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NITRIC OXIDE ALLEVIATED THE NEGATIVE IMPACT OF THE WATER DEFICIT AND HYPOXIA IN THREE CROP PLANTS.

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Nitric oxide is a bioactive signaling molecule involved in plant responses to several environmental stresses. The present investigation was carriedant to evaluate the effectiveness of different application methods of nitric oxide (seed soaking, soil application and foliar spraying) at different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM) on growth attributes of three crop plants (faba bean, cowpea and maize) grown under oxygen deficit (200% field capacity (FC)) and water- deficit stresses (70 and 50% FC). The data merely depicted the potential role of sodium nitroprusside, as a source of nitric oxide, on curtailing the deteriorations of both stresses on the three tested plants where the magnitude of crop responsive ranked as faba bean, cowpea and maize, respectively. The studied crops come in agreement that soaking was the influential practical method in application of nitric oxide. Dose- dependent response was attenuated by different crops where generally 0.1 mM was the most effective dose modulating adverse impacts of hypoxia and water- deficit stresses of different plants.

Key words: nitric oxide, sodium nitroprusside, oxygen deficit**,** water deficit, hypoxia, cow pea, faba bean and maize.

INTRODUCTION

Since the discovery of Nitric oxide (NO) as late as 1998, the research of plant NO biology spurted (Delledonne *et al*., 1998). The gaseous free radical NO performed a topic of plentiful debate within the scientific community due to its strike participation in a number of sequences managing plant responses varying from seed germination to plant senescence (Kong *et al*., 2014). Many investigations clarified multifunctional roles of NO in various physiological processes as root and shoot development, flowering, plant maturation and senescence, stomata movement, plant–pathogen interactions and programmed cell death (Mur *et al*. 2013). Conclusive evidence proved NO to be involved in many plant metabolic processes, such as mitochondrial (Zottini*et al*. 2002) and chloroplastic functionality, gravitropism and floral regulation (He *et al*. 2004). In addition, the cytoprotective role for NO in plants has clearly been tested with DNA, lipids, proteins, and chlorophyll (Lamattina et al. 2003). Nitric oxide may also affect biosynthesis, catabolism/ conjugation, transport, perception, and/or transduction of almost all the phytohormones, i.e. auxins, gibberellins, cytokinins, abscisic acid, ethylene, salicylic acid, jasmonates, and brassinosteroids (Freschi, 2013). Such NO- mediated plant responses advocated some researchers to coin NO as a plant growth regulator or non-traditional plant hormone (Wendehenne *et al*., 2004). However, NO is a reactive nitrogen species, and many studies have demonstrated that its effects on different cells are either protective or toxic, depending on its concentration and the position of action (Lamattina *et al*. 2003). Sodium nitroprusside (SNP) is often used as a NO donor in many studies. Its chemical reactions are mainly associated with the NO ligand (Coppens *et al*., 2002). Nonetheless, NO is soundly reported in mediating multiple responses to various abiotic and biotic stresses in plants (Xiong*et al*. 2009). NO had a protective effect in response to heavy metal stress, UV radiation stress (Shi *et al*., 2005) heat stress (Song et al,.2013) salinity (Siddiqui *et al*. 2017) and water-deficit (Silveira *et al*.2016).

Deficit-irrigation in agricultural soils elevated globally which is a potential threat to crop productivity and constrains a critical challenge to satisfy the ever-growing world population. Moreover, drought is a yield-limiting factor even if its deteriorations are not visible (Teimouri *et al*. 2014). Therefore, it is crucially important to figure out methods for increasing crops growth under harsh conditions.

Flooding, another abiotic stress, profoundly affects a wide range of ecosystems, from river forelands to farmlands, and it is one of the main causes of natural disasters worldwide. Moreover, as a consequence of climate change, the frequencies and intensities of floods are expected to increase in the future. Flooding is detrimental to many terrestrial plants as it generally hampers their growth and may ultimately cause their death (van Bodegom *et al*. 2008). The negative impact of flooding on plant growth and development may be ascribed to slow diffusion rates of gases in water compared to air and relatively low solubility of oxygen in water (Fan *et al*., 2014).

In the present study, since NO-mediated many physiological processes for different stresses as hormonal signaling, we investigated the incidence of exogenous NO supplied in different application methods at different concentrations on oxygen- and water- deficiency of three crop plants and searched a proper SNP concentration to alleviate these stresses.

MATERIALS AND METHODS:-

Experimental design:

Experiments were conducted at the greenhouse of Botany and Microbiology Department, Faculty of Science, Assiut University. Seeds of faba bean (cv. Giza 843); cowpea (cv. Caream 7) and maize (cv. Giza 186) were soaked in different concentrations of NO donor (SNP) (0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM) for 8 hours. Soaked and non- soaked seeds were sown at a depth of 1.5 cm in plastic pots containing 3 Kg clay soil and non-soaked seeds were divided into three groups (reference group, NO- soil supplemented group and NO- sprayed group). All pots of each group were irrigated with tap water around field capacity until the appearance of two true leaves (10 days), then each group was sub-divided into four treatments; pots irrigated around field capacity (100% FC), water-deficit was applied by decreasing water availabilities to 70 and 50% FC and oxygen deficiency was imposed by increasing irrigation water to 200% FC. The pots were weighed daily and watered to restore the appropriate moisture by adding the calculated amount of water and the pots were left for 1week (4 pots/treatment) and then the following applications have been done.
NO- soil supplemented group of different SNP concentrations (0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM) were added to the soil for each treatment.

NO- sprayed group: each pots of the last treatments were sprayed by nitric oxide donor (SNP) at different concentrations (0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM) each plant sprayed with 5ml of NO donor.

Reference and NO- soaked groups were irrigated with the corresponding water level with tap water without any further addition of NO.

The four plant groups were let grow for further three weeks when they were harvested and the following assessments have been conducted.

Growth: Lengths and fresh weights of roots and shoots of harvested plants were estimated then oven drying at 70°C for 72 hr to determine their dry weight (DW).

