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Role of Soluble Silica in Reducing Oxidative Stress in *Trigonella Foenum - Graecum* (Methi) Grown Hydroponically in Sewage Water

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Abstract **– Use of sewage water for growing plants hydroponically can be an important way to utilize nutrients present in sewage. However presence of some components in sewage like heavy metals can cause oxidative stress in plants grown hydroponically. Silica which is not considered as an essential nutrient for plants is proving to be very helpful for the growth of plant by decreasing the oxidative stress caused due to different biotic and abiotic factors. In the present study, *Trigonella foenum-graecum* plants were grown hydroponically in sewage and sewage enriched with silica. Oxidative stress in terms of Malondialdehyde content, Peroxidase activity and Proline content was measured. The study indicates insignificant increase in MDA and proline, and insignificant decrease in peroxidase activity in leaves of *Trigonella foenum-graecum* grown hydroponically using sewage as compared to control. MDA content significantly decreased in silica added sewage. Peroxidase activity increased insignificantly and proline content increased significantly in *Trigonella foenum-graecum* plant grown in sewage enriched with silica. It can be concluded from the present study that enrichment of sewage with silica used to grow plants hydroponically can overcome the drawbacks caused due to undesirable components of sewage.**

Keywords **– Khan River, Malondialdehyde, Peroxidase, Proline.**

I. Introduction

Silicon has been observed as a toxicity alleviating agent in plants via retaining heavy metals in roots and inhibiting their translocation to the shoots [14]. Silica gets deposited in the apoplast of the roots and on the leaf surface, forming a barrier to the apoplastic flow of metallic ions and to transpiration flux [13]. Increase in activity of antioxidant enzymes, reduction in lipid peroxidation and decrease in level of Arsenic in rice plants was observed when Silica was added to the growth medium [13].

Therefore, the present study was carried out to find whether silica in soluble form when supplied along with sewage to grow plants hydroponically can reduce oxidative stress caused due to presence of undesirable materials in sewage. Oxidative stress parameters studied are Malondialdehyde (MDA), Peroxidase activity and Proline content.

II. Materials and Methods

Sewage Sample

For the present study, sewage water was collected from Khan river near Vaishnavdevi temple, Indore (M.P.). Hoagland media was used as control to grow plants hydroponically.

Experimental Set

Seeds of *Trigonella foenum-graecum* commonly known as methi (fenugreek) were sterilized with 0.1% solution of mercuric chloride for 5 minutes to remove microbes, followed with repeated washings with sterilized double distilled water. Seeds were then allowed to grow for 25days.

Soluble Silica

Soluble Silica in the form of product Agribooster was mixed in sewage water, according to the pH of the sewage, 6ml silica was added to one liter of sewage to maintain favorable neutral pH for plants to grow hydroponically.

Malondialdehyde - It was estimated using thiobarbituric acid reagent by the method of Heath and Packer (1968). [15]

Peroxidase Activity - It was determined according to method of Summer and Gjessing (1943) using o-dianisidine. [6]

Proline - Proline was estimated using acid ninhydrin reagent following method of Bates *et al*., (1973). [7]

Statistical analysis: Values are expressed as mean ± SD, P-value was calculated to test significant difference.

III. Observations

Table I: Sewage analysis before and after growing Trigonella foenum - graecum hydroponically

Parameter	Normal Range	Sewage analysis before growing plants	Sewage analysis after growing plants	Sewage with silica analysis after growing plants
Total hardness (mg/l)	10-1000	196	360	170
Calcium (mg/l)	5-200	92	180	150
Magnesium (mg/l)	5-100	104	180	20
Chloride (mg/l)	5-1000	129.95	189.94	169.94
Sulphate (mg/l)	1-40	97.90	65.28	77.86
B.O.D (mg/l)	1-2000	3.5	2.8	3.3
C.O.D (mg/l)	5-1000	135.52	55	41
Cadmium (ppm)	0	0.004	0	0.003

Table II: Oxidative stress parameters in *Trigonella foenum-graecum* grown hydroponically in sewage, silica added sewage and control

