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Effect of sodium fluoride and sodium nitroprusside on *Cicer arietinum* and *Pisum sativum*

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ABSTRACT

In present study, the individual and combine effect of sodium fluoride (NaF) and sodium nitroprusside (SNP) on germination and biochemical parameters (pigments, sugar, protein, amino acid, and phenol) of Bengal gram (*Cicer arietinum*) and peas (*Pisum sativum*) has been studied. After three days of NaF treatment, reductions were observed in percentage of seed germination, root and shoot length, and pigment content with increasing concentration of NaF (1 to 4 mg L⁻¹). Seedlings treated with SNP, both alone and in combination of NaF, showed enhancement in seed germination as well as other growth parameters. NaF-treated seedlings were found to accumulate more soluble sugars and phenols, which were further increased by SNP treatment thereby indicating a synergistic effect of the possible reasons for the ameliorative effects of SNP in seedlings of *Pisum sativum* growing under NaF stress. Results also demonstrated that SNP application did not show any improvement in both morpho-physiologically and biochemically under sodium fluoride stress condition.

Keywords: Seedling growth, Biochemical parameters, Soil pollution, Phytointoxication, Ecological problem.

INTRODUCTION

Fluoride (F) is a strong electronegative element widespread in the environment that occurs in soil, air, water and the vegetation (Jha et al. 2009). The World Health Organization (WHO 1984) and Bureau of Indian Standards (BIS 2003) have laid down the maximum permissible limits of F in drinking water as 1.5 mg L⁻¹ and 1.0 mg L⁻¹, respectively, but there is no stringent threshold limit of F in soil and plants above which the ingestion may be detrimental to human health (Das et al. 2015). In acidic soil fluoride showed highest solubility due to its complexation with aluminum, but in alkaline condition, desorption of free fluoride due to repulsion by negatively charged surfaces. However, in neutral pH, fluoride readily bound to soil surface and is not available to plants (Das et al. 2015).

According to Arnesen (1997) plants can incorporate F from contaminated soil. The adsorbed F is translocated to the shoots causing physiological, biochemical, and structural damage and even cell death (Jha et al. 2009) depending on the concentration of cell sap. Some plants accumulate F and even at higher concentration up to 4 mg g⁻¹ without showing any signs of toxicity (Rahman et al. 2007). Most other plants show signs of toxicity at much lower concentration. The fluoride content of both leafy and root vegetables usually do not differ appreciably from those of cereals with an exception of spinach and onion (Jha et al. 2009) those usually enriched in fluoride and it is known as good accumulator of fluoride.

Phytotoxicity due to F is one of the severe ecological problems in the world (Saini et al. 2013). It is well documented (Das et al. 2015; Dey et al. 2013; Maitra et al. 2013) that F is absolutely nonessential element for plant growth and in higher concentration it is toxic for plants, especially for early seedling growth (Weinstein and Davison 2004). The importance of seed germination in plant growth is widely recognized and the effects of F toxicity on it have been studied by various researchers (Sabal et al. 2006; Saini et al. 2012; Maitra et al. 2013; Dey et al. 2013). Several physiological and biochemical processes are known to be markedly affected by F such as chlorosis and necrosis of leaf, inhibition of the nutrient uptake, reduction of plant growth, and enzyme activities (Chakrabarti and Patra 2013; Dey et al. 2012).

Nitric oxide is involved in germination and induction of lateral roots formation. It also modulates the influx of extracellular Ca²⁺ and actin filament organization during cell wall construction in *Pinus bungeana* pollen tubes (Wang et al. 2009). Recently, Wang et al. (2010) reported that NO induced programmed cell death (PCD) through mitochondrial pathway which is

regulated by Ca²⁺ in tobacco protoplasts. Exogenous application of NO also down-regulated xanthine oxidase mediated generation of O²⁻ in *Phalaenopsis* flowers (Tewari et al. 2009). Sun et al. (2007) reported that sodium nitropruside (SNP), an NO donor, partially reversed iron deficiency induced retardation of plant growth as well as chlorosis, suggesting a link between NO and iron metabolism. Batasheva et al. (2010), reported that export of assimilates from leaves might be regulated by NO signaling system. Freschi (2013) and Astier and Lindermayr (2012) have accounted that NO has direct involvement in plant hormone responses.

