The Pharma Innovation Journal 2023; SP-12(11): 2101-2105

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 2101-2105

www.thepharmajournal.com
Received: 01-08-2023
Accepted: 05-09-2023

Satish Kumar
Central University of Jharkhand,
Brambe, Ranchi, Jharkhand,
India

Amarendra Narayan Misra
Central University of Jharkhand,
Brambe, Ranchi, Jharkhand,
India

Corresponding Author:
Satish Kumar
Central University of Jharkhand,
Brambe, Ranchi, Jharkhand,
India

Ameliorative effect of sodium nitroprusside (SNP) on lead toxicity in wheat during vegetative stage

Satish Kumar and Amarendra Narayan Misra

Abstract

The impact of Lead (Pb) on the growth of wheat (*Triticum aestivum* L.) seedlings and the potential mitigation of lead toxicity through nitric oxide (NO) were examined. Exposure to 500µM of lead led to decreases in germination percentage, fresh weight, dry weight, chlorophyll, and carotenoid content. Nitric oxide (NO) is a highly reactive molecule involved in various physiological and biochemical pathways, demonstrating its protective role against oxidative stress. Treatment with the NO donor sodium nitroprusside (SNP) at 100 µM significantly alleviated lead-induced inhibition, resulting in increased shoot and root length, as well as higher chlorophyll and carotenoid levels compared to the control. However, higher concentrations of SNP (500 µM) displayed adverse effects, reducing photosynthetic pigment content in germinated seedlings. Application of 100 µM SNP in conjunction with 300 and 500 µM Pb effectively mitigated the toxic effects of lead on wheat seedlings. These findings underscore the protective function of SNP in counteracting in wheat seedlings, toxicity induced by lead.

Keywords: *Triticum aestivum* L, lead, sodium nitroprusside, chlorophyll

Introduction

A number of human interference, like as the disposing of sewage sludge and electronic waste, mining, and smelting, have resulted in higher concentrations of heavy metals in the natural environment. The detrimental impacts of heavy metals on plants are linked to the oxidative influence of metal ions, disrupting a variety of physiological and biochemical processes (Gratão *et al*., 2005) [1]. For example, lead (Pb) has been demonstrated to initiate the generation of reactive oxygen species (ROS) and alter the operation of enzymes in plant roots, including lupin, peas, rice, maize, and aquatic plants like *Wolffia arrhiza* and *Elodea canadensis* (Kaur *et al*., 2015) [4]. In a similar manner, the introduction of 0.10 mM sodium nitroprusside (a nitric oxide donor) and 17.50 mM calcium nitrate (Na/Ca = 5) has been proven to mitigate the reduction in chlorophyll content, root activity, and soluble sugar content. This treatment boosts the activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase, while decreasing electrolyte leakage, lipid peroxidation, hydrogen peroxide content, and superoxide generation rate (Xu *et al*., 2010; Tian *et al*., 2015) [13, 11]. Building upon this foundation, the proposition is that nitric oxide (NO) might alleviate the detrimental consequences of lead (Pb) on wheat. Kaur *et al*. (2015) [4] explored how NO functions as an antioxidant, managing oxidative harm caused by Pb in hydroponically grown wheat roots. Furthermore, the research scrutinized the impact of external NO on the creation of ROS (such as lipid peroxidation, hydrogen peroxide, conjugated dienes, superoxide ions, and hydroxyl ions), membrane integrity, ROS metabolism, variations in protein content, and changes in the molecular-level activities of SOD and CAT during the initial 0–8 hours of Pb exposure. Recently, a growing body of evidence proposes that nitric oxide (NO) also plays vital roles in supervising diverse physiological processes in plants. Beyond plant development, NO might also partake in adjusting plant defence responses against both biological and environmental pressures (Misra *et al*., 2011) [7]. It's noteworthy that the production of NO can be affected by biological and environmental pressures, and utilizing an NO donor (sodium nitroprusside; SNP) can heighten a plant's tolerance to specific stressors. Although NO is now acknowledged as a signaling molecule, there are only a few reports about the consequences of externally applied NO on plants undergoing water logging stress during initial growth phases. Additionally, the application of NO has been observed to influence chlorophyll metabolism by stimulating chlorophyll synthesis and curtailing chlorophyll degradation (Dong *et al*., 2014) [1]. Also, NO has been identified as a likely participant in the creation of parenchyma in response to hypoxia (Dordas *et al*., 2003) [2].