S. No.	Parameters	Control	Sewage	Silica added sewage	% Change
1.	MDA (mM/gm)	0.64 ± 0.15	0.69 ± 0.06 NS	0.38 ± 0.016*	a. 7.81 b. -40.6
2.	Peroxidase (Units/min/gm)	18.13 ± 1.40	17.46 ± 1.40 NS	19 ± 0.91 NS	a. -3.69 b. 4.79
3.	Proline (µM/gm)	0. 19 ± 0.017	0.22 ± 0.014 NS	0.23 ± 0.0069*	a. 15.78 b. 21.05

Control-Hoagland media, MDA - malondialdehyde, NS p-value>0.05 is not significant, * p-value<0.05 is significant as compared to control .a-signifies percent change in sewage compared with control, b-signifies percent change in silica added sewage compared with control.

NOTE - MDA level increased in sewage but decreased in silica added sewage. Peroxidase activity decreased in sewage but increased in silica added sewage. Proline content increased in both sewage and silica added sewage.

Table III: Effect of adding silica in sewage on oxidative stress parameters of *Trigonella foenum-graecum* grown hydroponically

S. No.	Parameters	Sewage	Silica added sewage	% Change
1.	MDA (mM/gm)	0.69 ± 0.06	0.38 ± 0.016*	-44.92
2.	Peroxidase (Units/min/gm)	17.46 ± 1.40	19 ± 0.91 NS	8.82
3.	Proline (µM/gm)	0.22 ± 0.014	0.23 ± 0.0069 NS	4.54

NS p-value >0.05 is not significant, * p-value <0.05 is significant as compared to sewage.

NOTE- MDA level was decreased in silica added sewage. Peroxidase activity and Proline content were increased in silica added sewage.

IV. Discussion

Rate of nutrient uptake from sewage was enhanced on addition of silica. Magnesium content, sulphate content, BOD and COD in silica added sewage after growing plants hydroponically decreased (Table I). This may be due to increased absorption of these components by *Trigonella foenum-graecum* which resulted in improved oxidative stress combating mechanism. However, increase in calcium and chloride amount may be due to dissolution of temporary hardness of sewage in presence of soluble silica. The decrease in total hardness in the presence of silica makes the uptake of nutrient easier from sewage for ***Trigonella foenum-graecum.*** Silica also prevent uptake of cadium through roots ,as observed in the present study,cadium is completey uptaken from sewage but when sewage is enriched with silica, cadium absorption is prevented(Table I)and still remains in detectable amount after growing ***Trigonella foenum-graecum*** hydroponically

Exposure of plants to heavy metals present in sewage results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the electron transport chain, or induction of lipid peroxidation. [10]

By decomposition of arachidonic acid and larger PUFAs, MDA is an end-product generated [5]. In the present study, MDA content an indicator of lipid peroxidation was insignificantly increased in *Trigonella foenum-graecum* plants grown hydroponically in sewage water as compared to control (table II). The results of present study are in line with Singh and Agarwal (2010) who showed that plants irrigated using waste water showed higher MDA concentration as compared to those irrigated using ground water.[3] As shown in table III, MDA level was significantly decreased in *Trigonella foenum- graecum* grown in soluble silica added sewage compared to sewage. This indicates that silica decreased the free radicals by enhancing the activity of antioxidative enzymes which were causing peroxidation of lipids present in membrane.

The application of silicon causes a decrease in H_2O_2 levels in leaf. This behavior can be associated to characteristic of this mineral to improve the capacity of plant defense against oxidative damages by increasing the activity of antioxidants enzyme. [8] Peroxidase is considered to be an important antioxidant enzyme which helps in removal of harmful free radicals.

The results of present study show that in *Trigonella foenum-graecum* peroxidase activity was increased insignificantly in silica added sewage (table II). Alberto *et al.,* (2013) showed that Silicon has been extensively researched in relation to the response of plants to biotic and abiotic stress, as an element triggering defense mechanisms which activate the antioxidant system.[3] Bafna et al (2014) showed sewage water significantly decreased activity of peroxidase enzyme in *Trigonella foenum-graecum.*.[1] Tale and Haddad (2011) observed decrease in activity of antioxidative enzymes under drought stress. However application of silica on such plants significantly enhanced the activities of SOD, CAT, APX and POD. [4]

Silica is believed to retain water inside the plants by forming a layer inside the cuticle of leaves of plants. This increased water availability helps plant to activate enzymes required to fight against harmful radicals generated by different stress conditions.

