelo teste de Tukey a 5% de significância. As médias obtidas nos tratamentos com e sem SNP foram comparadas pelo teste F a 5% de probabilidade. O ON devido à aplicação de SNP foi benéfico, proporcionando aumento na germinação, vigor e crescimento de plântulas. Houve aumento progressivo da atividade das enzimas antioxidativas no período de 0 a 24 horas, demonstrando organização do sistema antioxidante nas sementes de gergelim com o decorrer do tempo de germinação.

Termos para indexação: SNP, vigor, germinação, sistema antioxidativo.

Introduction

Problems related to the pollution and its harmful effects on living organisms are highly important. The heavy metals, currently mentioned as trace elements or persistent organic

pollutants are increasingly assuming a prominent role in the concern with the environment (Wani et al., 2012). Among these metals, cadmium (Cd) stands out for easily accumulating in living organisms (Bridgen et al., 2000).

The accumulation of these contaminating elements in plants

¹Submitted on 09/24/2015. Accepted for publication on 01/22/2016.

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may provoke several physiological damages, causing growth disorders (John et al., 2009), and structural and ultrastructural alterations in the plants (Kasim, 2006). In addition, high levels of cadmium may affect germination and the initial growth of seedlings, besides the oxidative stress and lipid peroxidation in plant tissues where they are found (Zhang et al., 2007).

With the intensification of the seeds metabolism during the germination process, there is usually production of reactive species of oxygen (EROs) that can be increased in the presence of stressing agents, such as cadmium. However, antioxidant enzyme systems act and are an important primary defense against the free radicals generated in seeds in stress conditions, such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), and ascorbate peroxidase (APX).

Studies show that chemical compounds, such as nitric oxide (NO) act in the protection of plants exposed to stress factors. The multifunctional molecule that acts in several physiological events is also cytoprotective, due to its capacity of regulating the level and toxicity of EROs. Sodium nitroprusside (SNP) is currently the most used donor of nitrogen oxides that produce NO.

Some results show the efficiency of NO in promoting germination. Seeds of *Plathymenia reticulata*, submitted to accelerated aging, had their germination increased when SNP was applied (Pereira et al., 2010a). In studies with seeds of yellow lupine (*Lupinus luteus*), Kopyra and Gwóźdz (2003) reported that SNP had a considerable effect on the promotion of germination in stress conditions, caused by the presence of lead and cadmium, indicating the efficiency of NO against the negative impact of the heavy metals on germination.

The introduction of crops of sesame in regions of Brazil is highly interesting, once it presents several nutritional and socio-economic advantages. However, studies related to the germination physiology of sesame seeds are important in order to favor the correct handling of the species and to get to know its tolerance and/or sensitivity to heavy metals, frequently found in environments where there was human intervention. The objective of this paper was to investigate the effect of NO as a protecting agent of sesame seeds submitted to different concentrations of cadmium, through evaluations of physiological characteristics and activity of the antioxidant enzymes.

Material and Methods

The research was carried out in the Forest Seeds Testing Laboratory of the Forest Science Department, at the Federal University of Viçosa. Sesame seeds (*Sesamum indicum*) harvested in 2013 were used.

Initially, preliminary tests were done to determine the

concentrations of cadmium chloride (CdCl_2), and the NO donor solution (Sodium nitroprusside - SNP) to be studied. The concentrations of cadmium were established so that they would interfere negatively in the germination of seeds, without killing them. The amount of SNP was the one capable of reversing or mitigating the actions of the metal. After that, the sesame seeds, in five replicates of 50, were put to germinate on a paper towel moistened with 3 mL of the solution, referent to the following treatments in Petri dishes: water (control), water increased by SNP and other treatments regarding the concentrations of cadmium and cadmium increased by SNP: 800 μM of CdCl_2 , 800 μM of CdCl_2 +200 μM of SNP, 600 μM of CdCl_2 , 600 μM of CdCl_2 +200 μM of SNP, 400 μM of CdCl_2 and 400 μM of CdCl_2 +200 μM of SNP, summing up to a total of eight treatments. The seeds of each treatment were kept in B.O.D. regulated with alternate temperature of 20-30 °C, with the presence of constant light (Brasil, 2009). The following evaluations were done:

Germination percentage: in the sixth day after sowing, the percentage of normal seedlings was evaluated.

First germination count: consisted of a record of the number of normal seedlings obtained in the third day after sowing, and the values were expressed in percentage.

Germination speed index: daily counts were done of the number of seeds that issued a radicle higher than 1.0 mm and were calculated according to Nakagawa (1999).

Length of the hypocotyl and the radicle: the seeds of each treatment, in five replicates of 25, were sown in gerbox, following the methodology described above for the germination test. A measurement of the length of hypocotyl was done in the seedlings classified as normal with the help of a graduated ruler. The results were expressed in cm. seedling⁻¹.