Leaves area: The leaf area of maize plants were determined by the equation
Leaf area= k (leaf length x leaf maximum width), where, K= 0.75 for maize (Norman and Campbell, 1989).
The leaflet area of broad bean plants was determined by using the linear measurements such as lamina length (L) and width (W) by (Peksen, 2007). The proposed leaf area LA estimation model is (LA = 0.919 + 0.682LW).
Leaf area measurement of cowpea achieved was by tracing the leaves on a graph paper and the total area per plant was obtained by measuring the maximum leaf length and breadth was multiplied by the correction factor 0.75 following the formula of Agbogidi and Ofuoku, (2005)

Photosynthetic pigments: The fractions of pigments (total chlorophyll and carotenoids) were estimated using the method recommended by Lichtenthaler (1987). 0.05 g fresh leaves sample was suspended in 5 ml of 95% ethyl alcohol and heated at 60-70°C in water bath, until being colorless. The total volume of extract was completed to 10 ml with 95% ethyl alcohol and absorbance was read using a spectrophotometer (Unico UV-2100 spectrophotometer) at wavelengths of 452, 644 and 663 nm against blank of pure 95% ethyl alcohol. Chlorophylls and carotenoids concentrations were calculated as mg/g FW.

Leaf area ratio (LAR)= Leaf area/ Total dry weight cm^2 g^{-1} (Evans 1972).

SI= Water content/ Leaf area.

Statistical analysis:

The data were subjected to two-way ANOVA of each treatment using the spss 17. Software program. Means and standard errors were calculated for 4 replicates. Means were compared by Duncan´s multiple range test and statistical significant was determined at 1% level.

Experimental results:-

The results clearly indicated the various responses of different organs not only to drought and hypoxia but also to the concentrations and application methods of SNP supplementation; such variations are species- dependent responses.

As illustrated in Fig(1), the shoot and root dry matter of maize (cv. Giza186) retarded significantly with increasing or decreasing water supply where the highest reduction was attenuated for 50% FC, the lowest for 70% FC and 200% was intermediate. The reduction in dry weight of shoot and root was to a large extent similar.

NO-soil supplemented maize plants induced small effect on well-watered and water-deficit treated plants with increasing percentage in average, 10% compared to non- treated plants. Whilst oxygen deficiency- treated plants recorded highest responses with percent increase of 28% at the level of 0.1 mM SNP. The optimal dose stimulated the highest increment of shoot and root dry weight was 0.1 mM for all studied stressors. In general highest concentrations of SNP manifested non- significant change in the dry matter acquisition of roots and shoots. In well-watered sprayed maize plants, 0.1 mM recorded the best results of growth enhancement. In water- deficit sprayed plants, as the concentration of NO increased the alleviating potential of NO was promoted where the maximal root and shoot growth were recorded at 0.5 mM SNP, but more so for roots and 70% FC compared to 50% FC and shoots. The opposite trend recorded for hypoxia- sprayed plants where maximal shoot and roots dry weight was attained at 0.05 mM SNP and the lowest response was recorded at 0.5 mM SNP. For soaked maize plants, the level of 0.1 mM was the most favorable dose restrained shoot and root dry weight under different water availabilities. Notably, soaking and to a large extent spraying were the best methods mitigating adverse effects of oxygen- and water- deficiency, whereas the lowest influence was for soil application.