Several studies have been conducted on the effects of F stress on seed germination, early seedling growth, and biochemical content (Chakrabarti and Patra 2013; Dey et al. 2013; Datta et al. 2012). However, no study has been done on reducing plant F toxicity by application of SNP. Therefore, the present investigation was undertaken to ascertain the influence of SNP on NaF-induced stress on early seedling growth of Bengal gram (*Cicer arietinum*) and peas (*Pisum sativum*) and some biochemical parameters viz, pigment, sugar, amino acid, protein and phenols.

MATERIAL AND METHODS

General information. Sterilized seeds of *C. arietinum* and *P. sativum* were germinated in individual petri dishes and labeled as control (distilled water) and respective treatment solutions (1, 2 and 4 mg L⁻¹ NaF solution, 1 mg L⁻¹ + SNP, 2 mg L⁻¹ + SNP, 4 mg L⁻¹ + SNP). Petri dishes were kept at 28 °C in a Biochemical Oxygen Demand (BOD) incubator. The germination percentage was recorded on the three days after seed sowing. After thirteen days of treatment, the root length, shoot length, fresh and dry weight and vigor index were measured and whole seedling were used for estimation of the photosynthetic pigments content (Arnon 1949), total soluble sugar (McCrary et al. 1950), amino acid (Moor and Stein 1948), protein (Lowry et al. 1951) and phenolic content (Malick and Singh 1980).

Sodium nitropruside and sodium fluoride solution.

A solution of 50 µM sodium nitropruside (MERC India limited, Mumbai) was prepared with proper dilution from 100 µM of SNP solution. A solution of 100 mg L⁻¹ sodium fluoride (MERC india limited, Mumbai) was prepared by dissolving 0.22 g sodium fluoride in one liter distilled water. The required concentration of fluoride solution was prepared by serial dilution of 100 mg L⁻¹ fluoride stock solution.

Germination percentage. Seed germination was recorded daily up to 3 days after the initial day of the

experiment. Seeds were considered as germinated when the radical reached a length of 1 mm (Kabir et al. 2008) and the germination percentage was calculated as per the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Root and shoot length (early seedling growth).

Length of root and shoot was measured with the help of a scale and reading was taken from both treated and controlled plants.

Biomass production. Root development in test plants was determined by counting the number of primary, secondary and tertiary roots.

Chlorophyll (a, b and total) analysis. Fresh young leaves (0.1 g) were selected from plants under each treatment at the last day of the experiment and washed with deionized water. The leaves were cut into small pieces. Chlorophyll fractions 'a', 'b' and total chlorophyll were determined in the acetone extract (80% v/v) (Arnon 1949) measured in a spectrophotometer at 645, 652 and 663 nm, respectively. The concentrations were expressed as mg chlorophyll g⁻¹ fresh weight with the following equations:

$$\text{Chl 'a'} (\text{mg} \cdot \text{g}^{-1} \cdot \text{f} \cdot \text{w}) = [12.7 \times D_{663} - 2.69 \times D_{645}] \times \frac{v}{1000 \times w} \quad (1)$$

$$\text{Chl 'b'} (\text{mg} \cdot \text{g}^{-1} \cdot \text{f} \cdot \text{w}) = [22.9 \times D_{645} - 2.69 \times D_{663}] \times \frac{v}{1000 \times w} \quad (2)$$

$$\text{Total Chl} (\text{mg} \cdot \text{g}^{-1} \cdot \text{f} \cdot \text{w}) = D_{652} \times 1000 \times \frac{v}{1000 \times w} \quad (3)$$

Here, D = absorbance, v = final volume of 80% acetone; w = mass of sample; fw = fresh weight of the sample.

Statistical analysis. The entire experiment was carried out in a completely randomized design (CRD) matrix. All Statistical calculation was performed with MINITAB version 13 software. Data were analyzed with the help of one way analysis of variance (ANOVA) to determine the significant effect of different treatments. The grouping was done at 5% level of significance and the treatments having the same letter are non-significant at the given level of significance.