Given these considerations, it becomes vital to examine if and how externally administered NO contributes to resistance against heavy metals, particularly in wheat crops. Thus, this investigation strives to delve into the combined effects of Pb and SNP on hydroponically cultivated wheat seedlings.

Materials and Methods

Seeds of the wheat variety PBW 343 (*Triticum aestivum* L.) known for their healthiness were procured from KVK Nawada (Bihar). To ensure cleanliness, the seeds underwent a surface sterilization process using a 0.1% solution of sodium hypochlorite for duration of 10 minutes. Subsequently, the seeds were thoroughly rinsed under flowing tap water, and this was succeeded by a meticulous washing procedure involving 5 to 6 repetitions using distilled water. After 24 hrs of hydroponics culture there were eight total treatments including control, with three replications. These includes (1) Control (Hoagaland solution) (2) Hoagaland solution + 100µM Pb (3) Hoagaland solution + 100µM SNP (4) Hoagaland solution + 100µM Pb + 100µM SNP (5) Hoagaland solution + 300µM Pb (6) Hoagaland solution + 300µM Pb +100µM SNP (7) Hoagaland solution + 500 µM Pb (8) Hoagaland solution + 500 µM Pb +100µM SNP. The treatment solution changed after two days to maintain constant treatment concentration. The plants were harvested after 7 days of treatment and measured for various physiochemical, biochemical and enzymatic changes.

Determination of physiological parameters

Wheat cultivar seeds, specifically PBW 343, were initiated into germination within laboratory settings. For this purpose, each Petri dish was lined with filter paper and subjected to autoclaving. Prior to germination, the seeds underwent surface sterilization using a solution of sodium hypochlorite (0.1%, w/v) for duration of 10 minutes. This was followed by five cycles of thorough washing with sterile distilled water. To evaluate the germination percentage, the number of germinated seeds in each Petri dish was tallied. To measure shoot length, a metric scale was employed to gauge the length in centimetres from the plant's base to the tip of the main shoot. This measurement was subsequently converted into centimetres. The shoot length of five plants taken from each replication was documented and averaged to ascertain the average length per plant in each treatment. Following a period of seven days, root length was evaluated. From each pot, five seedlings were chosen at random, cleaned, and delicately dried on Whatman paper. These seedlings were then separated into their root and shoot parts and their immediate fresh weight were determined. For determining dry weight, the shoot and root samples were subjected to 70 °C in an oven, and the resulting weight was expressed in milligrams.

Estimation of chlorophyll (chl) and carotenoid (car) contents

The quantification of chlorophyll and carotenoid levels (mg/g FW) followed the method outlined by Wellburn (1994) [12], with some minor adjustments. A fresh sample (0.5 g) from both the control and treated plants was finely diced and placed in dimethyl sulphoxide (DMF) in a 2 ml volume. The sample was then covered with aluminium foil and stored in darkness at a temperature of 4 °C for a span of 24 hours. As the liquid developed a green hue, its absorbance was gauged at wavelengths of 664 nm, 647 nm, and 480 nm using a UV-visual Perkin-Elmer double beam spectrophotometer, with DMF serving as the blank reference. The quantities of Chl a, Chl b, and carotenoids were computed employing appropriate experimental equations. The derived pigment content was subsequently expressed in micrograms per gram of fresh weight (µg g^{-1} FW).