In the present study proline content in *Trigonella foenum-graecum* was found to increase in sewage as compared to control. Nikolic *et al.,* (2014) showed that the accumulation of proline was stimulated by cadmium in *Triticum aestivm* L. [11] Aghaz *et al* (2013) also showed that accumulation of proline was stimulated by cadmium in Dill (*Anethum graveolens*).[2] As shown in table III proline content was found to be increased in plants grown in silica added sewage as compared to sewage. Proline is considered as nontoxic compound which shows protective role under osmotic stress condition. [8] Some of the studies are in contrast of present findings and show decre--ased content of proline upon addition of silica under salt

stress in soybean plants [18].

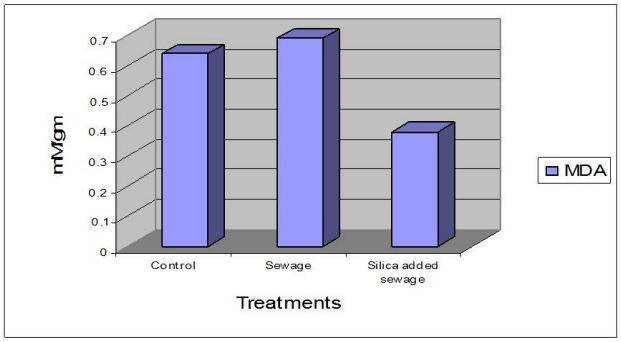

Figure no. 1: Effect of adding silica in sewage on MDA content of *Trigonella foenum-graecum* grown hydroponically

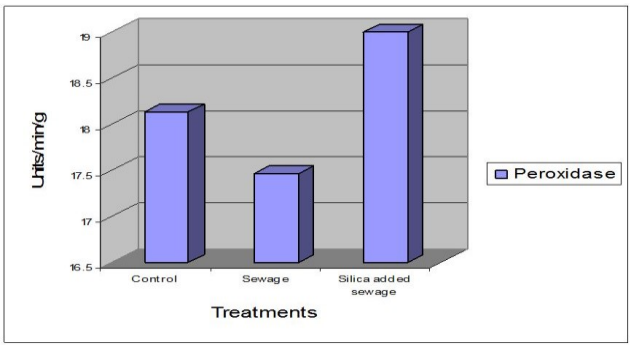

Figure no. 2: Effect of adding silica in sewage on peroxidase activity of *Trigonella foenum-graecum* grown hydroponically

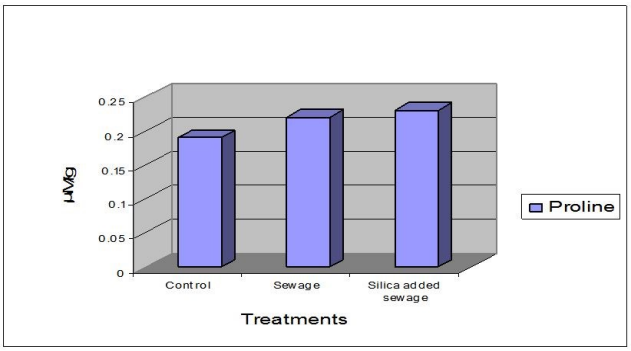

Figure no. 3: Effect of adding silica in sewage on proline content of *Trigonella foenum-graecum* grown hydroponically

V. Conclusion

From the present study it can be concluded that oxidative stress is induced in ***Trigonella foenum-graecum*** when grown hydroponically using sewage. As silica has ability to increase water retention in plant tissues, enrichment of sewage with silica may decrease harmful reactive oxygen species by raising availability of proton and by preventing cadium absorption. Thus Use of silica is suggested while growing plants hydroponically in sewage to prevent harm caused by sewage alone.

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