Dry matter of the hypocotyl and the radicle: the seeds used to measure the length of the hypocotyl and the radicle were separated in hypocotyl and radicle, and later dried in an oven for 72 hours at 65 °C. The results were expressed in mg. seedling⁻¹.

Activity of the main enzymes of the antioxidant system: determined by using the seeds soaked for 12 and 24 hours in water and in solutions of cadmium and cadmium increased by SNP. The enzyme extracts were obtained by maceration of 0.2 g of seeds in ice, followed by the addition of 2.0 mL of the following means of homogenizing: potassium phosphate buffer 0.1 M and pH 6.8, ethylenediaminetetraacetic acid (EDTA) 0.1 mm, phenylmethylsulfonyl fluoride (pmsf) 1 mm and polyvinylpyrrolidone (pvpp) 1% (p/v). After that, the extract was centrifuged at 15,000 g for 15 minutes at 4 °C and the supernatant was collected, where determinations were done on the activities of ascorbate peroxidase enzyme (APX), peroxidase (POX), catalase (CAT) and superoxide

dismutase (SOD).

The activity of peroxidase ascorbate (APX) was determined by the addition of 200 μL of raw enzyme extract at 2.9 mL of reaction medium of ascorbic acid 10 mM and H_2O_2 10 mM in potassium phosphate buffer 100 mM, pH 6.0. A decrease in the absorbance was observed at 290 nm, at 25 °C, during the first minute of the reaction. The enzyme activity was calculated using the molar extinction coefficient of 2.8 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ and expressed in $\mu\text{mol min}^{-1}\cdot\text{mg}^{-1}$ of protein (Nakano and Asada, 1981).

The activity of peroxidase (POX) was determined by the addition of 50 μL of raw enzyme extract at 2.97 mL of reaction medium composed of potassium phosphate buffer 100 mM and pH 6.8, pyrogallol 150 mM and hydrogen peroxide 125 mM (Kar and Mishra, 1976). The increase in the absorbance during the two first minutes of reaction at 420 nm at a constant temperature of 25 °C determined the production of purpurogallin. The enzyme activity was calculated using the molar extinction coefficient of 2.47 $\text{mM}^{-1}\cdot\text{cm}^{-1}$ and expressed in $\mu\text{mol min}^{-1}\cdot\text{mg}^{-1}$ of protein.

To quantify the CAT activity, 0.98 mL of sodium phosphate buffer 0.05 M pH 6.8, H_2O_2 0.0125 mM, dissolved in an adapted buffer of Madhusudhan et al. (2003) was added to a 30 μL enzyme extract. The enzyme activity was determined by the follow up of the absorbance drop at 240 nm, for 2 minutes, in intervals of 15 seconds, and calculated based on the extinction factor of 36 $\text{mM}^{-1}\cdot\text{cm}^{-1}$.

The activity of superoxide dismutase (SOD) was determined by the addition of 30 μL of raw enzyme extract at 2.95 mL of the reaction medium composed of sodium phosphate buffer 100 mM at pH 7.8, methionine 50 mM, p-nitro blue tetrazolium (NBT) 1 mM, EDTA 5 mM and riboflavin 100 mM. The reaction was conducted at 25 °C in a reaction chamber under lighting of a 15 W fluorescent lamp, kept inside a box internally coated with foil. After five minutes of exposure to the light, the lighting was interrupted and the blue formazan produced by NBT photo reduction was determined by the absorption at 560 nm in a spectrophotometer. A SOD unit was defined as the amount of necessary enzyme to inhibit the NBT photo reduction in 50%. The SOD activity was expressed in $\text{U min}^{-1}\cdot\text{mg}^{-1}$ protein. To determine the content of proteins, the method used was Bradford (1976) with a standard curve constructed with bovine serum albumin (BSA) as a reference protein.

For all determinations, the statistical design was entirely randomized with five replicates. The data was submitted to a variance analysis and the averages obtained for the treatments were compared by the Tukey test at a 5% significance. The averages obtained in the treatments

with and without SNP were compared by the F test at 5% probability and for the enzyme determinations the Tukey test was also used, at 5% significance.

Results and Discussion

The sesame seeds had their germination inhibited under stress by cadmium, with germination of 98.4% in great conditions (germination in water), 36.4% in high concentration of cadmium (800 μM of CdCl_2), 42.8% in intermediate concentration (600 μM of CdCl_2) and 56% in low concentration (400 μM of CdCl_2) (Figure 1A). Comparing the values of germination obtained in water and in greater cadmium concentration, a sharp reduction of 62 percentage points was seen.