RESULTS AND DISCUSSION

Root and shoot length. Root length of *C. arietinum* showed incremental pattern over control lower dose of fluoride up to 2 mg L⁻¹ (Table 1). Similarly, only application of SNP showed little improvement of root length over lower fluoride dose (1 mg L⁻¹). However, SNP does not show any improvement at higher dose of fluoride. Almost similar observation recorded for

shoot length also. But only application of SNP showed much improvement over lower dose of fluoride (1 mg L⁻¹). On the other hand, *P. sativum* showed similar variation of root length as *C. arietinum*. But shoot length of *P. sativum* was much less influenced by fluoride than *C. arietinum* (Table 1).

Fresh and dry weight. Effect of fluoride on *C. arietinum* clearly indicate the negative impact on both fresh and dry weight (Table 2). Application of SNP does not indicate any improvement on the above mentioned morpho-physiological trait. Results also demonstrated that water holding capacity of *C. arietinum* was also affected by application of different concentration of fluoride solution and SNP dose not shows any improvement (Table 2). The ratio of fresh weight to dry weight (FW/DW) also showed gradual reduction with increase of fluoride concentration with respect to control. However, only application of SNP showed little improvement over lower dose (1 mg L⁻¹) of fluoride application. Moreover, combined application of fluoride (1 mg L⁻¹) and SNP (50 μM SNP) showed the value of FW/DW as same as only application of 1 mg L⁻¹ fluoride application. On the other hand, effect of fluoride on fresh and dry weight of root and shoot, of *P. sativum* showed much lower impact than *C. arietinum* (Table 3). The effect of SNP on *P. sativum* showed little improvement of both fresh and dry weight of shoot over lower dose (1 mg L⁻¹) of fluoride application. However, combined application of fluoride (1 mg L⁻¹) and SNP (50 μM) showed much improvement of both fresh and dry weight of shoot (Table 2). At higher concentration (4 mg/L) of fluoride along with SNP (50 μM) application showed good improvement in dry weight of shoot and water content of *P. sativum*.

Pigment (Chl 'a', Chl 'b' and Total Chl) level.

Pigment concentration of *C. arietinum* and *P. sativum* in the form of Chl 'a', Chl 'b' and Total Chlorophyll has been presented in Figures 1, 2 and 3. It is clear that both *C. arietinum* and *P. sativum* were not equally affected by fluoride, so that *C. arietinum* is more intensely affected by fluoride than *P. sativum*. However, chlorophyll 'b' level showed opposite trend for both the plant species up to 2 mg L⁻¹ fluoride concentration (Figure 2). On the other hand, total chlorophyll level presented in Figure 3 and it shows that SNP has negative impact on total chlorophyll level for both the plant species. Moreover, total chlorophyll level linearly decreased with increasing fluoride level. This is probably due to higher accumulation of fluoride in leaves and subsequently it can bind readily with Mg²⁺, forming an MgF⁺ complex (Dey et al. 2012). In this way, this kind of complexation may destroy

the photosynthetic pigments, particularly the concentration of pigments (Trapp and McFarlane chlorophylls, thereby significantly decreasing the 1995).

Table 1. Effect of sodium fluoride (NaF) and sodium nitroprusside (SNP) and the combined dose of NaF+SNP on root and shoot length of *Cicer arietinum* and *Pisum sativum*.

Treatment	<i>Cicer arietinum</i>		<i>Pisum sativum</i>	
	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)
Control	2.0 ^c	6.5 ^a	2.5 ^b	7.5 ^b
1 mg L ⁻¹ NaF	2.5 ^b	5.7 ^b	2.5 ^b	7.5 ^b
2 mg L ⁻¹ NaF	6.0 ^a	5.0 ^c	2.0 ^c	7.5 ^b
4 mg L ⁻¹ NaF	1.0 ^d	3.5 ^d	5.0 ^a	8.0 ^b
Only SNP	2.6 ^b	6.5 ^a	2.0 ^c	8.5 ^a
1 mg L ⁻¹ NaF + 50 µM SNP	1.3 ^d	3.0 ^d	1.5 ^d	6.5 ^c
2 mg L ⁻¹ NaF + 50 µM SNP	2.1 ^c	2.5 ^e	2.0 ^c	3.5 ^d
4 mg L ⁻¹ NaF + 50 µM SNP	1.3 ^d	1.6 ^f	2.5 ^b	3.0 ^e

Each value is the mean of three replicate of each treatment. Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

Table 2. Effect of sodium fluoride (NaF) and sodium nitroprusside (SNP) and the combined dose of NaF+SNP on the fresh weight root (FWR) and shoot (FWS), the dry weight of root (DWR) and shoot (DWS) and the water content in *Cicer arietinum*.