$\text{Chla} = 11.65\text{OD}_{664} - 2.69\text{OD}_{647}$

$\text{Chlb} = 20.81\text{OD}_{647} - 4.53\text{OD}_{664}$

$\text{Car} = (1000 \times \text{OD}_{480} - 0.89 \times \text{chla} - 52.02\ \text{chlb})/245$

Results

The dominant abiotic stressor affecting agricultural productivity is heavy metal stress, which leads to substantial yield losses across various crops. With this concern in focus, the current study was conducted within a plant growth chamber at the Centre for Life Science. The objective was to examine the impact of lead-induced heavy metal stress, both in isolation and in conjunction with SNP (sodium nitroprusside), on plants. The experimental setup encompassed a total of 8 distinct treatments, with each treatment being replicated 3 times. The outcomes derived from this investigation are detailed in the subsequent section of this chapter.

Morphological Characters

Combined impact of Pb and SNP on seed germination

The exposure of Pb, germination percentage was decreased by 19.22%, 42.30% 51.92% at 100, 300 and 500 µM Pb concentrations respectively. However on SNP supplementation up to 100 µM increased 5.76% in germination relative to the control. Exogenous application of 100 SNP along with 100, 300 and 500 µM Pb enhance germination percentage by 14.28%, 22.00% and 4.87% in respectively in comparison to 100, 300 and 500 µM Pb treatments presented in Fig. 1.

Combined effect of Pb and SNP on shoot and root length

Comparing to control in 100 µM, 300 µM and 500 µM Pb stressed plants, the shoot length was reduced by 27.04%, 41.10% and 55.02% respectively whereas root length 49.61%, 65.09% and 72.58% respectively (Fig.1). Supplementation of 100 µM SNP with 100 µM Pb increased length by 21.13.% in shoot and 31.03% in root compare to 100 µM Pb treatment whereas a combination of 100µM SNP with 300 µM Pb increased shoot and root length by 11.76.% and 52.07% in compare to 300 µM Pb treatment. Exogenous application of 500 µM SNP along with 500 µM Pb enhances shoot length by 15.95% and root length by 29.06%relative to 500 µM Pb treatments.

Combined effect of Pb and SNP on shoot and root fresh weight

Comparing to control in 100 µM,300 µM and 500 µM Pb stressed plants, the fresh weight of shoot was reduced by 34.32%, 56.77% and 63.55% respectively whereas root fresh weight by 35.16%, 56.30% and 68.57% respectively (Fig.1). Supplementation of 100 µM SNP along with 100 µM Pb increased fresh weight by 29.03.% in shoot and 26.33% in root compare to 100 µM Pb treatment whereas adding with 300 µM SNP with 300 µM Pb increased shoot and root length by 20.50.% and 17.27% in compare to 300 µM Pb treatment. Exogenous application of 500 SNP along with 500 µM Pb enhances shoot fresh weight by 14.85% and root fresh weight 17.21% relative to 500 µM Pb treatment

Combined effect of Pb and SNP on shoot and root dry weight

Application of 100, 300 and 500 µM Pb stressed plants, the shoot dry weight was reduced by 42.08%, 54.56% and 65.67% respectively whereas root dry weight by 26.88%, 56.98% and 63.65% respectively relative to control (Fig.1). Supplementation of 100 µM SNP along with 100 µM Pb increased dry weight by 23.71% in shoot and 16.00% in root compare to 100 µM Pb treatment whereas a combination with 300 µM SNP with 300 µM Pb increased shoot and root length by 8.79.% and 14.79% in compare to 300 µM Pb treatment (Fig.1). Exogenous application of 500 SNP along with 500 µM Pb enhances shoot dry weight by 6.08% and root dry weight by 11.17% relative to 500 µM Pb treatment.