The application of SNP did not affect the germination of seeds in water, which was more or less expected, since the germination conditions were ideal (Figure 1A). The application of SNP in all treatments with Cd allowed the significant increase of germination regarding the SNP treatments. Therefore, in high, intermediate and low concentrations, SNP reversed the damaged caused by cadmium, providing an increase in germination of 13.2, 19.6 and 8.4 percentage points, respectively (Figure 1A).

Through the vigor, germination first count (FC) and germination speed index (GSI) tests, it is seen that the cadmium, in any of the tested concentrations, affected negatively the performance of the seeds, reducing germination speed (Figures 1B and 1C). In optimum conditions, the FC was 94.8%, and the GSI was 29.72, causing a significant drop under stress by Cd, with values of 24.4% and 9.99 for FC and GSI, respectively, in a greater concentration of cadmium, 31.6% and 10.35 for the intermediate concentration and 30.8% and 10.9 for the lowest concentration.

After the application of SNP, there was a significant increase in the values of FC in relation to the treatments without SNP, for all tested concentrations of cadmium (Figure 1B). On the other hand, the application of SNP did not cause a significant increase in the germination speed (GSI) when comparing to the treatments with cadmium (Figure 1C). This behavior may be related to a possible time of response of the SNP action in restoring or minimizing the damaging actions of the Cd.

Chugh and Sawhney (1996) associate the drastic effect of cadmium in germination of seeds to the reduction of the activity of α and β amylases, which compromises respiration, causing inhibition of the growth of the embryonic axis and the radicle. Moreover, the phytotoxic effect of cadmium promotes a disorder in the development and cellular differentiation, resulting in abnormal seedlings and decreasing the percentage of normal seedlings in germination (Rossi and Lima, 2001).

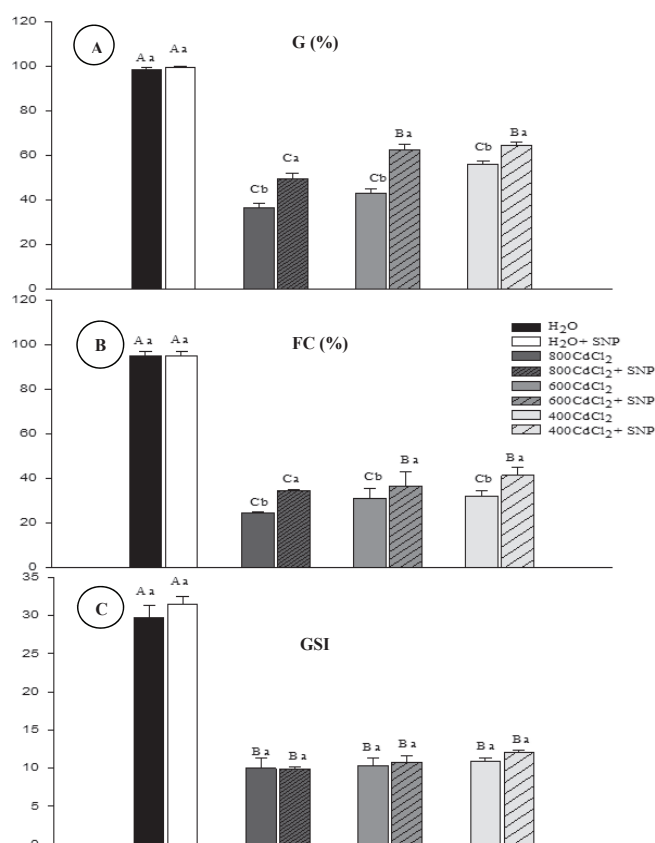


Figure 1. A- Germination (G%) of normal seedlings on the sixth day; B- Germination First Count (FC%), done on the sixth day; and C- Germination Speed Index (GSI) of sesame seeds in the tests conducted in substrate moistened with water (control), water + SNP, cadmium solution 800 μ M, and 800 μ M of CdCl₂ + SNP, 600 μ M of CdCl₂, and 600 μ M of CdCl₂ + SNP, 400 μ M of CdCl₂ and 400 μ M of CdCl₂ + SNP.

*Averages followed by the same uppercase letter do not differ among each other by the Tukey test at 5%. **Averages of each treatment with and without SNP followed by the same lowercase letter do not differ from each other by the F test at 5% probability. The bars correspond to the average standard deviation (n = 5).

According to Kopyra and Gwózdź (2003), NO stimulates germination in situations of high concentrations of heavy metals. Beligni and Lamattina (2002) reported that NO acts as an inductor of the germination process, increasing germination in the treatments with SNP. Exposure of seeds to the heavy metal caused an increase of the reactive species of oxygen produced due to some biological dysfunction caused by cadmium, such as the interference in the action of channel proteins or its negative action in the enzyme activity (Hasan et al., 2009). The use of SNP as a NO donor increased germination, probably due to its regulation capacity or elimination of these EROs, reducing the oxidative stress

and recovering germination partially. It is highlighted that the action of SNP is efficient in increasing germination, but not in restoring it before the application of cadmium.