Treatment	FWR (g)	FWS (g)	DWR (g)	DWS (g)	Water content (g ⁻¹ DW)	FW/DW
Control	0.144 ^a	0.089 ^a	0.013 ^a	0.010 ^b	0.021 ^a	10.130 ^a
1 mg L ⁻¹ NaF	0.049 ^c	0.053 ^b	0.010 ^b	0.009 ^b	0.008 ^c	5.368 ^d
2 mg L ⁻¹ NaF	0.073 ^b	0.051 ^b	0.011 ^b	0.006 ^c	0.011 ^b	7.294 ^b
4 mg L ⁻¹ NaF	0.036 ^d	0.031 ^d	0.007 ^c	0.006 ^c	0.005 ^d	5.154 ^e
Only SNP	0.027 ^e	0.085 ^a	0.010 ^b	0.016 ^a	0.009 ^c	5.894 ^c
1 mg L ⁻¹ NaF + 50 µM SNP	0.028 ^e	0.047 ^c	0.006 ^c	0.008 ^c	0.006 ^c	5.357 ^d
2 mg L ⁻¹ NaF + 50 µM SNP	0.021 ^f	0.018 ^e	0.005 ^c	0.005 ^c	0.003 ^f	3.90 ^g
4 mg L ⁻¹ NaF + 50 µM SNP	0.015 ^g	0.027 ^d	0.003 ^d	0.006 ^c	0.003 ^e	4.666 ^f

Each value is the mean of three replicate of each treatment. Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

Table 3. Effect of sodium fluoride (NaF) and sodium nitroprusside (SNP) and the combined dose of NaF+SNP on the fresh weight root (FWR) and shoot (FWS), the dry weight of root (DWR) and shoot (DWS) and the water content in *Pisum sativum*.

Treatment	FWR (g)	FWS (g)	DWR (g)	DWS (g)	Water content (g ⁻¹ DW)	FW/DW
Control	0.327 ^a	0.322 ^a	0.027 ^a	0.014 ^d	0.061 ^a	15.829 ^a
1 mg L ⁻¹ NaF	0.136 ^b	0.195 ^c	0.015 ^b	0.018 ^c	0.029 ^b	10.030 ^b
2 mg L ⁻¹ NaF	0.075 ^e	0.198 ^c	0.012 ^c	0.021 ^b	0.024 ^c	8.273 ^c
4 mg L ⁻¹ NaF	0.085 ^d	0.069 ^g	0.011 ^c	0.008 ^e	0.014 ^d	8.105 ^d
Only SNP	0.130 ^c	0.207 ^b	0.013 ^c	0.031 ^a	0.014 ^d	5.375 ^g
1 mg L ⁻¹ NaF + 50 µM SNP	0.041 ^g	0.131 ^d	0.016 ^b	0.016 ^c	0.012 ^e	6.273 ^f
2 mg L ⁻¹ NaF + 50 µM SNP	0.050 ^f	0.088 ^e	0.010 ^d	0.012 ^d	0.009 ^f	2.455 ^h
4 mg L ⁻¹ NaF + 50 µM SNP	0.029 ^h	0.079 ^f	0.006 ^e	0.009 ^e	0.029 ^b	7.659 ^e

Each value is the mean of three replicate of each treatment. Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

Biochemical parameters. Table 4 demonstrated the biochemical parameters of *C. arietinum* and *P. sativum* where we can observe that sugar level decrease in all level of fluoride treated in *C. arietinum*. However, *P. sativum* showed opposite trend in lower concentration

(1 mg L⁻¹ and 2 mg L⁻¹). Moreover, application of SNP does not improve the sugar level (Table 4). On the other hand, the level of protein and phenol showed similar results as sugar in *C. arietinum*, but in *P. sativum* the protein and amino acid level enhanced

under fluoride stress with respect to control. Results also clearly demonstrated that SNP showed little improvement especially in protein, amino acid and

phenol level for both *P. sativum* and *C. arietinum*. Under SNP treatment for both *P. sativum* and *C. arietinum* was much lower.

