Available online at http://www.journalijdr.com

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 3, Issue, 04, pp.018-020, April, 2013

Full Length Research Article

TOXICITY IMPACTS OF SEWAGE EFFLUENT ON THE AMYLASE ACTIVITY OF PIGEON PEA (*Cajanus cajan L.*) PLANT

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ARTICLE INFO

Article History:
Received 28th January, 2012
Received in revised form
08th February, 2013
Accepted 24th March, 2013
Published online 15th April, 2013

Key words:
Amylase,
Germination,
Sewage effluent,
Seedling growth.

ABSTRACT

Seed germination represents a crucial phase in the life cycle of angiosperms. Sewage is used for the irrigation of crops in field around most cities. Analysis of the sewage revealed presence of heavy metals which are known to suppress seed germination. Hence in the present investigation, the effect of sewage on the activities of total amylase, α-amylase and β-amylase in Pigeon pea plant has been studied. The activity of the enzymes was found to be significantly affected by higher concentration of sewage effluent due to unacceptable range of heavy metals in sewage effluent. The activity was found to be concentration dependent, activity being least in 100% sewage as compared to 50% sewage application. The study was found that the enzyme activity at each day was lower for the seedling treated with sewage effluent than control, activity and sugar production was found lowest for the highest concentration of sewage i.e. 100%.

INTRODUCTION

Over the last few decades due to rapid urbanization and industrialization coupled with an ever increasing population as resulted in production of huge amount of sewage. Treatment of sewage requires a large sum of money hence sewage is generally discharged without any treatment directly into the river, canal and agricultural field. Sewage serves as cheap source of plant nutrients. But Sewage water contains a large variety of wastes ranging from domestic to industrial; therefore, quality of such water is not suitable to irrigate any crop because of presence of many toxic chemicals (Furedy *et al*., 1999; Ghafoor *et al*., 2004). Sewage application although increase crop production because it contains agronomically important plant nutrients such as N, P, K, Zn, Mn, Cu, Fe. But with these it also contains hazardous heavy metals such as Cd^{2+}, Pb^{2+}, Al^{2+}, Ni^{2+} etc (Sanchez *et al*., 1999; Newaj *et al*., 1996). Thus the uncontrolled use of sewage water for irrigation can result in accumulation of some potentially toxic metals in soil and plants and cause adverse effects (Gupta *et al*., 1999). In India total sewage produced annually from cities is about 2600 mm^3 (Paroda and Mruthyunjaya, 1999). Out of which 70% of untreated sewage is applied on agricultural land and 30% discharge after treatment. Haryana produces raw sewage 485 million liter/day. It is estimated that it has the potential to contribute about 56.4 tones/day and 20.872 tones/day cumulatively of all nutrients.

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Seed is the beginning point of the next generation. Seed contains the food reserve in the form of carbohydrates, proteins and fats in more concentration than in plants. In present investigation the effect of sewage application on the amylase activity in Pigeon Pea plant has been studied. Pigeon Pea is the most important pulse crop in India both for vegetable and dry seed purpose. It is a cheaper source of protein. Several enzyme activities and other metabolic processes are known to be disturbed in plant by the presence of high concentration of toxic contaminants present in sewage (Hemalatha *et al*., 1997; Neelam and Jaganmohase, 2003). Amylase acts as growth enzyme and breaks the starch molecule in smaller unit that induce growth. High concentration of sewage causes inhibitory effect on amylase activity and growth.

MATERIALS AND METHODS

In the present study the seeds were collected form Hisar Agricultural University Krishi Vigyan Kendra, Rohtak. Sewage water samples were collected from main sewage discharge site of Rohtak in sterilized plastic bottles. Concentrated Nitric acid @ 5 ml was added at the time of sample collection to avoid adsorption of heavy metals on wall of bottles and to preserve it. Electric conductivity, pH were estimated in unacidified sample. All other parameters were analyzed by Standard methods given by APHA (1979). Heavy metals were estimated as such after filtering by digestion 100 ml of sample with diacid digestion and then determined by AAS.