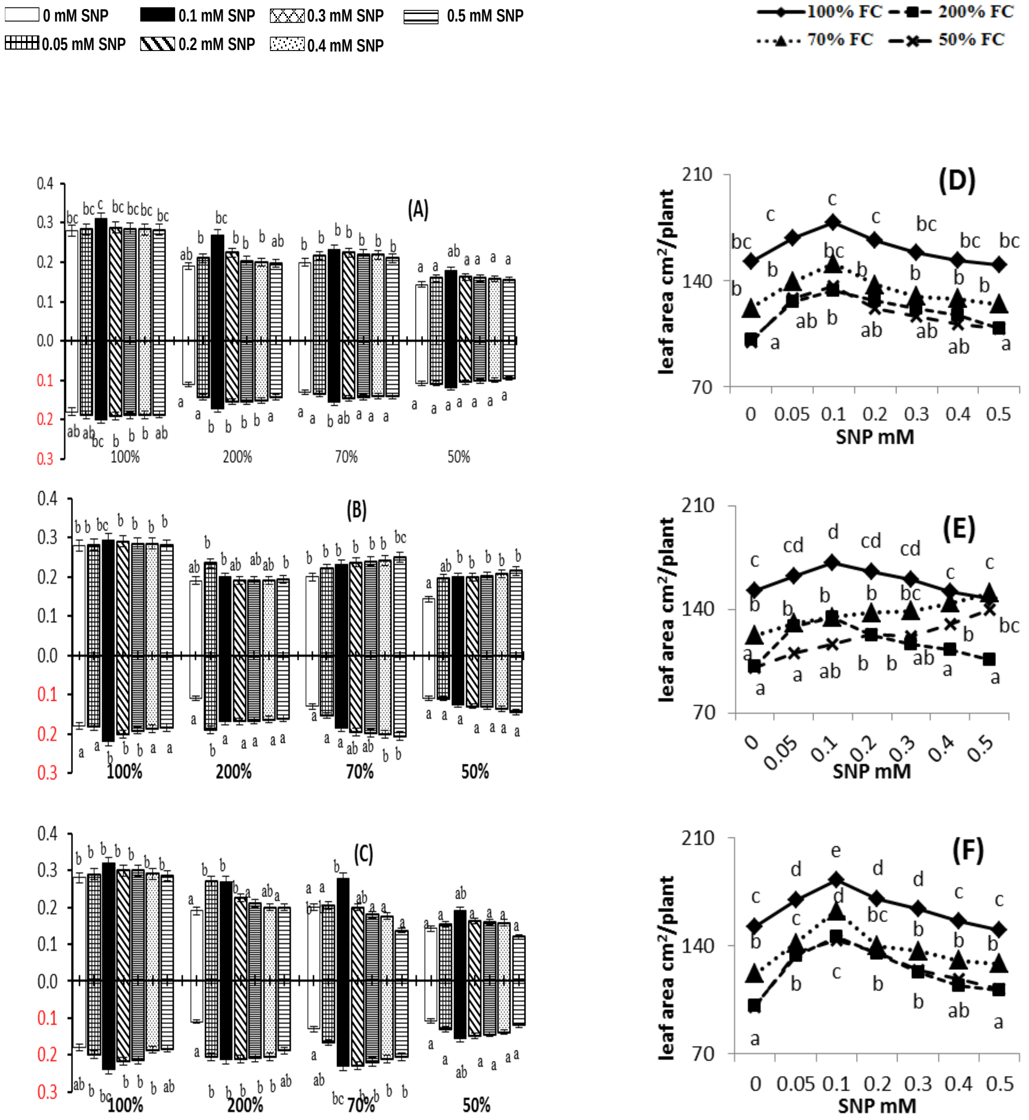
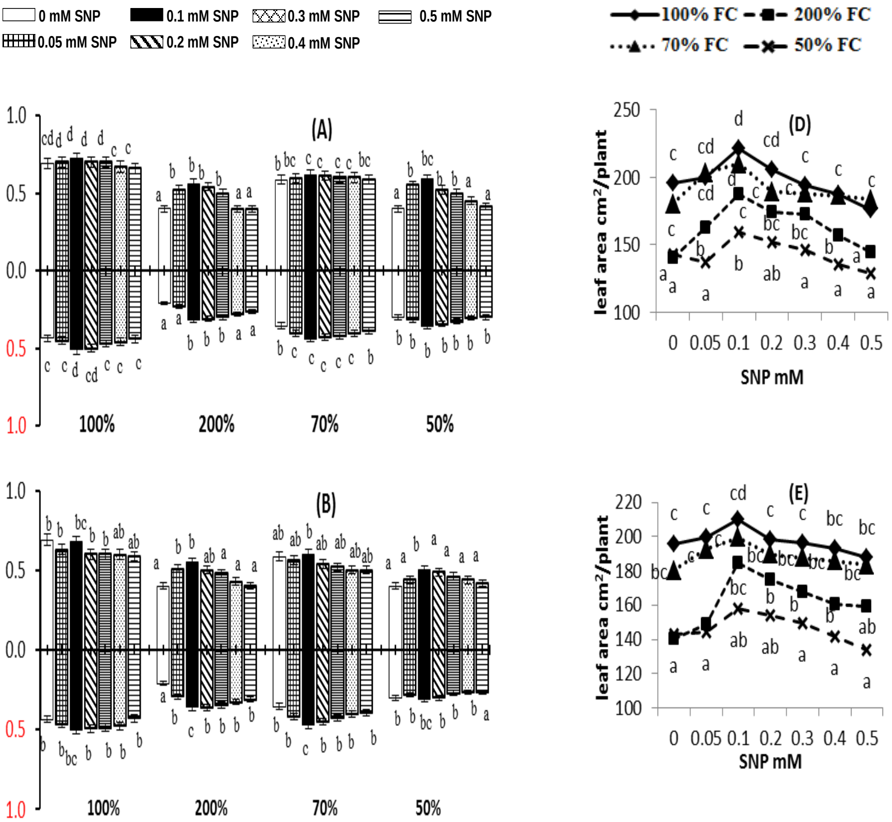


Fig (1) Shoot and root dry weight (A, B & C) as well as total leaves area (D, E & F) of maize plant grown under hypoxia (200% FC) and two levels of water-deficit (70 and 50% FC) exposed to different concentrations of sodium nitroprusside applied in (A) soil, (B) spraying and (C,) soaking method. Each value represents a mean value of three replicates and the vertical bars indicate ± SE.

In cowpea plants (cv.Caream 7), the dry weight of shoot and root were affected negatively with low irrigation regime as well as oxygen deficiency where the percent reduction of shoot and root compared to control plants was 51 and 63% for 200% FC, 23 and 22% for 70% FC as well as 42 and 50% for 50% FC, respectively. As illustrated in Fig (2), different SNP concentrations and application methods greatly improved the dry matter yield under water shortage and oxygen deficiency conditions, while amongst the studied doses 0.1 mM SNP was the most effective concentration given the best growth of shoot and root. Again soaking and spraying were the powerful application methods of SNP on growth enhancement.



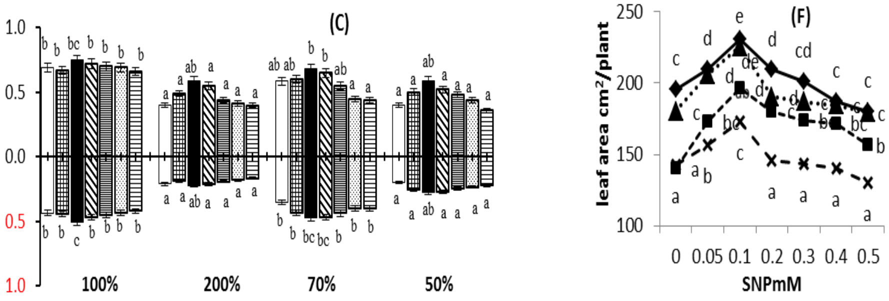


Fig (2) Shoot and root dry weight (A, B & C) as well as total leaves area (D, E & F) of cow pea plant grown under hypoxia (200% FC) and two levels of water-deficit (70 and 50% FC) exposed to different concentration of sodium nitroprusside applied in (A) soil, (B) spraying and (C) soaking method. Each histogram represents a mean value of three replicates and the vertical bars indicate ± SE.

The data in Fig (3) also discriminated that faba bean (cv. Giza, 843) under water scarcity and oxygen deficiency hampered the growth of shoot and root by about 42 and 24% for 200% FC, 11 and 12% for 70% FC as well as 42 and 37% for 50% FC in relation to their control. SNP application to droughted and water- logged faba bean plants restrained the decline of shoot and root growth whatever the dose used with tendency to promote the growth of shoot and root highly significantly at 0.1 mM SNP. Optimally irrigated plants reacted biophysically to different concentrations of SNP where quite response was recorded at lower doses especially at 0.1 mM as well as higher doses depressed the shoot and root growth, but non-significantly. Seeds priming recorded the favorable application method for faba bean plants also, and then foliar application and the lowest response was recorded for soil application. Collectively, the data vastly declared that faba bean was the most tolerant studied plant followed by maize while cowpea was the highly susceptible one and 50 % FC showed the highest damaging effect.

