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Effect of sewage on Peroxidase activity, Carbohydrate, Protein and Iron content of Seedlings of *Trigonella foenumgraecum* (Methi)

Bafna Angurbala, Pathrol Manisha and Maheshwari Rameshwar
Department of Biochemistry, Govt. Holkar Science College, Indore MP, INDIA

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Abstract

Undesirable changes in atmosphere, hydrosphere and lithospshere occuring continuouly due pollutants. Nowadays surface water bodies are getting polluted due to discharge of large amount of untreated sewage into them,a common practice of disposal. Farmers are using this sewage polluted water to irrigate their vegetable fields in city conurbations. Such irrigation practices are common to solve the problem of water shortage. The present research was done to study whether sewage water imposes raised oxidative stress condition and affects nutrients contents of crops. The study was done to observe effect of 75%, 50%, 25% diluted and 0% (undiluted) sewage of Krishnapura nallah Indore on Peroxidase Enzyme activity (marker of Stress) ,Carbohydrate, Protein and Iron content of Seedlings of Trigonella foenumgraecum (Methi). Significant reduction in peroxidase activity was observed at 50% and 75% dilution of sewage when compared with undiluted sewage i.e.0 % dilution. Reductions were also significant at all dilutions when compared with Tap water. Carbohydrate content was found to be significantly decreased at all dilutions of sewage when compared with tap water and also with undiluted sewage. Protein content was found to be significantly decreased at all dilutions of sewage when compared with tap water and also with undiluted sewage . Significant reduction in Iron content was found at 75% dilution of sewage when compared with undiluted sewage. Significant reduction in iron content was also observed with untreated sewage when compared with tap water but insignificant with 50 % and 25 % dilution of sawage. From the results of present study it is concluded that undiluted and diluted sewage were not imposing raised oxidative stress.Reduction in protein ,carbohydrate,and iron content might be due to higher amount of organic and inorganic material specially toxic heavy metals in sewage which are adversely affecting the enzymses of metabolic pathway.

Keywords: Trigonella foenumgraecum seedlings, conurbations, peroxidase activity Iron content.

Introduction

It is common practice to use municipal and industrial waste water to irrigate vegetable crops[1]. Since there is deficiency of adequate water for irrigation municipal waste water can be utilized as an important source around the city and industrial complexes[2]. Sewage water is rich in nutrients and toxic heavy metals which may either effect crop yield substantially and reduce the need for fertilizer or may have toxic effects on enzymes activity. Continuous irrigation with sewage waste water may create some adverse effects such as soil sickness, soil and ground water contamination and phytotoxicity[3]. Therefore the study was carried out to understand the effect of Krishnapura nallah sewage of Indore city. Peroxidase Enzyme activity, Carbohydrate, Protein and Iron content of *Trigonella foenumgraecum* seedlings treated with the sewage were estimated and compared with tap water treated seedlings.

Material and Methods

The sewage of Krishnapura nallah Indore was used for the present study. 75%, 50%, 25% dilution of sewage and 0% (undiluted) were used to treat the seedlings in the study and Tap water as control. Surface sterilization of healthy seeds was done with 0.1% $HgCl_2$ for 5 min and then seeds were washed with tap water. The seeds were then allowed to germinate for 24 hrs. Healthy germinated seeds were then transferred in Petri plates lined with Whatman filter paper no. 1. At the start of the experiment 3 ml of respective concentrations were added to moisten filter paper in each Petri plates and after that every day, 2 ml of respective concentration was added for consecutive 6 days in case of *Trigonella foenumgraecum*. Three sets in each concentration were maintained along with the control using tap water for comparison. On the seventh day Peroxidase Enzyme activity, Carbohydrate, Protein and Iron content were evaluated.

peroxidase activity was measured according to Summer *et.al*.,[4]. The enzyme activity is assayed using o-dianisidine as hydrogen donor and H_2O_2 as electron acceptor. The rate of formation of yellow orange colored dianisidine dehydrogenation product is a measure of Peroxidase activity and can be assayed spectrophotometrically at 430 nm. Carbohydrate content was determined by the method as given J.E.Hedge *et al.,* (1962)[5]. nm. carbohydrates are dehydrated by concentrated H_2SO_4 to form furfural.Furural condenses with anthrone to form a blue-green coloured complex which is measured colorimetrically at 630 Protein content was estimated by using the method given by

E.Layne.,[6]. In the method peptide bonds of protein reacts with copper ions in alkaline solution and produce a purple color complex. Intensity of purple colored complex is measured at 520 nm colorimetrically. Iron was estimated according to the method given by R. Hill *et.al.,*[7]. The sample is wet washed using H_2SO_4 , HNO_3 and $HClO_4$. An aliquot of the extract is treated with thioglycollic acid and the colour intensity is read in a spectrophotometer at 535nm

Figure-1
Showing appearance of sewage water of Krishnapura nallah Indore

Table-1
Shows the effect of different dilution of sewage water of Krishnapura nallah Indore on peroxidase enzyme activity, Carbohydrate, Protein and Iron content of *Trigonella foenumgraecum* seedlings