The radicle is the smallest mechanic resistance point and it is the first region to be in contact with the solution, being one of the main entryways of toxic metals. It is possible that this is the first organ to suffer damage because of these elements, and the other organs are harmed after the transportation of these metals, or as a consequence of its damaging effects in the radicle itself. A significant reduction was seen along the length of the radicle when the seeds were treated in a cadmium solution in relation to the seeds soaked in water (Figure 2B). It is observed that the development of the radicle was more compromised by the concentrations of the metal than the aerial part (Figures 2A, 2B). It is noted that there was no formation of roots in most part of the treated seedlings in relation to the control (Figures 3A and 3B, C and D). Accioly et al. (2004), working with seeds of *Eucalyptus camaldulensis* observed a greater content of Cd on the radicle in relation to the aerial part, being an indication of the non-translocation of this metal.

Long exposures to the radicle system of the plants to Cd lead to the manifestation of a set of symptoms that express the continuous effect of this ion on the growth of the radicular system, among them, the darkening of the radicle (Figure 3D).

These same symptoms of toxicity by Cd were observed in species of *Eucalyptus maculata* and *Eucalyptus urophylla* after a week of exposure to the stressing element, in which the radicles presented a smaller development and darkening in concentrations of 180 μ M of Cd (Soares et al., 2005).

A greater accumulation of the dry matter in the control seedlings that presented MSH of 20.07 mg.seedling⁻¹ and 19.54 mg. seedling⁻¹ of MSR (Figures 2C and 2D, respectively). In the highest concentration of cadmium, there was a reduction of 58.4% and 83% in the MSH and MSR regarding the control, respectively. The application of NO, through SNP, NO donor molecule, was able to partially reverse the reduction of MSR, only in the smallest concentration of cadmium.

Regarding the defense mechanism of the sesame seeds, a greater activity of the SOD, CAT, APX and POX enzyme (Tables 1 to 4, respectively) with a progressive increase of the soaking period of 0 to 24 hours, for basically all treatments. These results indicate an apparent organization of the antioxidant system in the sesame seeds as time goes by. However, it was seen that the period between 0 h and 12 h did not statistically differ the treatments in relation to the control for all the enzymes, with the exception of SOD, showing that these times are not enough for the organization of the antioxidant apparatus in sesame seeds submitted to stress by cadmium, or that possibly the toxic effect of cadmium

was not expressive until the 12-hour period. In the 24-hour period, it is possible to see the difference in the activity of the enzymes in relation to the control in basically all treatments. In the initial time (0 h), there was no contact of the seeds with

the solutions of cadmium or cadmium increased by SNP, so there is no contamination of the seeds. Therefore, the values of each enzyme were calculated, attributing a fixed value for all treatments in this time.

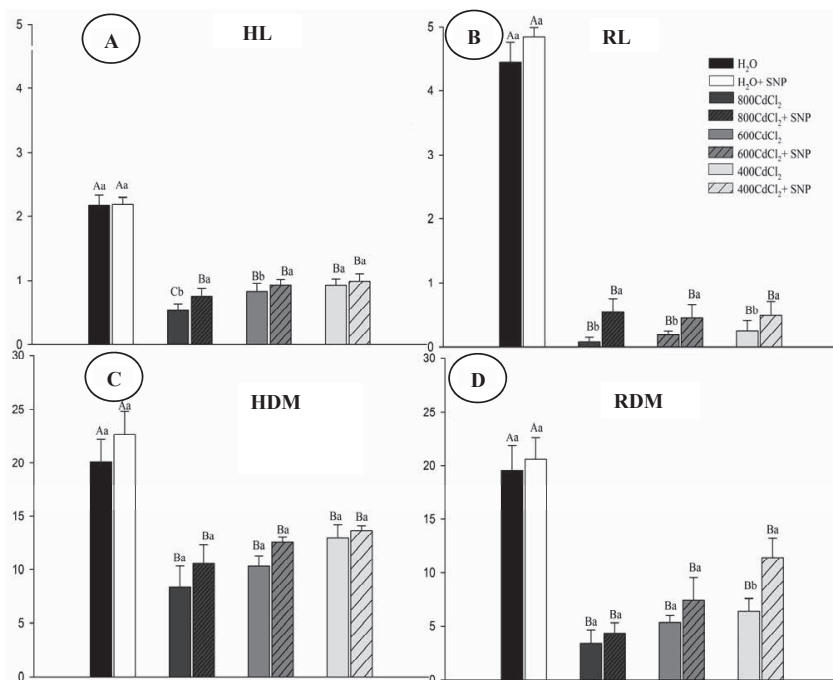


Figure 2. A- Hypocotyl length- HL (cm/normal seedling⁻¹); B- radicle length- RL (cm/normal seedling⁻¹); C- hypocotyl dry matter- HDM (mg normal seedling⁻¹); D- radicle dry matter- RDM (mg normal seedling⁻¹) of seedlings of *Sesamum indicum* in the tests conducted in substrate moistened with water (control), water + SNP, cadmium solution 800 µM, and 800 µM of CdCl₂ + SNP, 600 µM of CdCl₂ and 600 µM of CdCl₂ + SNP, 400 µM of CdCl₂ and 400 µM of CdCl₂ + SNP.