Regarding the data of total leaves area (Fig1,2&3), oxygen and water-deficit actuated inhibition of leaves area with percent reduction of 11, 4 and 16% for faba bean, 29, 8 and 27% for cowpea as well as 34, 20 and 34% for maize at 200, 70 and 50% FC, respectively in relation to control. SNP application by different methods lessened the reduction in assimilatory area by different magnitudes but in general 0.1 mM was the critical dose giving the highest results for the three crops except for SNP sprayed- drought treated maize plants where 0.5 mM was their optimal dose.

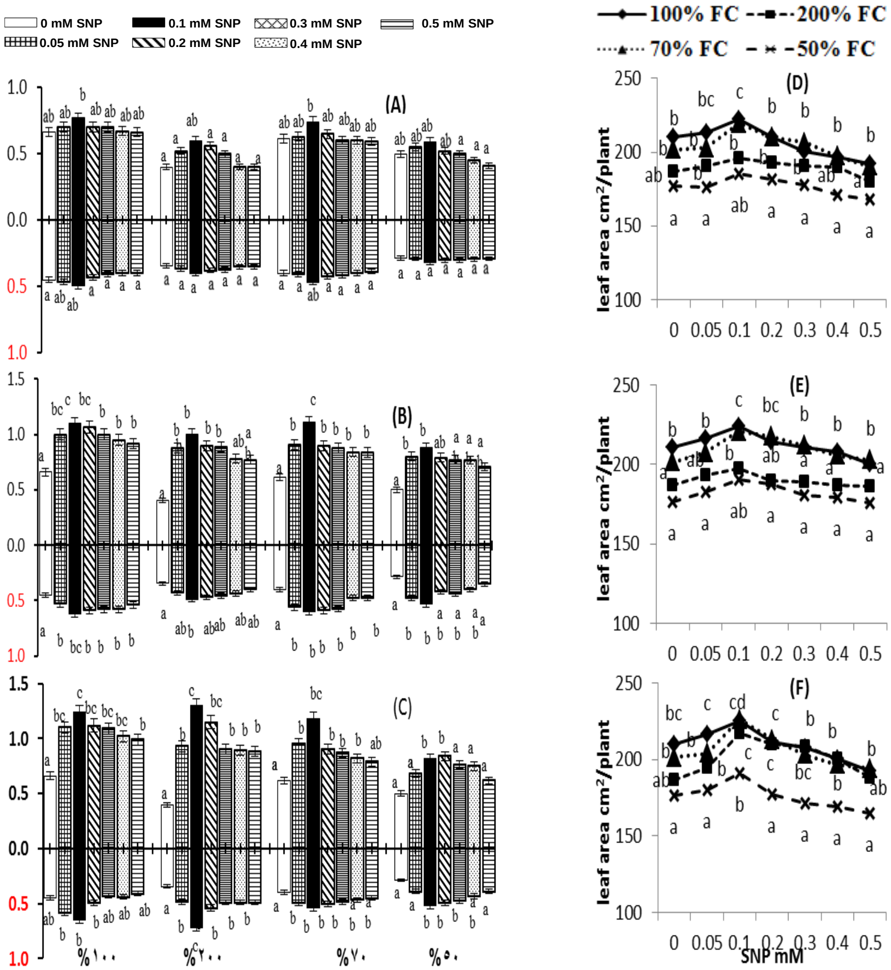
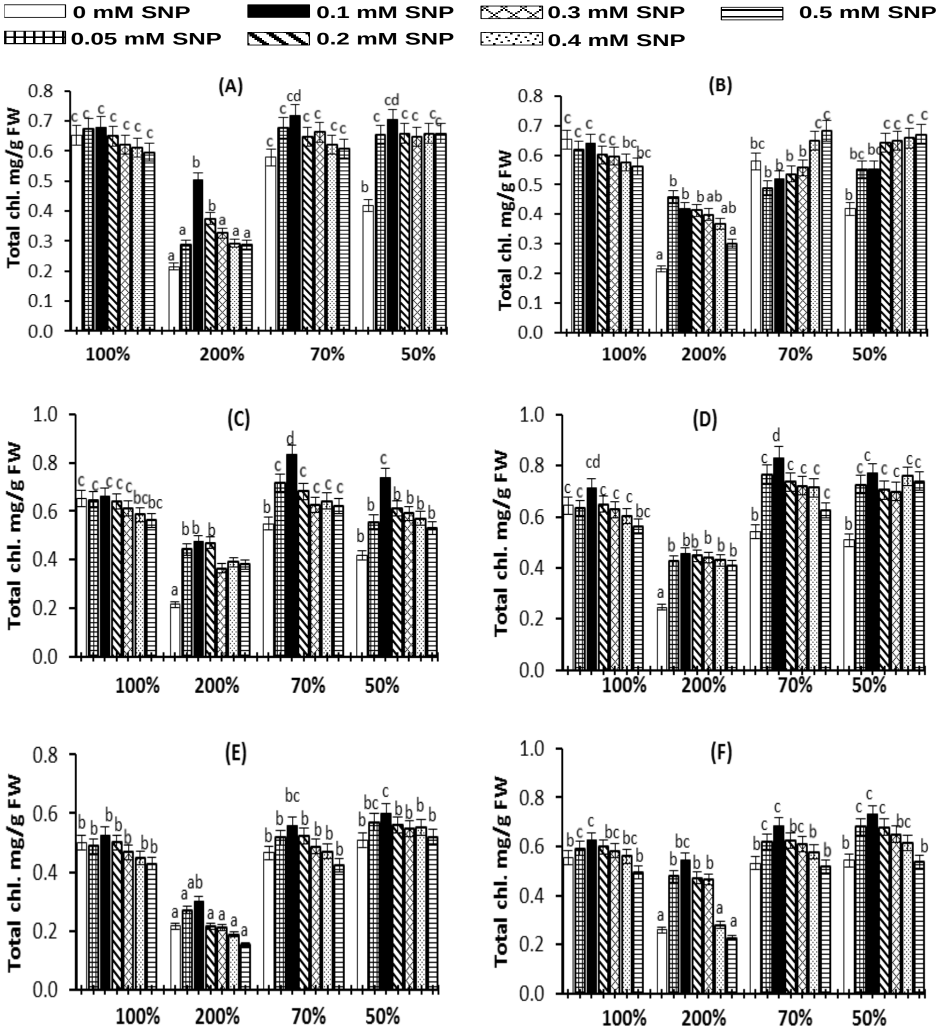