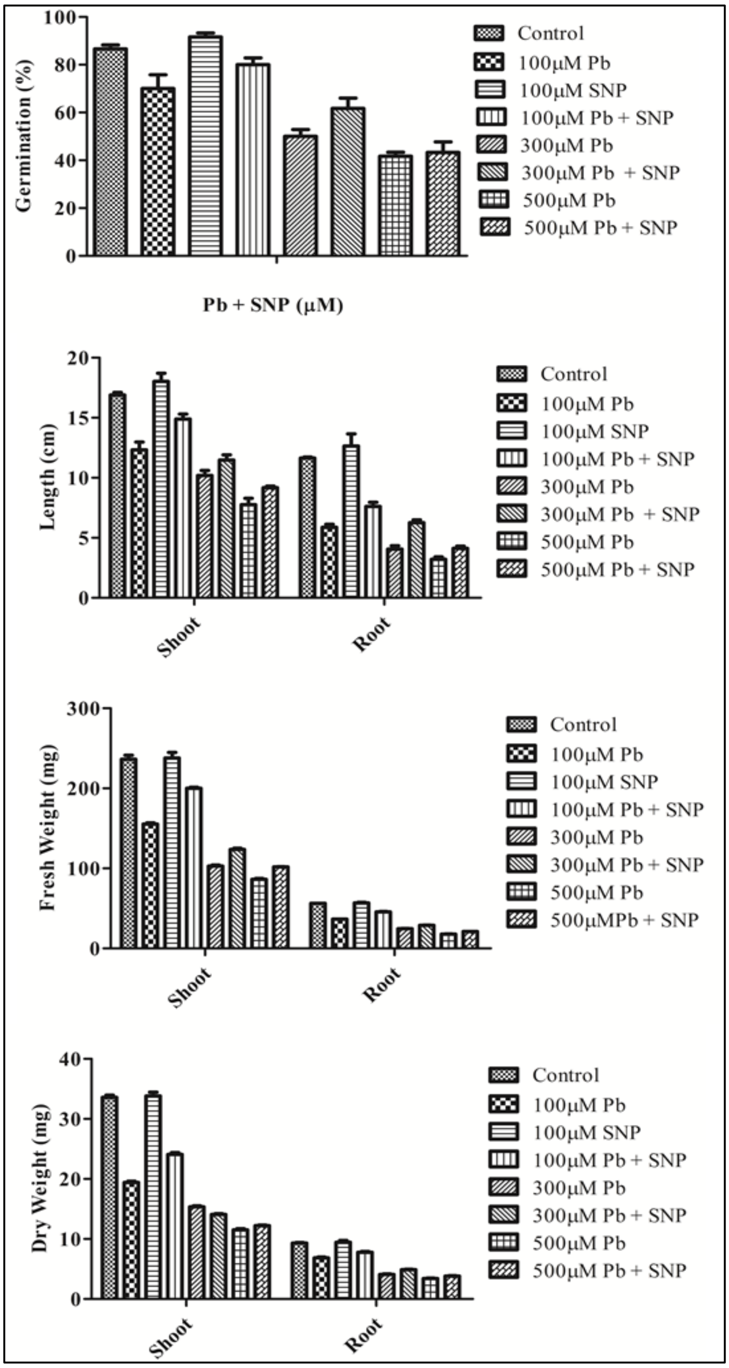

Fig 1: The impact of Sodium nitroprusside (SNP) on alterations induced by lead (Pb) exposure was examined in terms of germination, shoot and root length, as well as the fresh and dry weight of wheat seedlings. The provided data presents the average values (±SE) obtained after each treatment in three biological replications. Notably, all recorded values exhibited significant distinctions at a significance level of $p \leq 0.05$, as confirmed through post hoc Tukey's test.

Biochemical Parameters

Chlorophyll 'a'content: Previous research has documented that wheat subjected to lead stress experiences diminished photosynthesis and compromised plant growth. Notably, plants exposed to lead exhibited a notable reduction in their chlorophyll a content. In 100, 300and 500 µM Pb stressed plants; the chlorophyll a, content was reduced by 30.32%, 48.37% and 69.67% respectively (Fig.2). Supplementation of 100 µM SNP along with 100 µM Pb increased chlprophyll a, content by 45.33% compare to 100 µM Pb treatment whereas supplementation with 300 µM SNP with 300 µM Pb increased by 32.51% compare to 300 µM Pb treatment. Exogenous application of 500 SNP along with 500 µM Pb enhances chlorophyll a by 20.83% relative to 500 µM Pb treatment.