Amylolytic Activity

Different concentration of sewage effluent was taken to assess the amylolytic activity. Amylolytic activity was assessed in control, 50% sewage and 100% sewage in petriplates. The extraction and assay procedure used for this enzyme was described by Swain and Dekker (1996). Germinating seeds were extracted in 0.02 M tris-HCl (pH 7.4) containing 1mM $CaCl_2$ and 5mM β-mercaptoethanol, the cell free extract was dialysed at 4°C against extraction buffer for 4 hrs. Total amylase activity was assayed after adding 19mM $CaCl_2$ to the dialyzed extract to bring the total concentration of Ca^{2+} ion to 20mM. For determining individual activities of α and β amylase, the dialyzed extract was subjected to different pretreatment in order to selectively inactivate one of these enzymes. Activity of α amylase was determined after rendering β amylase inactive by complete removal of face pre-incubating the dialyzed extract containing 20mM $CaCl_2$ with 7.5 mM p-hydroxymercuric benzoate at 20°C for 20 min. For selectively assaying β amylase activity the dialysed extract which contained only 1mM $CaCl_2$, was pre-incubated with 10mM EDTA at 20°C for 20 min. Under these conditions α amylase was rendered non-functional due to its absolute requirement for Ca^{2+} ions.

RESULT AND DISCUSSION

The physicochemical characteristics of sewage water presented in Table 1 indicate that pH; EC and BOD were within permissible limit. Table 1 also indicates the heavy metal concentrations present in sewage. Except Cadmium and Lead all heavy metals were found within permissible range when compared with irrigation water standards given by Indian Standards.

Table 1. Average physico-chemical characteristics of sewage effluent

Parameters		Sewage Effluent
pH		7.4
Conductance	(ds/m)	1.7
BOD	(mg/l)	250
Cadmium	(mg/l)	0.065
Chromium	(mg/l)	0.183
Nickel	(mg/l)	0.195
Lead	(mg/l)	0.595
Copper	(mg/l)	0.0674
Zink	(mg/l)	0.238

Amylolytic Activity

It was found that different concentration of sewage effluent cause a very interesting effect on the activity of total amylase, α amylase and β amylase. Enzyme activity showes very little increase in the log phase of imbibitions or day 2-4. After day 4 the amylase activity showed a steep rise in the activity of amylase. Total amylase activity of seedling under two gradients of sewage effluent showed constant rise from day 4-8 after imbibitions (Fig 1). In 50% sewage treatment the total amylase activity was found to be more than 100% treatment from day 2-8 after imbibitions i.e. on day 2 it was found to be 83% and 46% of control respectively and on day 8 it was 87% and 51% of control respectively due dilution of heavy metals present in the solution (Fendri *et al.*, 2012). The activity was found to be highest between days 6-8 (Fig 1). Amylase activity was highest in control unit due to non availability of heavy metals. Same trend was found in the activity of α amylase and β amylase as shown in (Fig 2 and 3). On day 2-4 activity was found to be low and then after 4 days there was a steep rise in activity. The α-amylase activity was 38% and 14% of control on day 2 for 50% and 100% sewage treatment respectively and on day 8 it was 17% and 13% of control for α amylase and β amylase respectively (Fig 2). The β-amylase activity was also diminished in sewage treatment as compare to control as on day 8 of imbibition i.e. 60% and 37% for 50% and 100% treatment respectively (Fig 3). The study showed that the amylolytic activity is dose dependent. The enzyme activity at each day was lower for the seedling treated with sewage effluent; activity was lowest for the highest concentration of the sewage i.e. 100% and highest for the control treatment. Sewage water along with nutrients contained heavy metals in varying concentration. So at 50% sewage treatment inhibitory effect was less than the 100% treatment, it may be due to the dilution of toxic substances including heavy metals at 50% treatment, which may interfere in the metabolism of seedling. While at 100% sewage effluent treatment inhibitory effect was more pronounced at the load of toxic substances increase which in term diminished levels of starch also interferes with starch biosynthesis and hydrolysis. Same observations were also documented by Fendri *et al.* (2012).