Table 4. Effect of sodium fluoride (NaF) and sodium nitroprusside (SNP) and the combined dose of NaF+SNP on the level of sugar, protein, amino acid and phenol of *Cicer arietinum* and *Pisum sativum*.

Treatment	Sugar (mg g ⁻¹)		Protein (mg g ⁻¹)		Amino acid (mg g ⁻¹)		Phenol (mg g ⁻¹)	
	Gram	Pea	Gram	Pea	Gram	Pea	Gram	Pea
Control	33.3 ^a	36.11 ^c	34.64 ^a	25.77 ^b	44.25 ^e	26.55 ^g	0.049 ^c	0.036 ^c
1 mg L ⁻¹ NaF	4.49 ^e	48.26 ^b	21.06 ^g	38.76 ^a	63.9 ^c	44.25 ^e	0.006 ^e	0.060 ^b
2 mg L ⁻¹ NaF	6.54 ^d	50.31 ^a	33.14 ^d	25.86 ^c	70.80 ^a	88.52 ^b	0.090 ^d	0.060 ^b
4 mg L ⁻¹ NaF	14.45 ^b	10.36 ^f	41.23 ^b	23.72 ^d	68.15 ^b	70.81 ^c	0.070 ^d	0.030 ^c
Only SNP	3.14 ^f	17.45 ^e	41.23 ^b	24.72 ^d	53.11 ^d	61.63 ^d	0.179 ^a	0.060 ^b
1 mg L ⁻¹ NaF + 50 μM SNP	9.27 ^c	3.54 ^g	27.61 ^e	18.21 ^f	33.70 ^f	97.37 ^a	0.135 ^b	0.040 ^c
2 mg L ⁻¹ NaF + 50 μM SNP	4.49 ^e	31.08 ^d	25.67 ^f	25.86 ^c	35.41 ^f	35.41 ^f	0.132 ^b	0.050 ^b
4 mg L ⁻¹ NaF + 50 μM SNP	2.37 ^g	17.45 ^e	30.79 ^c	20.41 ^e	17.16 ^g	61.96 ^d	0.090 ^d	0.070 ^a

Each value is the mean of three replicate of each treatment. Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates were taken.

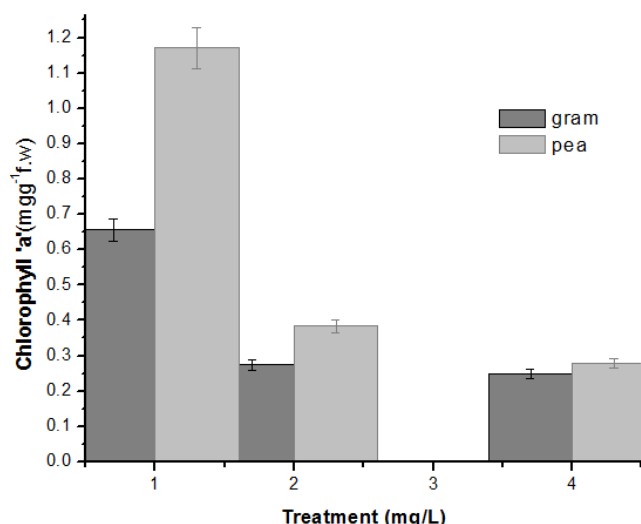


Figure 1. Level of Chlorophyll 'a' in *Cicer arietinum* (gram) and *Pisum sativum* (pea) exposed to sodium fluoride.

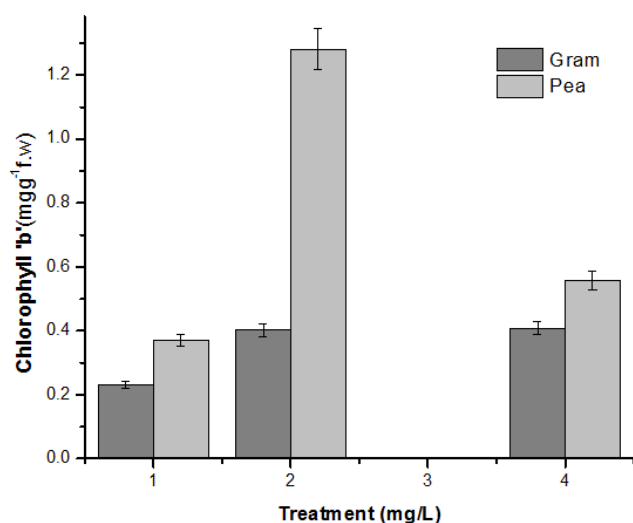


Figure 2. Level of Chlorophyll 'b' in *Cicer arietinum* (gram) and *Pisum sativum* (pea) exposed to sodium fluoride.