*Averages followed by the same uppercase letter do not differ from each other by the Scott-Knott test at 5%. **Averages of each treatment with and without SNP followed by the same lowercase letter do not differ from each other by the F test at 5% probability.

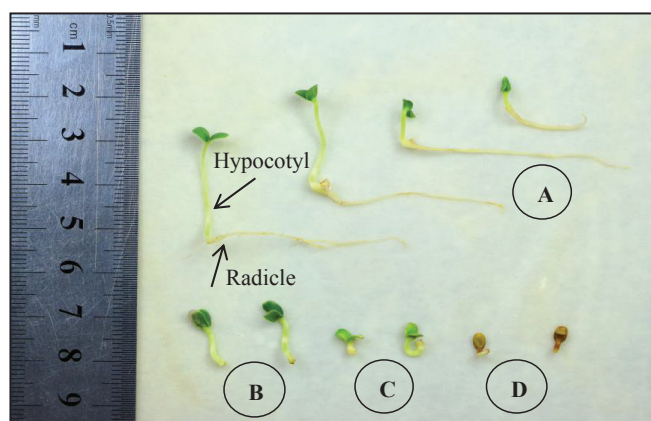


Figure 3. A- The normal seedlings of *S. indicum* obtained in the seeds germinated in water on the sixth day; B- abnormal seedlings of *S. indicum* with total inhibition of the radicle and partial of the hypocotyl obtained in the germinated seeds in solutions of cadmium on the sixth day; C- abnormal seedlings of *S. indicum* with total inhibition of radicle and total of the hypocotyl obtained from seeds germinated in solutions of cadmium on the sixth day; and D- abnormal seedlings of *S. indicum* darkened by the toxic effect of the stressing element on the sixth day of germination.

For all the enzymes in the interval of 24 hours, it is seen that the application of Cd solutions stimulated the antioxidant system of the seeds, and this increase is more expressive in higher concentrations of the solution. However, a higher activity of the enzymes may be seen in the treatments increased by SNP, which suggests detoxifying activity of

these molecules in seeds submitted to stress by cadmium (Tables 1 to 4, respectively).

SOD is considered the first antioxidant line of defense against EROs (Valko et al., 2006), which explains the increase of the activity in relation to the control in the first 12 hours of soaking (Table 1).

Table 1. Activity of the enzyme superoxide dismutase (SOD) in seeds of *S. indicum* after 0, 12 and 24 hours of soaking in different concentrations of cadmium added or not by SNP.

Treatment	SOD (U min ⁻¹ .mg ⁻¹ .protein)		
	0 h	12 h	24 h
Test	0.38 ± 0.01 Ba	0.58 ± 0.02 Ba	1.16 ± 0.02 Ba
800 CdCl ₂	0.38 ± 0.01 Ba	3.13 ± 0.01 Ba	4.25 ± 0.05 Ae
800 CdCl ₂ + SNP	0.38 ± 0.01 Ba	0.69 ± 0.02 Bd	8.21 ± 0.07 Aa
600 CdCl ₂	0.38 ± 0.01 Ca	2.40 ± 0.01 Bb	4.93 ± 0.01 Ad
600 CdCl ₂ + SNP	0.38 ± 0.01 Ba	0.91 ± 0.05 Bd	7.39 ± 0.01 Ab
400 CdCl ₂	0.38 ± 0.01 Ca	1.89 ± 0.03 Bc	2.64 ± 0.01 Af
400 CdCl ₂ + SNP	0.38 ± 0.01 Ba	0.92 ± 0.08 Bd	5.83 ± 0.06 Ac
VC (%)	-----	22.97	20.45

*Averages followed by the same lowercase letter in the column do not differ among each other by the Tukey test at 5% probability. **Averages followed by the same uppercase letter in the row do not differ from each other by the Scott Knott test at 5% probability. Average ± standard deviation.

Table 2. Activity of the catalase enzyme (CAT) in seeds of *S. indicum* after 0, 12 and 24 hours of soaking in different concentrations of cadmium added or not by SNP.