Chlorophyll 'b' content
Existing literature has already highlighted that wheat cultivated under the influence of lead stress experiences a decline in both photosynthetic activity and overall plant growth. Furthermore, an observable reduction in the content of Chlorophyll b has been documented in plants subjected to lead treatment. Application of 100, 300 and 500 µM Pb stressed plants; the chlorophyll b, content was reduced by 9.01%, 53.52% and 61.21% respectively (Fig.2). Supplementation of 100 µM SNP along with 100 µM Pb increased chlprophyll b, content by 18.88% compare to 100 µM Pb treatment whereas supplementation with 300 µM SNP with 300 µM Pb increased by 16.36% compare to 300 µM Pb treatment. Exogenous application of 500 SNP along with 500 µM Pb enhances chlorophyll b by 10.94% relative to 500 µM Pb treatment.

Total chlorophyll content
There was a significant drop were observed in total chlorophyll amount in Pb treated plants. In 100, 300 and 500 µM Pb stressed plants; the total chlorophyll content was reduced by 22.08%, 50.32% and 66.37% respectively (Fig.2). Supplementation of 100 µM SNP along with 100 µM Pb increased total chlprophyll content by 33.42% compare to 100 µM Pb treatment whereas supplementation with 300 µM SNP with 300 µM Pb increased by 26.32% compare to 300 µM Pb treatment. Exogenous application of 500 SNP with 500 µM Pb enhances total chlorophyll b by 16.33% relative to 500 µM Pb treatment.

Carotenoid content
Application of 100µM, 300 µM and 500 µM Pb treated plants; the carotenoid content was reduced by 44.59%, 60.62% and 77.70% respectively (Fig.2). Supplementation of 100 µM SNP with 100 µM Pb increased carotenoid content by 28.93.% compare to 100 µM Pb treatment whereas supplementation with 300 µM SNP with 300 µM Pb increased by 24.77% compare to 300 µM Pb treatment. Exogenous application of 500 µM SNP along with 500 µM Pb enhances carotenoid content by 17.18% relative to 500 µM Pb treatment.