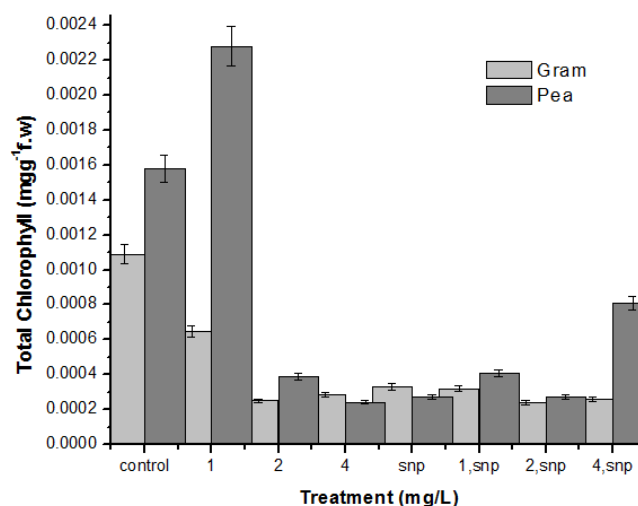


Figure 3. Level of total chlorophyll in *Cicer arietinum* (gram) and *Pisum sativum* (pea) exposed to sodium fluoride.

CONCLUSION

The results of the present study showed that both *P. sativum* and *C. arietinum* were affected by the fluoride but *C. arietinum* is more intensely affected than *P. sativum*. Results also suggest that SNP has no beneficial role towards improvement of morpho-physiological and biochemical traits of both *P. sativum* and *C. arietinum* under fluoride stress condition. However, pigment level dose not affected equally for both the tested species. Biochemical study clearly demonstrated that SNP has some role towards improvement of protein, amino acid and phenol level for both *P. sativum* and *C. arietinum*. The level of sugars was much lower under SNP treatment for both *P. sativum* and *C. arietinum*. Finally it can be concluded that fluoride has tremendous negative

impact on both morphophysiology and biochemistry of *P. sativum* and *C. arietinum* and SNP has no role towards overcome the negative impact of fluoride.

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RESUMO

Efeito de fluoreto de sódio e nitroprussiato de sódio sobre *Cicer arietinum* e *Pisum sativum*. No presente estudo, estudou-se o efeito individual e combinado de fluoreto de sódio (NaF) e nitroprussiato de sódio (SNP) na germinação e parâmetros bioquímicos (pigmentos, açúcar, proteínas, aminoácidos e fenol) de grão-de-bico (*Cicer arietinum*) e ervilha (*Pisum sativum*). Depois de três dias de tratamento com NaF, foram observadas reduções na porcentagem de germinação das sementes, no comprimento de raízes e de parte aérea e no teor de pigmento com o aumento da concentração de NaF (1 a 4 mg L⁻¹). As plântulas tratadas com SNP, tanto isoladamente como em combinação de NaF, mostraram melhoria na germinação de sementes, bem como nos outros parâmetros de crescimento. Plântulas tratadas com NaF acumularam mais açúcares e fenóis, os quais foram ainda aumentadas pelo tratamento com SNP, indicando assim um efeito sinérgico que pode indicar efeito melhorador do SNP em mudas de *Pisum sativum* que crescem sob estresse com fluoreto de sódio. Os resultados também demonstraram que a aplicação SNP não apresentou qualquer melhoria tanto para características morfo-fisiológica quanto bioquímica na condição de estresse com fluoreto de sódio.

Palavras-chave: Crescimento de plântula, Parâmetros bioquímicos, Poluição do solo, Fitointoxicação, Problema ecológico.

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