Treatment	CAT (μmol.min ⁻¹ .g ⁻¹ .protein)		
	0 h	12 h	24 h
Test	15.64 ± 1.24 Ca	22.51 ± 3.11 Ba	35.44 ± 3.45 Ae
800 CdCl ₂	15.64 ± 1.24 Ca	23.96 ± 3.67 Ba	29.33 ± 2.78 Af
800 CdCl ₂ + SNP	15.64 ± 1.24 Ca	24.08 ± 2.43 Ba	52.19 ± 4.12 Ac
600 CdCl ₂	15.64 ± 1.24 Ca	26.50 ± 2.12 Ba	37.56 ± 3.28 Ae
600 CdCl ₂ + SNP	15.64 ± 1.24 Ca	26.76 ± 2.18 Ba	58.23 ± 2.13 Ab
400 CdCl ₂	15.64 ± 1.24 Ca	27.59 ± 3.12 Ba	48.69 ± 2.17 Ad
400 CdCl ₂ + SNP	15.64 ± 1.24 Ca	27.82 ± 1.56 Ba	64.90 ± 1.76 Aa
VC (%)	-----	17.14	18.34

*Averages followed by the same lowercase letter in the column do not differ among each other by the Tukey test at 5% probability. **Averages followed by the same uppercase letter in the row do not differ from each other by the Scott Knott test at 5% probability. Average ± standard deviation.

Table 3. Activity of the ascorbate peroxidase enzyme (APX) in seeds of *S. indicum* after 12 and 24 hours in different concentrations of Cd and Cd added by SNP.

Treatment	APX (μmol.min ⁻¹ .g ⁻¹ .protein)	
	12 h	24 h
Test without soaking	0.12 ± 0.01 Ba	1.06 ± 0.02 Ag
800 CdCl ₂	0.09 ± 0.02 Ba	4.71 ± 0.01 Ac
800 CdCl ₂ + SNP	0.06 ± 0.01 Ba	7.73 ± 0.14 Aa
600 CdCl ₂	0.16 ± 0.02 Ba	3.30 ± 0.02 Ad
600 CdCl ₂ + SNP	0.09 ± 0.02 Ba	6.76 ± 0.23 Ab
400 CdCl ₂	0.20 ± 0.04 Ba	2.12 ± 0.22 Af
400 CdCl ₂ + SNP	0.11 ± 0.04 Ba	2.87 ± 0.78 Ae
VC (%)	19.43	21.76

*Averages followed by the same lowercase letter in the column do not differ among each other by the Tukey test at 5% probability. **Averages followed by the same uppercase letter in the row do not differ from each other by the Scott Knott test at 5% probability. Average ± standard deviation

CAT is responsible for removing the hydrogen peroxide present in high concentrations in peroxisomes, protecting the cells from oxidation damage (Kibinza et al., 2011). On Table 2, an increase is seen in the activity of CAT in relation to the control in all treatments of 24 hours; however, in opposition to what happened to the SOD, there was a decrease in the activity of the enzyme in higher concentrations of the metal, as well as in the treatments with SNP. Several factors can affect the catalase activity, such as the stressing toxic agent, its used concentration, the time of exposure and the plant species, which, to a certain extent, can make this enzyme activity be submitted to great variations (Pereira et al., 2010b). This decrease in the CAT activity can also be related to the inhibition of the synthesis of the enzyme in the presence of cadmium.

The APX acts against the reactive intermediate of oxygen,

degrading the H_2O_2 in water in the presence of ascorbate, specific donor of electrons (Noctor and Foyer, 1998). Given the importance of this enzyme in the antioxidant defense of the plants, the increase of its activity has been presented by several species when exposed to different toxic agents (Moller et al., 2007). On Table 3, APX activity can be verified. At 0 h, there was no enzyme activity (data not presented) possibly due to some

methodology mistake during the determination of its activity. In the interval of 24 hours after the beginning of soaking, similarly to what happened to the SOD (Table 1), the activity of peroxidase of ascorbate (APX) and peroxidase (POX) (Tables 3 and 4, respectively) increase in the higher concentrations of cadmium. It was also possible to see greater activity in both the enzymes in the treatments increased by SNP.

Table 4. Activity of the peroxidase enzyme (POX) in seeds of *S. indicum* after 0, 12 and 24 hours of soaking in different concentrations of cadmium added or not by SNP.