Fig (3) Shoot and root dry weight (A, B & C) as well as total leaves area (D, E & F) of faba bean plant grown under hypoxia (200% FC) and two levels of water-deficit (70 and 50% FC) exposed to different concentration of sodium nitroprusside applied in (A) soil, (B) spraying and (C,) soaking method. Each histogram represents a mean value of three replicates and the vertical bars indicate + SE.

Histograms represented at Fig (4) revealed that increasing oxygen- and water- deficit in growing substrate induced reduction of chlorophyll a and chlorophyll b, which was much more so at 200% FC and for chlorophyll b compared to chlorophyll a as well as maize relative to cowpea. Whilst faba bean showed increment of chlorophylls whatever the stress imposed.



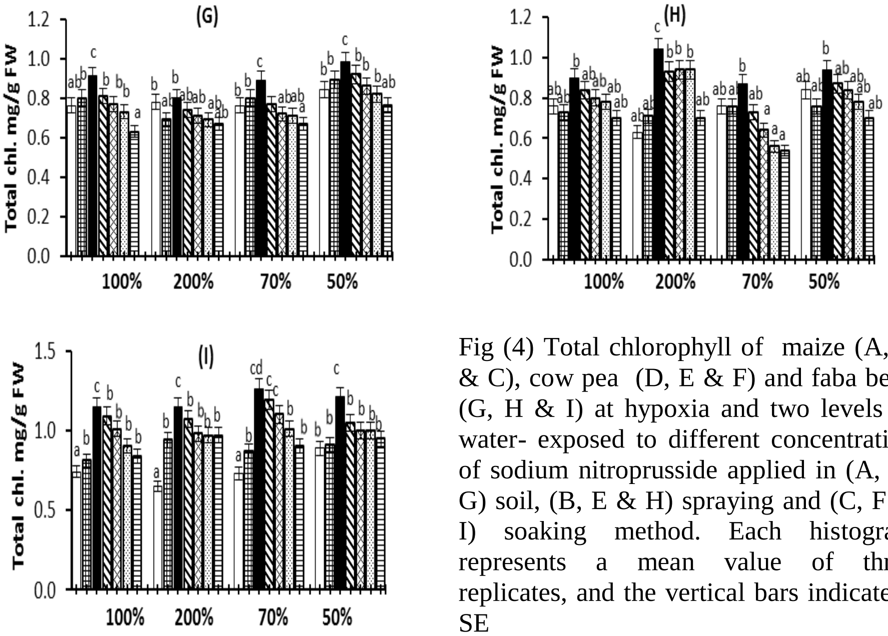
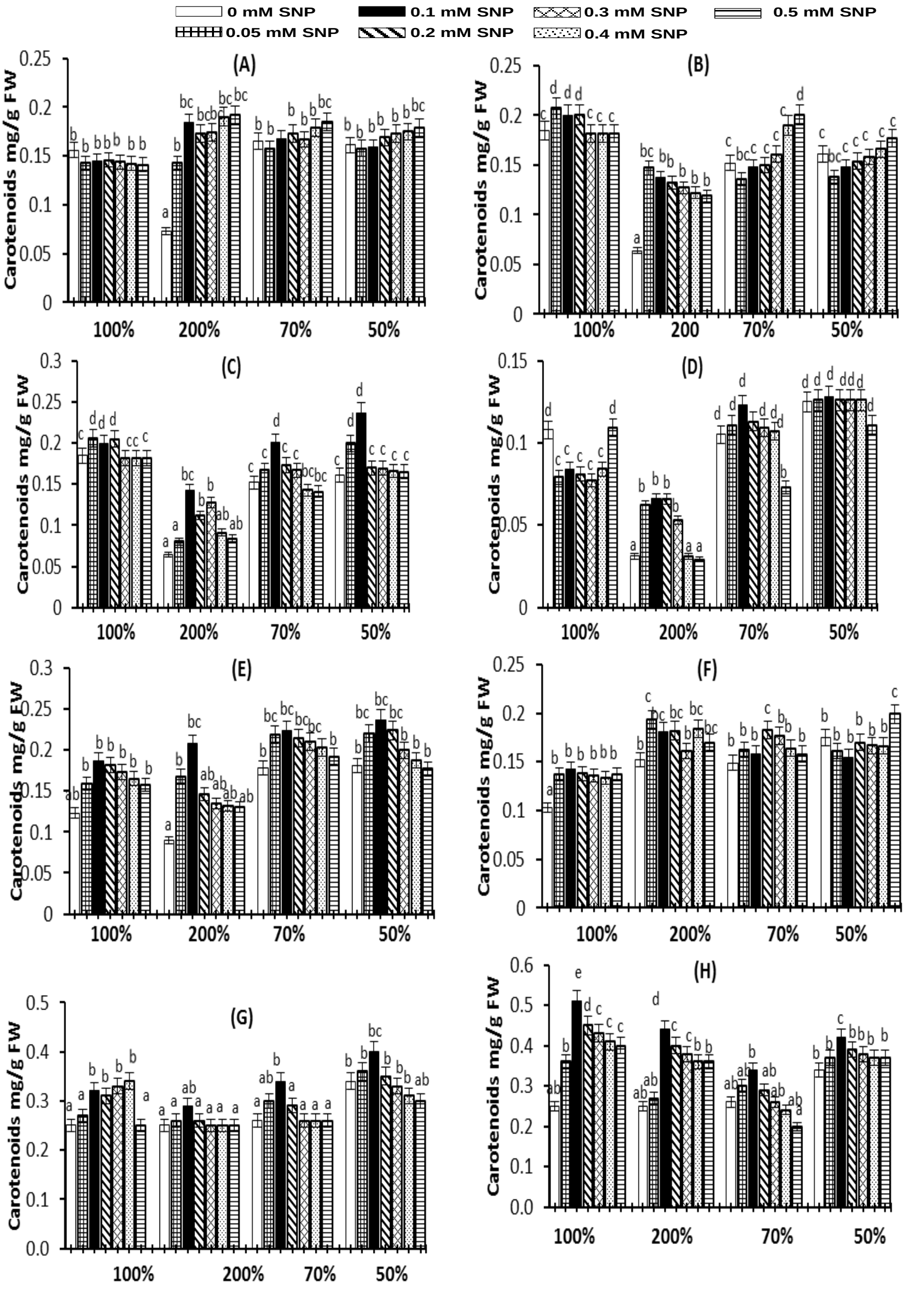