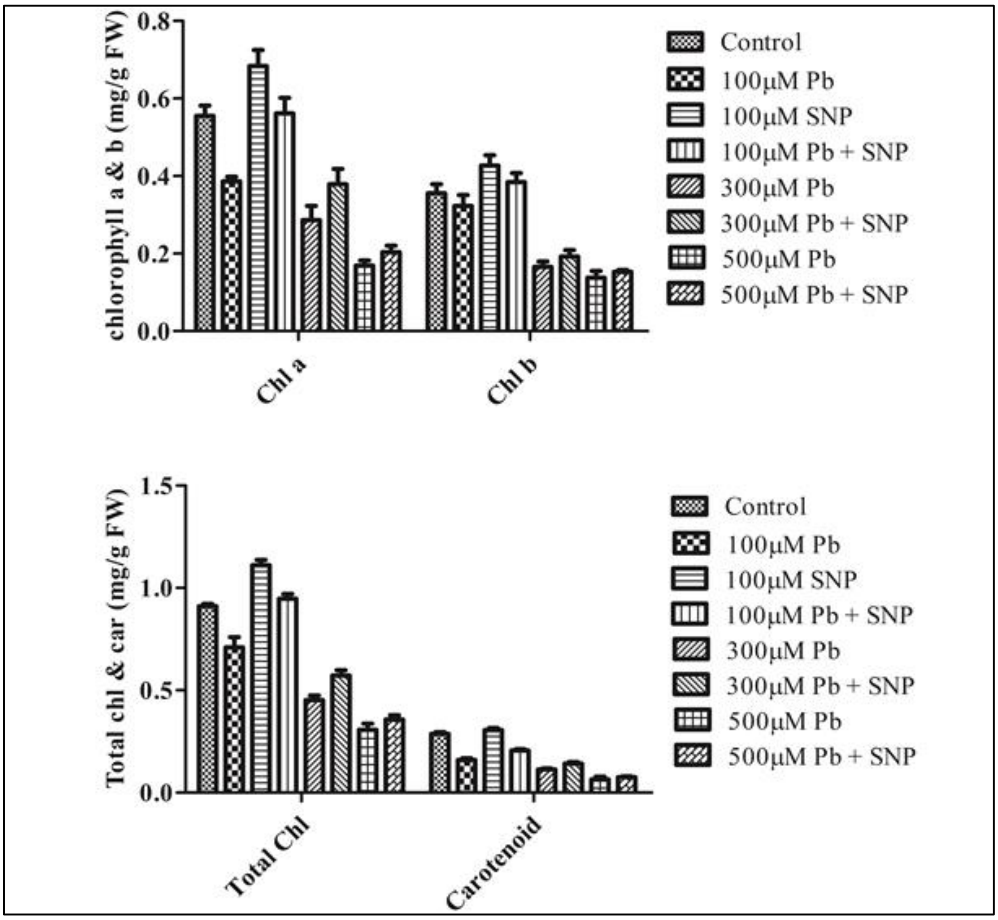

Fig 2: Effects of Sodium nitroprusside (SNP) on the Pb-induced changes in, photosynthetic pigments (Chlorophyll a, Chlorophyll b, Total chlorophyll and Carotenoids) wheat seedlings after exposure to Pb. Data represent the mean (±SE) was calculated after each treatment in three biological replications. All values are significantly different at $p \leq 0.05$ applying post hoc Tukey's test.

Discussion
In this study, we have provided evidence of lead (Pb) toxicity and the role of externally applied nitric oxide (NO) in mitigating the adverse effects of Pb stress on wheat seedlings. Similar findings were also reported by Nayyar *et al.* (2005) [9], who observed a reduction in root length of *Cicer arietinum* under water stress. This reduction in root length could be attributed to factors such as decreased root-hair diameter and distortion, and a decrease in plasmolysis percentages due to Pb exposure. The exposure to different concentrations of Pb led to reduced shoot and root lengths. Comparable results were noted by Nayyar *et al.* (2005) and Khan *et al.* (2017) [9, 16], specifically in root length of *Cicer arietinum* under water stress and pea seedlings under cold stress. The detrimental impacts were particularly pronounced at higher Pb concentrations as shown in Fig. 1. Inhibition of germination

percentage, as well as reduced shoot and root lengths, has been reported in various plants by other researchers (Lamhamdi *et al.*, 2011) [6]. Interestingly, the introduction of exogenous sodium nitroprusside (SNP) into Pb-treated nutrient solutions demonstrated a mitigating effect on Pb-induced stress in terms of germination, shoot, and root lengths. Exogenous SNP application has previously been identified as providing protection against Pb toxicity in wheat (Kaur *et al.* 2015; Nasibi and Kalantari, 2009; Zhao *et al.* 2011) [4, 8, 15]. The fresh and dry weights of plants experienced reductions due to Pb exposure, with the extent of reduction being more pronounced at higher concentrations. Treatment with SNP up to 100 µM resulted in increased weights, which then decreased beyond that point. Remarkably, the co-application of 100 µM SNP with Pb concentrations of 100, 300 and 500 µM resulted in increased fresh weight of both shoot and root. A common manifestation of Pb toxicity is leaf chlorosis. Photosynthetic pigments like total chlorophyll and carotenoids are valuable indicators for assessing lead toxicity in plants. In this study, a significant decrease in chlorophyll pigments was observed in Pb-treated plants. However, the application of 100 µM SNP effectively reversed the detrimental effects of Pb treatment. Notably, the total chlorophyll and carotenoid content increased at the 100 µM SNP treatment level, compared to the control. The same observations were made by Dong *et al.* (2014) [1] in peanut seedlings under cadmium stress, as well as by Yang *et al.* (2011) [14] in Chrysanthemum and Tan *et al.* (2008) [10] in wheat seedlings.

Conclusion

The findings from both the previous study by Kaur *et al.* (2015) [4] and the current investigation shed light on the potential mechanisms underlying the alleviation of lead (Pb) toxicity through the influence of nitric oxide (NO). The application of sodium nitroprusside (SNP) demonstrated the ability to decrease Pb uptake, enhance chelation processes, and mitigate oxidative stress in seedlings exposed to Pb stress. While SNP doesn't directly scavenge reactive oxygen species (ROS), it effectively improves the antioxidant system. This enhanced tolerance to Pb stress in wheat plants was notably evident through improvements in germination, growth, and the content of photosynthetic pigments.