Treatment	POX ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}\cdot\text{protein}$)		
	0 h	12 h	24 h
Test	8.54 \pm 1.03 Ba	11.67 \pm 0.12 Ba	20.18 \pm 0.24 Ag
800 CdCl ₂	8.54 \pm 1.03 Ca	20.16 \pm 0.32 Ba	30.73 \pm 0.13 Ad
800 CdCl ₂ + SNP	8.54 \pm 1.03 Ca	19.17 \pm 0.27 Ba	46.73 \pm 0.16 Aa
600 CdCl ₂	8.54 \pm 1.03 Ca	16.72 \pm 0.12 Ba	25.29 \pm 0.17 Ae
600 CdCl ₂ + SNP	8.54 \pm 1.03 Ca	18.17 \pm 0.31 Ba	42.73 \pm 0.17 Ab
400 CdCl ₂	8.54 \pm 1.03 Ba	12.78 \pm 0.27 Ba	23.83 \pm 0.17 Af
400 CdCl ₂ + SNP	8.54 \pm 1.03 Ca	18.69 \pm 0.25 Ba	34.83 \pm 0.37 Ac
VC (%)	-----	16.56	21.32

*Averages followed by the same lowercase letter in the column do not differ among each other by the Tukey test at 5% probability. **Averages followed by the same uppercase letter in the row do not differ from each other by the Scott Knott test at 5% probability. Average \pm standard deviation

As mentioned by Moller et al. (2007), the action of these toxic agents such as the Cd affects the action of enzymes of oxidative stress. Consequently, these elements can interfere in the cellular metabolism during germination, causing damages that can compromise the vigor and the quality of the seeds. We believe that more efforts must be done in the sense of evaluating the complexity of these damages and finding a way to mitigate them, once the contamination of soils by toxic elements is an ever-increasing concern for agriculture.

Conclusions

The increase in the concentration of cadmium in the soaking solution reduces germination and vigor of the sesame seeds and the initial growth of the seedlings, showing a possible toxic effect of this element to the seeds. The SNP seems to partially reverse the damage caused by the heavy metal.

Mitigation of a damaging action of cadmium by the use of SNP occurs due to the increase of the activity of the antioxidant enzymes, showing an elimination system of the species reactive of oxygen which occurs after the application of the NO donor in response to exposure to heavy metal.

Acknowledgments

We thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Foundation of Research Support of the State of

Minas Gerais - FAPEMIG), the Coordination of the Higher Education Personnel Training (CAPES) and the National Board of Technological and Scientific Development (CNPq) for the financial support and grant of scholarships.

References

- ACCIOLY, A. M. A.; SIQUEIRA, J. O.; CURI, N.; MOREIRA, F. M. S. Amenização do calcário na toxidez de zinco e cádmio para mudas de *Eucalyptus camaldulensis* cultivadas em solo contaminado. *Revista Brasileira de Ciência do Solo*, v.28, n.4, p.775-783, 2004. <http://www.scielo.br/pdf/rbcs/v28n4/21800.pdf>
- BELIGNI, M. V.; LAMATTINA, L. Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. *Plant Cell Environment*, v.25, p.737-74, 2002. <http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00857.x/full>
- BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, v.72, p.248-254, 1976. http://ac.els-cdn.com/0003269776905273/1-s2.0-0003269776905273-main.pdf?_tid=cb19ee2e-c37b-11e5-83d6-00000aabb0f26&acdnat=1453737396_8c39b9bfe5174426b1f9e0387d7c13d
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA-ACS, 2009. 395p. http://www.agricultura.gov.br/arq_editor/file/2946_regras_analise_sementes.pdf
- BRIDGEN, K.; STRINGER, R. LABUSKA, I. Poluição por organoclorados e metais pesados, associada ao fundidor de ferro da Gerdau em Sapucaia do Sul, Brasil, Rio Grande do Sul, Greenpeace, 2000.