S. No	Seedlings Treatment used	Peroxidase enzyme activity (Units/min/g)	Carbohydrate content (mg/100mg)	Protein content (mg/g)	Iron content (mg/100g)
1.	Tap water	75.66±5.34	0.66±0.057	136.66±42.5	0.49±0.44
2.	75% Diluted Sewage	65.5±15.5 (-13.4%) A^{**} B^{**}	0.26±0.05 (-60.6%) A^{**} B^{**}	132.5±8.66 (-3.04%) A^{**} B^{**}	0.21±0.09 (-57.14%) A^{**} B^{**}
3.	50% Diluted Sewage	65.5±0.5 (-13.4%) A^{**} B^{**}	0.46±0.15 (-30.30%) A^{**} B^{**}	117.5±28.39 (-14.02%) A^{**} B^{**}	0.44±0.19 (-10.20%) A^{ns} B^{**}
4.	25% Diluted Sewage	68.66±4.72 (-9.25%) A^{**} B^{**}	0.26±0.20 (-60.6%) A^{**} B^{**}	115.83±18.08 (-15.24%) A^{**} B^{**}	0.45±0.23 (-8.16%) A^{ns} B^{**}
5.	Undiluted Sewage	67.33±9.60 (-11%) A^{**}	0.5±0.26 (-24.2%) A^{ns}	123.33±20.81 (-9.75%) A^{**}	0.38±0.19 (-22.44%) A^{**}

Note: A, stands for comparison of sewage treatment with distilled water treatment, B stands for comparison of undiluted sewage with diluted sewage treatment, **= changes are very significant change ($p < 0.01$) and ^{ns} = changes are not significant ($p > 0.05$) Values in the brackets indicate the % decrease in the studied parameters of *Trigonella foenumgraecum* when sewage treatment is compared with distilled water.

Results and Discussion

Results: Peroxidase enzyme activity of seedling treated with tap water was 75.66±5.34 units /min/g and with 75%, 50%, 25% diluted and undiluted sewage was 65.6±15.5, 65.5±0.5, 68.66±4.72, 67.33±9.60 units/min/g respectively. Highest significant decrease in enzyme activity was observed with 75% and 50% (13.4%) diluted sewage. From these observations it was concluded that dilution of sewage results in reduction of oxidative stress.

Carbohydrate content in seedlings treated with tap water was 0.66±0.057 % mg/100 mg. Carbohydrate content with 75%, 50%, 25%, diluted and undiluted sewage treated seedling was found to be 0.26± 0.05, 0.46 ± 0.15, 0.26 ± 0.20 and 0.5 ±0.26 % mg/100 mg respectively. Carbohydrate content of treated seedling was significantly decreased and maximum decrease in carbohydrate content was found with 75% and 25% (60.6%) diluted sewage. These results indicate that dilution of sewage has no regular pattern of effect on carbohydrate content

Protein content in seedlings treated with tap water was 136.66±42.5 mg/g. At 75%, 50%, 25%, diluted and undiluted sewage treatment was found to be 132.5 ±8.66, 117.5± 28.39, 115.83 ± 18.08, and 123.33 ± 20.81 mg/g respectively. Significant decrease in protein content was observed in seedlings either compared with diluted sewage or with undiluted sewage.

Iron content in control was 0.49±0.44 mg/100g. At 75%, 50%, 25%, diluted and undiluted sewage treatment was found to be 0.21±0.09, 0.44±0.19, and 0.45±0.23 and 0.38±0.19 mg/100 g respectively. From observation of iron content it can be suggested that undiluted sewage can be used for irrigation.

Discussion: The present study result showed that in *Trigonella foenumgraecum* the activity of peroxidase was significantly decreased when seedlings were treated with sewage. Hence the sewage was not imposing raised oxidative stress in seedlings. The results of present study in case of *Trigonella foenumgraecum* were not in the agreement of findings of Singh *et al*[8] who concluded that peroxidase enzyme activity was increased in plants irrigated with wastewater as compared to those irrigated with ground water.

Total carbohydrate content of the seedlings of *Trigonella foenumgraecum* was decreased at all the level of dilutions of sewage treated seedlings as compared to the tap water. The results of present study were not in agreement of Bamniya *et al* (2010) [9] who reported increased carbohydrate content in crops irrigated with waste water than ground water.

The present study showed a decrease in the protein content of *Trigonella foenumgraecum* at all dilutions of sewage as compared to tap water. The result was in contradictory with the work done by Yagmur *et al* (2005) [10], who suggested that sewage sludge and mineral fertilizers treated plants had higher protein content than control seeds.

Iron content of the *Trigonella foenumgraecum* was significanly decreased in the sewage treated seedlings as compared to the tap water and dilution of sewage has no significant effect. These decreases were insignificant. The result of present study was in contradiction with the work done by Girisha *et al* (2009) [11], who observed higher iron that in K-134 variety of groundnut (Arachis hypogea. L) irrigared with municipal water compared to ground water.

Conclusion

From the study it was concluded that sewage of Krishnapura nallah Indore can be used for irrigation

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