Fig (4) Total chlorophyll of maize (A, B & C), cow pea (D, E & F) and faba bean (G, H & I) at hypoxia and two levels of water- exposed to different concentration of sodium nitroprusside applied in (A, D, G) soil, (B, E & H) spraying and (C, F & I) soaking method. Each histogram represents a mean value of three replicates, and the vertical bars indicate ± SE

Carotenoids (Fig 5) mainly have not been altered by different irrigation availabilities compared with non- stress environments. In the present study, SNP treatment increased not only the morphological parameters, but also chlorophylls and carotenoids content as compared to control irrespective of concentrations of SNP especially the lowest concentrations. The data greatly reflected variation in tolerance of the studied plants; faba bean was the most tolerant plant, followed by cowpea and maize as the hypersensitive ones. Soaking in general was the effective method for application of SNP compared to the other methods and faba bean was the upmost responded plant to SNP.

The data represented in Fig (6), displayed that water deficit and flooding reduced succulence index according to the following order 200%>50%>70% compared to control for maize and cowpea. On the other hand succulence index (SI) has not been affected by water deficit in faba bean, but SI reduced significantly for water logging treatment. SNP was effective in increasing SI for the three tested plants, but the lowest response was for faba bean.


0 mM SNP
0.05 mM SNP
0.1 mM SNP
0.2 mM SNP
0.3 mM SNP
0.4 mM SNP
0.5 mM SNP
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
Carotenoids mg/g FW
100%
200%
200
70%
50%
0
0.05
0.1
0.15
0.2
0.25
0.3
0.0
0.1
0.2
0.3
0.4
0.5
0.6

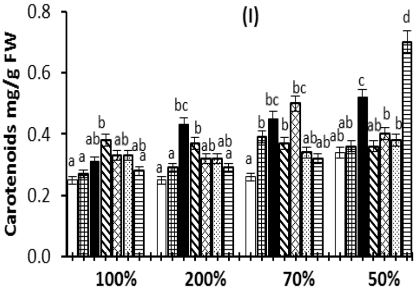


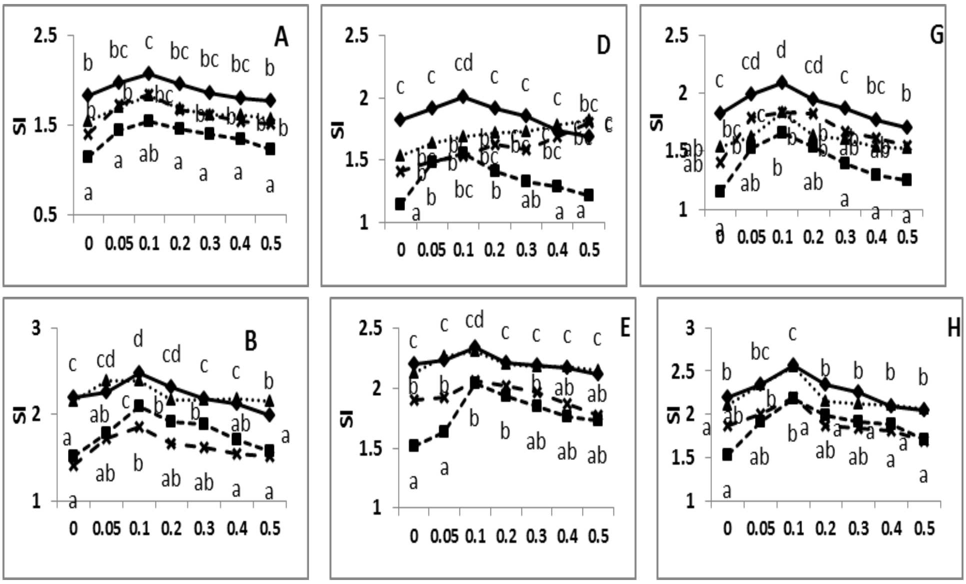
Fig (5) Carotenoids of maize (A, B & C), cow pea (D, E & F) and faba bean (G, H & I) at hypoxia and two levels of water- deficit exposed to different concentration of sodium nitroprusside applied in (A, D, G) soil, (B, E & H) spraying and (C, F & I) soaking method. Each histogram represents a mean value of three replicates, and the vertical bars indicate ± SE.

Leaf area ratio was represented in Fig (6), it denoted that LAR was elevated via water and oxygen deficiency where the upmost effect was for 50% FC compared to other water levels. SNP effectively reduced the values of LAR to be lower than the corresponding treatment, but faba bean recorded the highest reduction values of LAR under SNP treatment.