- CHUGH, L. K.; SAWHNEY, S. K. Effect of cadmium on germination, amylases and rate of respiration of germinating pea seeds. *Environmental Pollution*, v.92, p.1-5, 1996. <http://www.sciencedirect.com/science/article/pii/0269749195000933>
- HASAN, S. A.; FARIDUDDIN, Q.; ALI, B.; HAYAT, S.; AHMAD, A. Cadmium: Toxicity and tolerance in plants. *Journal of Environmental Biology*, v.30, n.2, p.165-174, 2009. https://www.researchgate.net/publication/41396456_Cadmium_toxicity_and_tolerance_in_plants_J_Environ_Biol
- JOHN, R.; AHMAD, P.; GADGIL, K.; SHARMA, S. Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production*, v.3, n.3, p.65-76, 2009. https://www.researchgate.net/publication/237724355_Heavy_metal_toxicity_effect_on_plant_growth_biochemical_parameters_and_metal_accumulation_by_Brassica_juncea_L_Int_J_Plant_Prod_366-75
- KAR, M.; MISHRA, D. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiology*, v.57, p.315-319, 1976. <http://www.plantphysiol.org/content/57/2/315.long>
- KASIM, W. A. Changes induced by copper and cadmium stress in the anatomy and grain yield of *Sorghum bicolor* (L.) Moench. *International Journal of Agriculture and Biology*, v.8, n.1, p.123-128, 2006. http://www.fspublishers.org/published_papers/64929_.pdf
- KIBINZA S.; BAZINA J.; BAILLY C.; FARRANT J. M.; CORBINEAU O.; BOUTEAU H. Catalase is a key enzyme in seed recovery from ageing during priming. *Plant Science*, v.181, p. 309-315, 2011. https://www.researchgate.net/publication/51496832_Catalase_is_a_key_enzyme_in_seed_recovery_from_ageing_during_priming_Plant_Sci
- KOPYRA, M.; GWÓZDZ, E.A. Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology Biochemistry*, v.41, p.1011-1017, 2003. <http://www.sciencedirect.com/science/article/pii/S098194280300175X>
- MADHUSUDHAN, R.; ISHIKAWA, T.; SAWA, Y.; SHIGEOKA S.; SHIBATA, H. Characterization of an ascorbate peroxidase in plastids of tobacco BY-2 cells. *Physiologia Plantarum*, v.117, p.550-557, 2003. <http://onlinelibrary.wiley.com/doi/10.1034/j.1399-3054.2003.00066.x/epdf>
- MOLLER, I. M.; JENSEN, P. E.; HANSSON, A. Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*, v.58, p.459-481, 2007. <http://www.annualreviews.org/doi/pdf/10.1146/annurev.arplant.58.032806.103946>
- NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: KRZYZANOSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B (Ed.). *Vigor de sementes: conceitos e testes*. ABRATES, Londrina, p.2-1-2.21, 1999.
- NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, v.22, p.867-880, 1981. <http://pcp.oxfordjournals.org/content/22/5/867.full.pdf+html>
- NOCTOR, G; FOYER, C. H. Ascorbate and Glutathione: Keeping Active Oxygen Under Control. *Annual Review of Plant Physiology and Plant Molecular Biology*, v.49, p.249-279, 1998. <http://www.annualreviews.org/doi/pdf/10.1146/annurev.arplant.49.1.249>
- PEREIRA, B.L.C.; BORGES, E.E.L.; OLIVEIRA, A.C.; LEITE, H.G.; GONÇALVES, J.F.C. Influência do óxido nítrico na germinação de sementes de *Plathymenia reticulata* Benth com baixo vigor. *Scientia Florestalis*, v.38, n.88, p.629-636, 2010a. <http://www.ipef.br/publicacoes/scientia/nr88/cap09.pdf>
- PEREIRA, F. J.; MAGALHÃES, P. C.; SOUZA, T. C.; CASTRO, E. M.; ALVES, J. D. Atividade do sistema antioxidante e desenvolvimento de aerênquima em raízes de milho 'Saracura'. *Pesquisa Agropecuária Brasileira*, v.45, p.450-456, 2010b. <http://www.scielo.br/pdf/pab/v45n5/03.pdf>
- ROSSI, C.; LIMA, G. P. P. Cádmiio e a atividade de peroxidase duante a germinação de sementes de feijoeiro. *Scientia Agricola*, v.58, n.1, p.197-199, 2001. <http://www.scielo.br/pdf/sa/v58n1/a30v58n1.pdf>
- SOARES, C. R. F. S.; SIQUEIRA, J. O.; CARVALHO, J. G.; MOREIRA, F. M. S. Fitotoxidez de cádmio para *Eucalyptus maculata* e *E. urophylla* em solução nutritiva. *Revista Árvore*, v.29, n.2, p.175-183, 2005. <http://www.scielo.br/pdf/rar/v29n2/a01v29n2.pdf>
- VALKO, M.; RHODES, C.J.; MONCOL, J. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico Biological Interaction*, v.160, p.1-40, 2006. http://ac.els-cdn.com/S0009279705004333/1-s2.0-S0009279705004333-main.pdf?_tid=cb6f97a8-c37f-11e5-beeb-00000aacb361&acdnat=1453739114_ab3e2366f88da46b7e4c1d505f6b29e0
- WANI, P. A.; KHAN, M. S.; ZAIDI, A. Toxic effects of heavy metals on germination and physiological processes of plants. In: ZAIDI, A.; WANI, P.A.; KHAN, M.S. Toxicity of heavy metals to legumes and bioremediation. *Springer*, p.45-66, 2012. http://link.springer.com/chapter/10.1007%2F978-3-7091-0730-0_3
- ZHANG, F. Q.; WANG, Y. S.; LOU, Z. P.; DONG, J. D. Effect of heavy metal stress on antioxidant enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorhiza*). *Chemosphere*, v.67, p. 44-50, 2007. http://ac.els-cdn.com/S004565350601335X/1-s2.0-S004565350601335X-main.pdf?_tid=0e07c766-c380-11e5-ac0d-00000aab0f6c&acdnat=1453739226_252a4fe2b03c7c3a33fc7a109719340