These results hold significant promise in the context of mitigating heavy metal contamination and enhancing wheat production. The insights gained from this study may contribute to strategies aimed at reducing the negative impacts of heavy metal exposure, ultimately leading to more sustainable agricultural practices and improved crop yields.

Reference

1. Dong Y, Xu L, Wang Q, Fan Z, Kong J, Bai X. Effects of exogenous nitric oxide on photosynthesis, antioxidative ability, and mineral element contents of perennial ryegrass under copper stress. Journal of plant interactions. 2014;9(1):402-411.
2. Dordas C, Hasinoff BB, Igamberdiev AU, Manac'h N, Rivoal J, Hill RD. Expression of a stress-induced hemoglobin affects NO levels produced by alfalfa root cultures under hypoxic stress. The Plant Journal. 2003;35(6):763-770.
3. Gratão PL, Polle A, Lea PJ, Azevedo RA. Making the life of heavy metal-stressed plants a little easier. Functional plant biology. 2005;32(6):481-494.
4. Kaur G, Singh HP, Batish DR, Mahajan P, Kohli RK, Rishi V. Exogenous nitric oxide (NO) interferes with lead (Pb)-induced toxicity by detoxifying reactive oxygen species in hydroponically grown wheat (*Triticum aestivum*) roots. PLoS One. 2015;10(9):e0138713.
5. Ahmed AA, El-Hag Ahmed MA, Abouhaddaf RM. Density functional investigation of the adsorption of nitric oxide on palladium clusters (PDN n=1-6). Int. J Adv. Chem. Res. 2020;2(1):05-08. DOI: 10.33545/26646781.2020.v2.i1a.15
6. Lamhamdi M, Bakrim A, Aarab A, Lafont R, Sayah F. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. Comptes rendus biologies. 2011;334(2):118-126.
7. Misra AN, Misra M, Singh R. Nitric oxide ameliorates stress responses in plants. Plant, Soil and Environment. 2011;57(3):95-100.
8. Nasibi F, Kalantari KM. Influence of nitric oxide in protection of tomato seedling against oxidative stress induced by osmotic stress. Acta physiologiae plantarum. 2009;31:1037-1044.
9. Nayyar H, Kaur S, Singh SKJ, Dhir KK, Bains T. Water stress-induced injury to reproductive phase in chickpea: Evaluation of stress sensitivity in wild and cultivated species in relation to abscisic acid and polyamines. Journal of Agronomy and Crop Science. 2005;191(6):450-457.
10. Tan J, Zhao H, Hong J, Han Y, Li H, Zhao W. Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. World J Agric. Sci. 2008;4(3):307-313.
11. Tian X, He M, Wang Z, Zhang J, Song Y, He Z, *et al*. Application of nitric oxide and calcium nitrate enhances tolerance of wheat seedlings to salt stress. Plant Growth Regulation. 2015;77:343-356.
12. Wellburn AR. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of plant physiology. 1994;(3):307-313.
13. Xu J, Wang W, Yin H, Liu X, Sun H, Mi Q. Exogenous nitric oxide improves antioxidative capacity and reduces auxin degradation in roots of Medicago truncatula seedlings under cadmium stress. Plant and Soil. 2010;326:321-330.
14. Yang L, Wei DD, Chen Z, Wang JS, Kong LY. Reversal of multidrug resistance in human breast cancer cells by *Curcuma wenyujin* and *Chrysanthemum indicum*. Phytomedicine. 2011;18(8-9):710-718.
15. Zhao L, Sun YL, Cui SX, Chen M, Yang HM, Liu HM, *et al*. Cd-induced changes in leaf proteome of the hyperaccumulator plant *Phytolacca americana*. Chemosphere. 2011;85(1):56-66.
16. Khan EA, Misra M, Sharma P, Misra AN. Effects of Exogenous Nitric Oxide on Protein, Proline, MDA contents and Antioxidative System in Pea Seedling (*Pisum sativum* L.). Research Journal of Pharmacy and Technology. 2017;10(9):3137-3142.