DISCUSSION

Plant growth is influenced by various internal and external environmentally stresses. Due to the raised indecision of extreme climate conditions, hypoxia has become striking limiting to crop production globally, which causes diminution of soil oxygen and performed diminishing crop plants growth. In addition to water scarcity, which is the detrimental factor for plant cultivation in arid and semi-arid regions. The three studied crop plants have been negatively influenced by both stresses, where they displayed distinct variation in their tolerance to drought and hypoxia stresses indicating that the crops were not uniformly susceptible to different water regime. The quantity and quality of plant growth depend on cell division, enlargement and differentiation and all of these events are affected by water stress (Kusaka *et al.*, 2005). This might be the reason for the reduced growth of plants under water deficit stress. When plants are flooded, the aerobic energy-generating systems were sharply reduced and the functional relationship between roots and shoots is disturbed as was reported by (Vartapetian and Jackson, (1997). Of the prospective roles of the applied SNP on the studied plants was not only recovered the inhibitory effect of drought and hypoxia on dry matter of shoot and root (for maize and cowpea) but also increased its values above that of non- stressed plants especially for the highly responded plant, faba bean. This was associated to the role of NO on the relaxation of the cell wall which acts on the phospholipids bilayer, increase the fluidness of the membrane, and induce cell enlargement and plant growth (Leshem and Haramaty, 1996). Also, NO-induced increase in plant growth parameters may be due to the role of NO in cell elongation, cell division and tissue differentiation (Fernández-Marcos et al., 2012). NO is also known to be involved in vegetative growth processes of the shoot (An et al., 2005) and xylem differentiation (Gabaldon et al., 2005). Also, the dry matter acquisition under the interactive effect of NO and water stress related to enhanced root growth where the activation of root organogenesis (Pagnussat et al. 2002), the adventitious roots proliferation (Pagnussat et al., 2003), lateral root development (Correa Aragunde et al., 2004) and root hair formation was induced by NO.

The analysis of leaf chlorophyll revealed that typical symptoms of flooding and water-deficit toxicity as yellowing and depletion on the leaves area were encountered merely for susceptible species (cowpea and maize) specially water- logged plants. On the other hand, faba bean showed increment of total chlorophylls which may be the main cause of its tolerance to both stresses. Drought caused cell shrinkage, reducing cellular volume which makes cellular contents more viscous. Consequently,



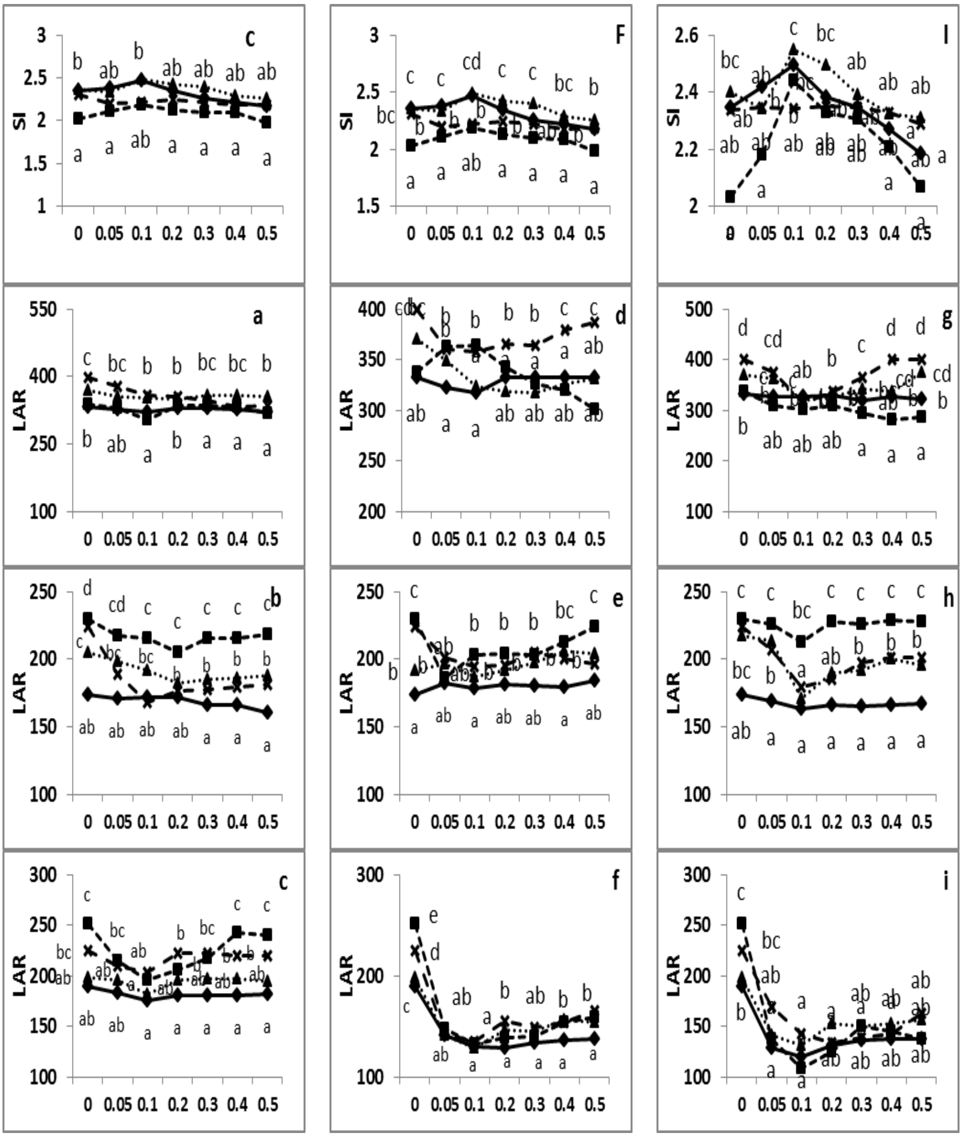


Fig (6) SI and LAR under interactive effect of different water levels (200, 100, 70&50 %FC)and sodium nitroprusside applied by soil, spraying and soaking method, respectively on maize (A, a, B,b&C,c), cow pea (D,d, E,e&F,f) and faba bean (G,g, H,h&I,i), respectively. Each line represents a mean value of three replicates and the vertical bars indicate + SE.