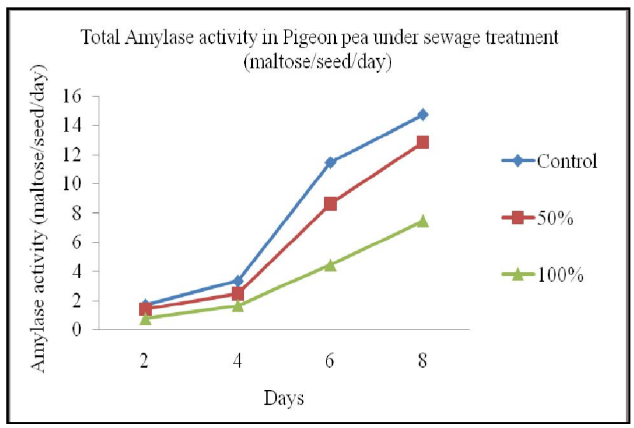

Fig. 1. Total Amylase activity in Pigeon pea under sewage treatment (maltose/seed/day)

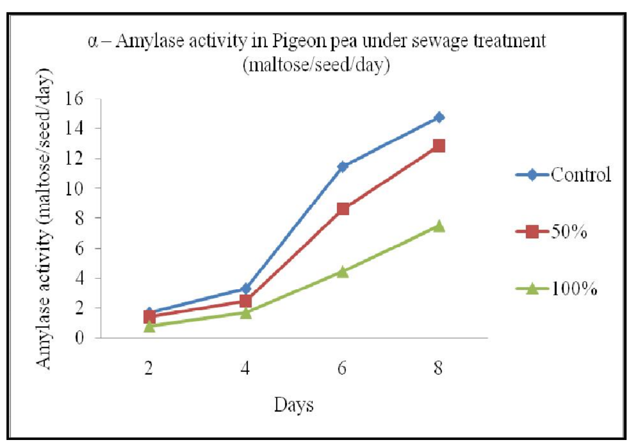

Fig. 2. α – Amylase activity in Pigeon pea under sewage treatment (maltose/seed/day)

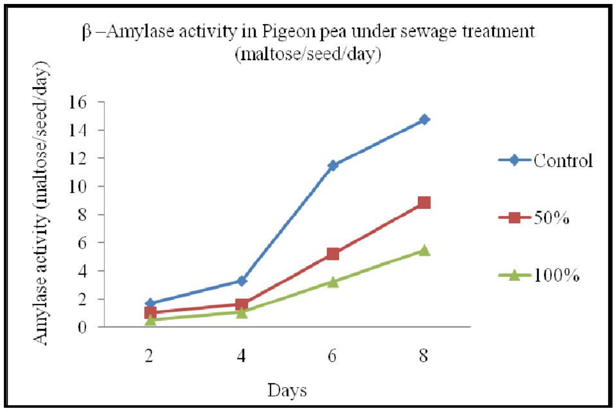

Fig. 3. β – Amylase activity in Pigeon pea under sewage treatment (maltose/seed/day)

CONCLUSION

The present investigation showed that the sewage concentration inhibit the enzyme activity. It was conclude that the amylase activity in present study is dose dependent. The amylolytic activity was found highest in control treatment and lowest in the 100% of treatment. Sewage tends to hasten the appearance of α-amylase during initial stage but its activity was considerably diminished as the germination proceeds. Activity of β-amylase was also suppressed by the high sewage concentration particularly during early stag. After day 4 β-amylase activity had, just begun to increase in seeds. The results showed that the activity increase as the time of imbibition increase and diminished with increased sewage concentration.

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