protien-protein interaction elevated leading to their aggregation and denaturation. Solutes accumulation increased viscosity of the cytoplasm that may become toxic and may be deleterious to the functioning of enzymes, including those of the photosynthetic machinery (Hoekstra et al., 2001). On the other hand the increase of pigments as in the case of faba bean was declared by Vahidi et al., (2013) who stated that leaf chlorophyll content increased in two sugar beet (Beta vulgaris L.) genotypes owing to the larger cells per unit leaf weight at water stress. Waterlogging sensitivity of the studied cultivars has been affirmatively related to photosynthetic depression which may be due to deficit production of ATP under O_2 deficiency which retarded root growth (Armstrong and Drew 2002), damaging of nutrient uptake and interception due to reduction of plasma membrane H^+-ATPase activity (Jackson et al. 2003) leading to limited nutrient transport to leaf tissues, therefore damaging of chlorophyll and photosynthesis was encountered (Meyer et al. 1987). The striking regulatory role of SNP was discriminated in restraining the damaging impacts of water-logging and drought on depletion of chlorophyll content especially plant grown under hypoxia. This may be due to attenuation of metal-induced impairment of the electron transport chain in photosynthesis by NO in plants (Yang et al. 2012; DONG Y. et al. 2017) as well as stimulation electron transport through photosystem (PS) II (Wodala et al. 2008). Additionally, NO stimulated a slow and continuous increase of the non-photochemical quenching of fluorescence, a well-known photo-protective mechanism (Ördög et al. 2013). Moreover, the assimilatory area was enhanced for SNP treated plants whatever the method of application or the water level imposed compared to control plants. Carotenoids are necessary for photo-protection of photosynthesis and they play an important role as a precursor in signaling during plant development under abiotic/biotic stress. Growth improvement in plants under stressful environment has been widely reported to be due to the significant role of zeaxanthin in alleviating oxidative damage of membranes (Isaksson and Andersson, 2008; Asma J. et al 2016). The studied plants merely conserved carotenoids content under water deficit treatments, but depleted for water-logged plants drawing the bad impacts of water-logging compared to deficit-irrigation. The protection role of NO was attributed partially to its activation of carotenoids especially for the highly NO- responded plant, faba bean revealing the photoprotection role of enhanced carotenoids under NO treatment in maintaining the photosynthetic apparatus more efficient than the non-treated stressed plants.

In the respect, 0.1mM SNP induced maximal stimulation of growth criteria at all the tested plants. Concentrations between 0.05–0.2mM SNP had been demonstrated to be effective in a number of biological systems (Garcia-Mata and Lamattina, 2001).The low tested concentrations of SNP (0.05, 0.1& 0.2 mM) were able to partially prevent growth inhibition of the three tested plants caused by hypoxia or water deficit, compared to the control. Higher SNP concentrations did not induce significant effect on growth (maize and cowpea), whereas faba bean growth decreased much more that imposed by hypoxia and water deficit without SNP. These results are agreement with Wang (2015) who recommended that low concentrations of NO can have ameliorative effect on plants, while high levels inhibited many plant processes. Soaking was the best application method of SNP that may give better adaptation and produce vigor plants from germination stage compared to soil application and foliar spraying. Also, the provided data revealed that highest leaves area and pigmentation was recorded under soaking treatments which confer higher photo assimilates to be allocated to both shoot and root, especially when we take into consideration that NO- application, irrespective to the application method, enhanced equally shoot and root growth.

LAR which is a morpho-physiological trait gives information about the efficiency of assimilatory area with regard to plant dry weight. Both water stressors increased LAR highly significantly, but much more so for water-logged plants, thus lighter leaves. This may be due to decline of leaf chlorophyll content (Koryo 2006) and smaller lamina surface of individual leaf and thus lighter leaves (Khalil et al. 2011). As a regulatory point of NO application on plants, decline of LAR was concomitant with increased photosynthetic pigments as well as leaf area. Furthermore, NO application promoted the efficiency of assimilatory area to produce higher dry matter, thereby LAR vastly decreased relative to the corresponding treatment, thus heavier leaves. This behavior was more ascertained for faba bean compared to cowpea and maize.

In the present investigation, water relations in terms of SI was negatively affected by different water levels supplemented, but the highest reduction was observed at flooding stress.The tolerant cultivar, faba bean, showed least water status disturbances where SI has not been affect for water- deficit stressed plants and slight reduction was encountered for oxygen-deficit plants. NO adjusted water status via increasing SI concomitant with its role in curtailing the reduction of roots growth that enabled the plants to maintain water status, there by sustaining water supply to shoots. Similar reduction of SI under water stress was advocated by Qi et al., (2009).

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خفف أكسيد النيتريك الأثر السلبي لنقص الماء ونقص الأكسجين في ثلاث محاصيل.

اكسيد النيتريك هو جزئ إشاره حيوى نشط بين الخلايا يعمل على استجابة النباتات تحت عديد من التأثيرات البيئية المختلفه. البحث الحالى يختبر مدي تأثير إضافة أكسيد النيتريك بطرق مختلفه مثل (نقع البذور، إضافة الى التربه ،بالرش على الأوراق)بتركيزات مختلفه وهى (٠.٠٥، ٠.١، ٠.٢، ٠.٣، ٠.٤، ٠.٥ مم) لنمو ثلاثة محاصيل نباتيه وهم (فول، لوبيا و ذرة) تحت تاثير نقص الأكسجين وهو بزيادة كمية الماء فى التربه(٢٠٠%)عن السعه الحقليه وكذلك نقص الماء (٧٠% و٥٠%) من السعه الحقلية. النتائج تصور الدور الفعلى لنيتروبروسيد الصوديوم، كمصدر لأكسيد النيتريك ،فى الحد من تدهور نمو النباتات الثلاثة تحت تأثير كلاً من (نقص الماء والأوكسجين)حيث تم ترتيب استجابة النباتات لها كالآتى الفول ثم اللوبيا ثم الذرة. إتفقت المحاصيل النباتية المختبره على إضافة أكسيد النيتريك بطريقة نقع البذور . التركيز النموذجى من الماده الذى عمل على الحد من تدهور نمو معظم النباتات تحت تأثير نقص الماء وكذلك الأكسجين وهو ٠.١ مم.